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JAG1 MUTATION SPECTRUM IN CASES WITH ALAGILLE SYNDROME FROM TURKIYE

TÜRKİYE'DE ALAGILLE SENDROMLU OLGULARDA *JAG1* MUTASYON SPREKTRUMU

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ABSTRACT

Objective: Alagille syndrome (ALGS), known as arteriohepatic dysplasia, is an autosomal dominant multisystem disorder primarily linked to *JAG1* gene variants. It features distinctive anomalies in the liver, heart, eyes, spine, and facial morphology.

Material and Method: Patients diagnosed with ALGS and referred to Istanbul Faculty of Medicine, Department of Medical Genetics between January 2016 and December 2022 were included in the study. The clinical, radiological, cytogenetic, and molecular findings of the patients as well as their families were re-assessed retrospectively. Karyotype, fluorescence in situ hybridization (FISH), array comparative genomic hybridization (aCGH), and *JAG1* gene sequencing utilizing next-generation and Sanger sequencing methodologies were conducted.

Result: The presence of both large deletion and small variants associated with Alagille syndrome was detected in all cases. In karyotype and aCGH analysis of a single case, a 20p deletion was identified. Subsequent next-generation sequencing (NGS) of the *JAG1* gene revealed the following findings: a heterozygous pathogenic variant c.2122_2125del/p.(Gln708Valfs*34), a heterozygous likely

ÖZET

Amaç: Arteriyohepatik displazi olarak da bilinen Alagille sendromu (ALGS), çoğunlukla *JAG1* genindeki mutasyonların neden olduğu otozomal dominant kalıtılan bir multisistem hastalığıdır. Karaciğer, kalp, göz, vertebra ve yüz morfolojisinde belirgin anomaliler içerir.

Gereç ve Yöntem: Çalışmamıza Ocak 2016-Aralık 2022 tarihleri arasında ALGS tanısı alan ve İstanbul Tıp Fakültesi Tıbbi Genetik Anabilim Dalı'na sevk edilen hastalar dahil edildi. Hastalar ve ailelerinin klinik, radyolojik, sitogenetik ve moleküler bulguları retrospektif olarak yeniden değerlendirildi. Karyotip, floresan in situ hibridizasyon (FISH), karşılaştırmalı genomik hibridizasyon (aCGH) yöntemi ile yeni nesil ve Sanger dizileme teknolojisi kullanılarak yapılan *JAG1* geni moleküler ve moleküler sitogenetik sonuçları incelendi.

Bulgular: Tüm vakalarda Alagille sendromuyla ilişkili büyük delesyon veya nokta mutasyonlarının varlığı tespit edildi. Karyotip ve aCGH analizi ile tek bir vakada *de novo* 20p delesyonu saptadı. *JAG1* geninin yeni nesil dizileme analizi aşağıdaki bulguları ortaya çıkardı; heterozigot patojenik c.2122_2125del/p.

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pathogenic variant c.1754_1755del/p.(Asn585Argfs*4), a heterozygous pathogenic variant c.2026del/p.(Cys676Alafs*67), a heterozygous pathogenic variant c.753C>A/p.(Cys251*), and a heterozygous likely pathogenic variant c.2458+2_2458+4delTAAinsGAC/p.(?). In one case, FISH analysis revealed a 20p deletion inherited from the mother. Analysis of available family members further indicated that three variants were inherited within the family. One of the two novel truncating variants, the c.1754_1755del variant was identified as *de novo*, while the other c.2458+2_2458+4delTAAinsGAC variant was determined to be familial.

Conclusion: In summary, the research effectively identified various *JAG1* gene alterations and underlined the significance of incorporating molecular cytogenetic analysis in conjunction with sequence analysis of the *JAG1* gene for accurate genetic diagnosis and counseling. Furthermore, study highlights the valuable outcome of screening parents, siblings, and children to clarify the genetic etiopathogenesis, as there is a remarkable intra- and inter-familial phenotypic variability in patients with ALGS.

Keywords: Alagille syndrome, *JAG1*, arteriohepatic dysplasia

(Gln708Valfs*34), heterozigot olası patojenik c.1754_1755del/p.(Asn585Argfs*4), heterozigot patojenik c.2026del/p.(Cys676Alafs*67), heterozigot patojenik c.753C>A/p.(Cys251*), heterozigot olası patojenik c.2458+2_2458+4delTAAinsGAC/p.(?) varyantları olduğu anlaşıldı. Bir vakada ise FISH analizi ile anneden kalıtılan 20p delesyonu saptandı. Mevcut aile üyelerinin analizi sonrasında üç varyantın ailesel olduğunu anlaşıldı. İki novel varyanttan biri olan c.1754_1755del varyantı *de novo* olarak tanımlanırken, diğer c.2458+2_2458+4delTAAinsGAC novel varyantının ailesel olduğu belirlendi.

Sonuç: Özetle, araştırmamız farklı *JAG1* geni varyantlarını tanımlamakla birlikte ve doğru genetik tanı ve genetik danışmanlık için *JAG1* geninin dizi analizi ile birlikte moleküler sitogenetik analizi birleştirmenin önemli olduğunun altını çizdi. Ayrıca çalışmamız, ALGS'li hastalarda dikkate değer aileler arasında ve aile içi fenotipik değişkenlik olduğundan, genetik etiyopatogenezi netleştirmek için ebeveynleri, kardeşleri ve çocukları taramanın katkısını vurgulamaktadır.

Anahtar Kelimeler: Alagille sendromu, *JAG1*, arteriyohepatik displazi

INTRODUCTION

Alagille syndrome (ALGS), is an autosomal-dominant, multisystem disorder caused by a defective NOTCH signaling pathway with a broad spectrum of clinical variability, spanning from severe life-threatening cardiac or liver complications to mild clinical manifestations. Its prevalence is estimated at 1:30,000 to 1:50,000 live births (1). ALGS is defined by the presence of three of the following five major clinical features along with hepatic bile duct paucity; cholestasis presented with cholestatic liver disease, congenital heart disease (CHD) (commonly pulmonic stenosis), skeletal abnormalities (typically butterfly vertebrae), ocular findings (especially posterior embryotoxon), and recognizable facial features (2). Typical facial features include a prominent broad forehead, deeply set eyes, long nose with a bulbous tip and a pointed chin, giving the appearance of a triangular face. Less frequently, clinical involvement of renal and vascular abnormalities, growth failure, developmental delay and delayed puberty can also occur (3, 4). The clinical phenotype associated with Alagille syndrome manifests considerable interfamilial and intrafamilial variation (5). Clinical findings in heterozygous individuals are unpredictable and can vary from subclinical manifestation to severe liver disease. Early diagnosis and treatment are important for mortality and morbidity in ALGS. The prognosis is related to the severity of bile flow obstruction and scarring of the liver and cardiovascular involvement (3,6). Ninety-four percent of patients exhibit monoallelic pathogenic alterations in the Notch signaling ligand, JAGGED1 (*JAG1*), while 2.5% of patients carry monoallelic pathogenic variants in the Notch signaling receptor NOTCH2 (7, 8). Small sequence variants within the *JAG1* gene account for roughly 85% of the identified pathogenic alterations in ALGS. Further molecular diagnoses, amounting to approximately 9%, are facilitated by employing techniques such as fluorescence in situ hybridization (FISH), array comparative genomic hybridization (aCGH), or multiplex ligation-dependent probe amplification (MLPA) to detect deletions or duplications (8). No genotype-phenotype correlations for *JAG1* and *NOTCH2* pathogenic variants causing ALGS were noted. Only a very small number (3.2%) of individuals clinically diagnosed with ALGS exhibit an identified disease-causing mutation in either of the two genes (8). The *JAG1* gene encodes a transmembrane protein that is a ligand of Notch receptors involved in cell differentiation. So far, 766 variations in the *JAG1* gene have been reported in the Human Gene Mutation Database (HGMD) Professional subscription (November 2022). The pathogenic variants protein truncation which include frameshift, nonsense, exon level deletions and splice site are frequently found in patients with ALGS, although missense variants and whole gene deletions were also demonstrated in the cases. In this study, we conducted a comprehensive analysis of the molecular and clinical characteristics of seven ALGS cases from Turkiye. Notably, one of these cases was diagnosed with ALGS prenatally. We further compared the genetic profiles of these cases with those documented in existing literature.

MATERIAL and METHODS

Patients

The study encompassed cases that had received a confirmed diagnosis of ALGS and had been referred to the clinic of the Department of Medical Genetics at Istanbul Faculty of Medicine, Istanbul University between the years

2016 and 2022. The clinical, biochemical, cytogenetic and molecular findings of the patients were retrospectively reviewed. The study includes probands and their affected family members with ALGS. The study underwent an extensive review process and received approval from the institutional review board at Istanbul University (Date: 07.10.2022, No: 18). Additionally, prior to conducting genetic testing, written informed consent was appropriately obtained from all parents or legal guardians of the participating patients. The research involved reviewing the family history, clinical details, biochemical analyses, pathological assessments, and radiological examinations of the individuals.

Genetic tests

DNA extractions were performed using a kit (MagNaPure, Roche). Genetic testing was carried out using a sequential methodology. Initially, cytogenetic and molecular cytogenetic tests were conducted, and individuals with normal results proceeded to undergo molecular genetic investigation. All encoded exons and exon-intron regions of the *JAG1* gene (NM_000214.3) were sequenced using next generation sequencing (NGS) technology (Miseq, Illumina Inc., San Diego, CA, USA) and confirmed by Sanger sequencing as well as segregated in available parents, except for Case 3 whose parents were also sequenced with *JAG1* by NGS. The variants were checked with the ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and HGMD (http://www.hgmd.cf.ac.uk/ac/) databases. Single nucleotide polymorphism (SNP) and novel variants were assessed by using dbSNP, gnomAD (https://gnomad.broadinstitute.org/). In silico prediction software, Mutation Taster (https://www.mutationtaster.org/), Sorting Intolerant from Tolerant (SIFT, https://sift.bii.a-star.edu.sg), and PolyPhen-2 HumVar, (http://genetics.bwh.harvard.edu/pph2/) were used to predict the pathogenicity. The splice site affect was searched from Splice AI (9). The classification of the variants according to The American College of Medical Genetics and Genomics was based on the Varsome (10) and Franklin databases (https://franklin.genoox.com/). The Karyotype and aCGH analysis were performed in Case 1 due to the diagnosis of multiple congenital anomalies (MCA). A Karyotype analysis was performed using G-banded chromosomes at 500-550 band levels. The aCGH was performed using the Agilent SurePrintG3 CGH+SNP Microarray Kit 4x180K (Agilent Technologies Inc, Santa Clara, CA, USA) according to the manufacturer protocol. The 20p12 band was investigated with FISH using a *JAG1* specific probe (Diagen, Ankara, Turkiye) and was performed in Case 7 with a clinical diagnosis of ALGS whose *JAG1* gene sequence analysis was normal.

RESULTS

Patient characteristics

Seven of the index patients were from distinct families. Among them, two were males and five were females. The clinical, radiological, and molecular findings are summarized in Table 1. In three families, there was a history of individuals similarly affected by the condition (Case 4, Case 6, and Case 7). All cases exhibited facial dysmorphism consistent with ALGS, along with the cardiovascular complications and skeletal abnormalities (as shown in Figure 1). Furthermore, posterior embryotoxon was observed in each postnatal case that underwent eye examination.

Clinical findings

Case 1; a female fetus was the first child of a non-consanguineous couple. A Gestation (G)1 Medical Abortion (MA)1, was terminated at the 20th Gestation Week (GW) due to MCA. The first trimester ultrasound revealed cystic hygroma. Polyhydramnios, a single umbilical artery, hypoplastic left heart syndrome (HLHS), scoliosis, suspicion of hemivertebrae, bilateral pes equinovarus, and shortness of all tubular bones in the ultrasound performed at the 20thGW. The postmortem evaluation revealed characteristic facial findings (hypertelorism, depressed nasal root, bulbous nasal tip, wide columella, thin lips) associated with ALGS and X-ray findings (rib fusion, hemivertebra, vertebral cleft, butterfly vertebra).

Case 2; a 9-year-old male patient, the third child born to non-consanguineous parents (G3P3), was delivered at the 38thGW by cesarean section (C/S) but due to prolonged labor the birth weight was 2550 g (-1.41 SD). His birth length measured 48 cm (-0.05 SD) and his head circumference (HC) was 33 cm (-0.91 SD). The family history did not reveal any notable conditions. His parents and siblings were in good health. He presented dysmorphic facies (sparse hair, broad forehead, triangular face, deep-set eyes, hypertelorism, long nose with a bulbous tip, pointed chin). He was evaluated for jaundice at two months

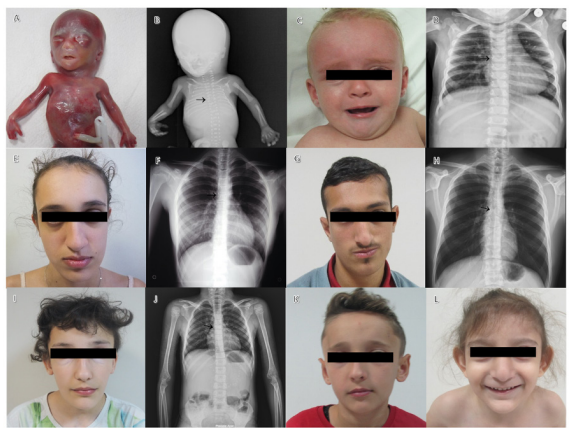

Figure 1: Characteristic facial dysmorphism and skeletal anomalies of ALGS cases
Case 1 (A and B), Case 3 (C and D), Case 4 (E and F), Case 5 (G and H), Case 6 (I and J), Case 2 (K), Case 7 (L). Arrows indicate butterfly vertebrae.

Table 1: Summary of clinical and molecular findings of *JAG1* gene related ALGS

Case	Affecting family members	Liver (biopsy)	Eye	Cardiac	Skeletal	Variant type • dbSNP • Clinvar	Genetic results • nucleotide • peptide
Case I [♀]	none *de novo*	unknown	unknown	hypoplastic left heart	scoliosis, hemivertebrae	gross deletion NA NA	20p13p12 deletion NA NA
Case II [♂]	none *de novo*	cholestasis, bile duct paucity	posterior embryotoxon	PPS	butterfly vertebrae	small deletion rs727504412 VCV000177941.29 **SCV004023385**	c.2122_2125del p.(Gln708Valfs*34)[23]
Case III [♀]	none *de novo*	cholestasis, mild bile duct proliferation	posterior embryotoxon, peripapillary atrophy	BAV, aortic stenosis, mild AR, PDA	butterfly vertebrae	small deletion NA **SCV004023386**	c.1754_1755 del p.(Asn585Argfs*4)
Case IV [♀]	paternal	unknown	posterior embryotoxon	PPS, peripheral pulmonary vascularization	scoliosis, butterfly vertebrae	small deletion NA **SCV004023387**	c.2026 del p.(Cys676Alafs*67)[22]
Case V [♂]	none unknown	bile duct paucity	posterior embryotoxon	PPS	butterfly vertebrae, scoliosis	Nonsense NA **SCV004023388**	c.753C>A p.(Cys251*)[21]
Case VI [♀]	maternal	unknown	posterior embryotoxon	PPS	butterfly vertebrae, scoliosis	splicing small deletion NA **SCV004023389**	c.2458+2_2458+4delTAAinsGAC p. (?)
Case VII [♀]	maternal	bile duct paucity	posterior embryotoxon	PPS	butterfly vertebrae	gross deletion	20p12.2 deletion

PPS: Peripheral pulmonary stenosis, BAV: Bicuspid aortic valve, PDA: Patent ductus arterious, AR: Aortic regurgitation, NA: Not available, VCV: (Variation ClinVar record), SVC: Submitted record in ClinVar

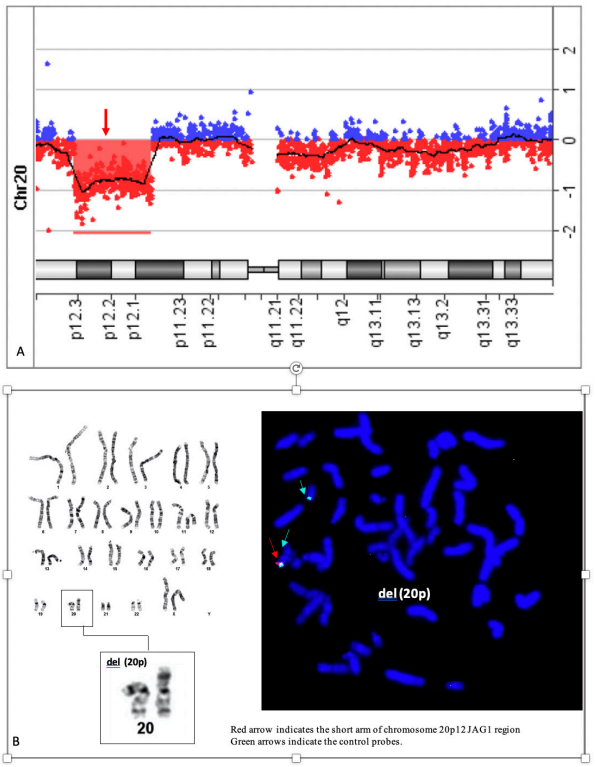

Figure 2: Cytogenetic and molecular cytogenetic results of Case 1 and Case 7

A aCGH showed a 9.5 Megabase (Mb) deletion in the 20p13p12.1 region, including the *JAG1* gene [arr[GRCh37] 20p13p12.1(4709272_14175391)x1] in Case 1.

B Full and partial karyotype and FISH study using JAG1 specific probe showed a deletion on 20p12.2. Red signal is absent on one chromosome 20 in case 7.

of age. The echocardiography revealed peripheral pulmonary stenosis (PPS). He was found to have butterfly vertebrae. A Histopathological examination of the liver at five months of age revealed cholestasis which is associated with the loss of the intrahepatic bile ducts (paucity of interlobular bile ducts). His eye examination showed bilateral posterior embryotoxon, and he underwent a hepaticojejunostomy at the age of two. His growth parameters and motor developmental milestones were normal.

Case 3; a 7-year-old female, the first child born to non-consanguineous parents from the same village (G1P1). The family history was unremarkable. Prenatal ultrasound at the first trimester showed nasal bone hypoplasia and nuchal translucency (NT). Cytogenetic analysis of amniotic fluid cells performed for pathologic ultrasound revealed a normal karyotype. The NT was normal in further scans. She was delivered at term via C/S due to oligohydramnios with a birth weight of 2760 g (-0.84 SD) and a birth length of 47 cm (-1.03 SD). Postnatally, she showed cholestasis (conjugated hyperbilirubinemia with

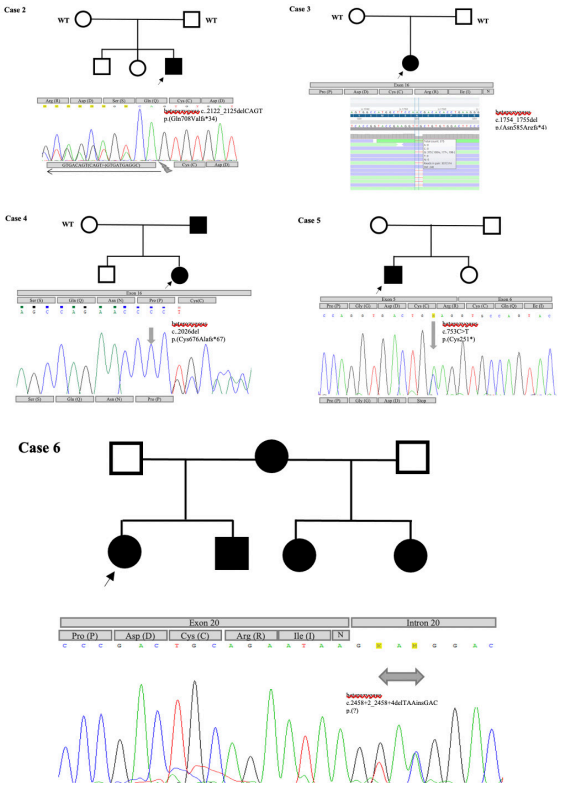

Figure 3: Pedigree analysis and molecular results of Case 2-6. Sanger sequencing of Case 2, 4, 5, 6 and next generation sequencing of Case 3 and her parents revealed *de novo/novel* heterozygous c.1754_1755 del p.(Asn585Argfs*4) variant. WT: Wild Type

high hepatic enzymes) which manifested as jaundice. The echocardiography revealed a mild aortic regurgitation (AR) with aortic stenosis, bicuspid aortic valve, and patent ductus arteriosus. A histopathological examination done at 3.5 months old, revealed cholestasis and mild ductal proliferation. At nine months of age, an eye examination identified posterior embryotoxon and the presence of peripapillary atrophy. Butterfly vertebra in the thoracolumbar region was detected. A physical examination at 10 months of age revealed failure to thrive with a weight 6600 g (-2.53 SD), the length 66 cm (-2.36 SD), and OFC: 44 cm (-0.76 SD). Her physical examination was remarkable for mild jaundice and dysmorphic facial dysmorphic features (pronounced forehead, deeply located eyes, slightly narrow palpebral fissures, flattened nasal root, thin lips, fish mouth, high palate). Her motor developmental milestones were normal.

Case 4; an 18-year-old female patient was the second child born to non-consanguineous parents (G2P2). She was born via C/S (due to history of previous C/S) at the 38th GW with a weight of 3130 g (0.12 SD), a height of 49 cm (-0.05 SD) and a head circumference of 33 cm (-0.91 SD). Her father had unilateral renal hypoplasia. There was

a history of jaundice that did not require postnatal phototherapy. At the age of eight, an increased level of liver enzymes was detected during a routine screening. The echocardiography revealed PPS. Thoracal kyphoscoliosis secondary to butterfly vertebra was detected in a vertebral X-ray. On a thorax MR angiography, bilateral peripheral pulmonary vascularization was slightly decreased. Posterior embryotoxon was detected in an eye examination. Facial dysmorphic findings (broad forehead, deeply located eyes, prominent nasal root, malar/midfacial hypoplasia, short philtrum, wide columella) were observed in the physical examination. Her growth parameters and motor developmental milestones were normal.

Case 5; a 19-year-old male patient was the second child born to non-consanguineous parents (G2P2). His parents and his older brother were healthy. He was delivered via normal vaginal delivery (NVD) at the 34th GW with a weight of 2870 g (2.22 SD). Direct hyperbilirubinemia was found at the postnatal on the third day. An Echocardiography revealed pulmonary stenosis, a doppler ultrasound revealed the absence of bile ducts, and an ophthalmic examination revealed posterior embryotoxon. A liver biopsy revealed an absence of bile ducts in the portal area. At 3.5-years-old the patient presented with complaints of severe itching, jaundice, and swelling in the abdomen. A liver transplant was performed at the age of eleven because of persistent itching. The patient was found to have hyperglycemia on the fourth day after the transplant, and was diagnosed with Type 1 DM in the follow-ups and insulin therapy was started. Butterfly vertebrae and mild thoracolumbar scoliosis were found on vertebral radiographs at nine years of age. He had an operation for urinary incontinence at the age of 11. He was diagnosed with a hepatitis B virus (HBV) infection at the age of 15, received interferon treatment for a duration of one year and subsequently underwent lamivudine treatment. Aseptic necrosis was detected in the femoral head at the age of 15. A physical examination at almost 19 years of age showed growth retardation [Weight: 50 kg (-2.7 SD), Height: 159.5 cm (-2.63 SD)] was present. Facial dysmorphic findings (bitemporal flattening, prominent glabella, synophyria, upward slanted palpebral fissures, malar hypoplasia, prominent nasal arch, inferior columella, short philtrum, prognathia, simple/slightly posteriorly located ears, high palate, posteriorly located upper lateral incisors) were present. His motor developmental milestones and mental development were normal.

Case 6; a 17 year old female patient was the first child of non-consanguineous parents. She was born via NVD at term with a weight of 2950 g (-1.01 SD). There was a family history of the mother affected with posterior embryotoxon and BAV and sisters and a brother affected with vertebral anomalies and hyperbilirubinemia. She was evaluated for direct hyperbilirubinemia at the postnatal on the fourth day of life. A liver biopsy revealed the absence of bile ducts. An abdominal ultrasound performed at the age of seven found a left renal cortical cyst. An eye examination revealed posterior embryotoxon. Pulmonary stenosis was detected on an echocardiography. Butterfly vertebra and thoracolumbar scoliosis were found on an X-Ray. A prominent broad forehead, up upslanting palpebral fissures, hypertelorism, deep set eyes, malar hypoplasia were noted as dysmorphic facial features.

Case7; A 2-year-old female patient was the third child of non-consanguineous parents. Her twin sister and brother were diagnosed with ALGS and her brother died at the age of 2.5 years after a liver transplant. Her mother had facial dysmorphism compatible with ALGS, including renal anomaly (focal ectasia), cardiac anomalies (mild mitral insufficiency, and patent foramen ovale). She was born via C/S (due to history of previous C/S) at the 37th GW with a weight of 2500 g (-0.93 SD). She was admitted at 15 months because of the family history. Facial dysmorphic findings (prominent forehead, upslanting palpebral fissures, malar hypoplasia, hemangioma on the left side of the nose, bulbous nasal tip, antevert ears) were present. Her growth parameters were all normal. Butterfly vertebra was detected in the vertebral X-ray. An Echocardiography revealed pulmonary stenosis. An increased level of liver enzymes was detected. Her growth parameters and motor developmental milestones were normal.

Genetic results

An examination of the available parents' genetic profiles showed that three of the pathological variants were inherited (Case 4-paternal, Case 6-maternal, Case 7-maternal), while the remaining three were *de novo* (Case 1, Case 2, and Case 3) occurrences. Segregation analysis was not feasible for Case 5 because the parents would not consent to undergo testing themselves.

Case 1 had a heterozygous gross deletion on chromosome 20p, which was detected on fetal karyotype analysis. aCGH showed a 9.5 megabase (Mb) deletion in the 20p13p12.1 region, including the *JAG1* gene (Figure 2A). Karyotype analysis of the parents was normal. It was determined that the case was a *de novo* novel gross deletion. Next generation sequencing of the *JAG1* gene revealed a heterozygous c.2122_2125del/p.(Gln708Valfs*34) in Case 2, a heterozygous *novel* c.1754_1755del/p.(Asn585Argfs*4) in Case 3, a heterozygous c.2026del /p.(Cys676Alafs*67) in Case 4, a heterozygous c.753C>A/p.(Cys251*) in Case 5, and a heterozygous novel c.2458+2_2458+4delTAAinsGAC/p.(?) in Case 6, in *JAG1* (Figure 3). In Case 7, *JAG1* gene sequencing yielded normal results. However, upon conducting a FISH examination, a 20p12 deletion was detected. The karyotype and FISH analysis was interpreted as follows: 46,X,del(20)(p12.2).ish del(20)(p12.2)(JAG1-) (Figure 2B). This deletion was also observed in

the affected mother and affected sister, indicating that the variant was inherited within the familial.

All variants were truncating types of mutations including three small deletions and one small deletion insertion resulting in frameshift mutation, and one nonsense mutation (Figure 3). The previously reported pathogenic variants were identified in a total of three individuals (c. c.2122_2125del, c.753C>A, c.2026del). The two variants (c.1754_1755del and c.2458+2_2458+4delTAAinsGAC) were novel truncating and predicted as likely pathogenic [PVS1 (pathogenic very strong), PM2 (pathogenic moderate)] according to ACMG criteria. The deep learning splicing prediction analysis (SpliceAI) of c.2458+2_2458+4delTAAinsGAC was predicted to abolish the Splice Donor site of the pre-mRNA of the *JAG1* showing a maximum pathogenicity score of 1.0.

DISCUSSION

In this study, our objective was to elucidate the clinical, radiological, cytogenetic, and molecular attributes of seven index cases with Alagille syndrome, which also goes by the name arteriohepatic dysplasia. This autosomal dominant multisystem disorder arises from pathogenic variants occurring in the *JAG1* gene. The diagnosis of ALGS in infants and children primarily hinges on their clinical manifestations. Chronic cholestasis stands out as the most common initial symptom, while distinctive facial features play a pivotal role as reliable indicators for ALGS diagnosis.

All six individuals diagnosed postnatally exhibited all five major clinical features. Since it is difficult to detect liver findings, facial dysmorphism and ocular findings in the prenatal period, ALGS must be considered in fetal cases with multiple anomalies including the heart and vertebra. Especially, fetal hemivertebrae detected by detailed ultrasound scan may be a clue for ALGS (11). In Case 1, which had a gross heterozygous deletion of 9.5 Mb, including the *JAG1* gene region, the vertebral anomalies accompanying the hypoplastic left heart syndrome were noted in the prenatal period. In the literature, the diagnosis of ALGS in the prenatal period is rare and is often found with a history of affected parents (12-15). However, a case of HLHS with a de novo gross 20p deletion has been reported, as in our case (16). HLHS is a rare severe CHD characterized by underdevelopment of left heart components and dysfunction of the left ventricle. Our case is a second fetal ALGS case with HLHS in the prenatal period, which was found to have a deletion of the 20p and contributed to the literature in this respect.

Liver disease including biliary tract failure and cholestasis, one of the five main clinical findings of ALGS, is an important suggestive finding for ALGS documented by liver biopsy. The characteristic hepatic pathology in ALGS is defined by a paucity of interlobular bile ducts, accompanied by an absence of significant bile ductular reaction. Currently, a liver biopsy may not be mandatory if there are other characteristic major findings of ALGS syndrome, including cholestasis (17). In our case series, a liver biopsy was done in four of the six postnatal diagnosed cases (Case 2, 3, 5 and 6). The histopathological findings of all cases were consistent with ALGS, except in Case 3, who underwent liver biopsy in the neonatal period.

Case 3, who presented with cholestasis, posterior embryotoxon and bicuspid aortic findings, ductal proliferation, received a liver biopsy in the neonatal period. Although progressive insufficiency of the intrahepatic bile ducts are considered to be the most important and constant feature of ALGS, paucity of the intrahepatic bile ducts may not always be seen in infants younger than six months of age, and a normal ratio of portal tracts to bile ducts, bile duct proliferation, or neonatal giant cell hepatitis may be observed (18). Since ductal proliferation is also seen in a liver biopsy in biliary atresia, it is important to evaluate the liver biopsy results of patients with ALGS in the neonatal period with clinical findings.

About 20% to 70% of ALGS cases may require a liver transplant by age 18 due to liver failure or intense itching. A liver transplant (LT) is advised for pediatric ALGS patients. Selecting appropriate donors for the living donor LT (LDLT) is crucial due to ALGS's autosomal dominant nature. Although genotype-phenotype correlation is unclear, potential asymptomatic donors are often overlooked. Donors can be screened using liver function tests and imaging; if normal, first-degree relatives may serve as LDLT donors. Genetic studies' role in ALGS-related organ donation remains undefined (19). ALGS, a genetic disorder, can lead to early childhood liver transplants with high morbidity and mortality. In Case 7, a sibling history exists: a liver transplant at 2.5 years resulted in fatality. The prognosis is worse for neonatal cholestatic jaundice cases.

The clinical history and findings in ALGS patients underscore notable inter- and intra-familial phenotypic variability, including liver severity and manifestations in other organs. While affected cases often display three or more main features, our case series, excluding prenatal cases, commonly meets all criteria. Affected relatives typically present a single additional criterion besides shared facial appearance. Case 4, Case 6, and Case 7 are familial: one paternal and two maternal inheritance patterns. Case 4 exemplifies intra-familial variation, with an affected father displaying renal hypoplasia and mild facial dysmorphism. Similarly, Case 6 and Caser 7 feature mildly affected siblings and mothers.

In our series of cases, even in the absence of established genotype-phenotype correlations for *JAG1*, we have

observed instances of ALGS patients exhibiting supplementary anomalies such as developmental delay, hearing loss, and autism. Remarkably, these individuals were identified to possess a larger deletion encompassing *JAG1* on chromosome 20p12 (20). Similarly, the presence of HLHS in the fetal case (Case 1) with a 9,5 Mb deletion supports the prediction that large deletions may be associated with additional anomalies in ALGS. In accordance with the literature, it is suggested that microscopic and submicroscopic chromosomal deletions should be excluded before *JAG1* gene sequence analysis, since developmental delay and/or the presence of additional anomalies in addition to the common features in ALGS in our case series may increase the suspicion of chromosomal deletion.

Analysis of available family members revealed that three variants were familial inherited, and two were *novel* single nucleotide variation. The variants (c.753C>A, c.2026del, c.2122_2125del) are rare variants previously reported as a single case report in the literature (21-23). Case 3 and Case 6 had *novel* variants, whereas Case 4, Case 6, and Case 7 were familial.

In this study, a noteworthy fraction of familial cases was noted, with three out of seven patients originating from familial clusters. In ALGS, roughly 30%-50% of cases inherit the pathogenic variant from an affected family member, while the remaining 50%-70% exhibit a *de novo* pathogenic variant.

The presence of multiple affected individuals within Case 6 and Case 7 accentuates the critical role of genetic counseling. In these instances, the diagnosis of ALGS was established in more than one child of a mildly affected mother, leading to significant implications for the family, given the pronounced morbidity and mortality associated with this disorder. Deciphering the underlying molecular etiopathogenesis of the condition in its early stages, combined with well-directed genetic counseling, is poised to enhance the management of familial cases of this nature.

CONCLUSION

Our study highlights the significance of comprehensive assessment of parents, siblings, and children of affected cases, particularly after elucidating the genetic etiopathogenesis. This methodology is crucial due to the notable intra- and inter-familial phenotypic variability observed in patients with ALGS. Such an approach not only deepens the comprehension of the disorder but also holds substantial implications for clinical management strategies and genetic counseling.

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