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CLINICAL CASE

Alagille syndrome associated to *JAG1* gene deletion. An unusual etiology

Síndrome de Alagille por deleción del gen *JAG1*. Una causa poco frecuente

Diana Avila-Jaque[a], **Catherine Díaz**[b], **Rosa Pardo**[c]

[a]Sección de Genética, Hospital San Juan de Dios, Santiago, Chile.
[b]Unidad de Genética, Hospital Roberto del Río, Santiago, Chile.
[c]Sección de Genética y Unidad de Neonatología, Hospital Clínico Universidad de Chile, Santiago, Chile.

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What do we know about the subject matter of this study?

Alagille syndrome is an autosomal dominant genetic syndrome, associated with mutations in the JAG1 and NOTCH2 genes, which usually manifests with cholestasis, cardiac, ocular, skeletal, vascular, and renal anomalies, and distinctive facial features, requiring multidisciplinary management.

What does this study contribute to what is already known?

We describe the clinical and paraclinical features of a patient diagnosed with Alagille syndrome, caused by a copy number variant (arr[GRCh37] 20p12.2(10414643_10792802)x1), which includes the deletion of the entire JAG1 gene, and which has not been previously reported in the literature, thus constituting this report as a relevant tool for the analysis and interpretation of genetic test results.

Abstract

Alagille syndrome (ALGS) is an autosomal dominant, multisystem disorder that typically presents with cholestasis, cardiac, ocular, skeletal, vascular and renal abnormalities, and distinct facial features. Most cases are due to variants in the *JAG1* gene, with only a small percentage involving a complete gene deletion. **Objective:** to contribute to the phenotype delineation and interpretation of a microdeletion not previously described in the literature on chromosome 20. **Clinical Case:** A 4-month-old female patient was diagnosed with a heart murmur. An echocardiogram revealed pulmonary artery stenosis, which, combined with a prominent forehead observed on physical examination, determined her referral to clinical genetics. Because ALGS was suspected, complementary studies were performed, revealing butterfly vertebras and a genetic panel identified a pathogenic heterozygous deletion, encompassing the entire coding sequence of the *JAG1* gene. To rule out a more extensive deletion, a chromosome microarray was performed, confirming a pathogenic microdeletion on chromosome 20 of 378 kb (arr[GRCh37] 20p12.2(10414643_10792802)x1). **Conclusions:** A targeted sequencing panel followed by confirmation with a chromosome microarray allowed the identification and delineation of a pathogenic microdeletion not previously reported in the literature, including the complete *JAG1* gene in a Chilean patient whose phenotype is consistent with ALGS.

Keywords:
Alagille Syndrome;
JAG1 Gene;
Chromosome Microdeletion;
Pulmonary Artery Stenosis

Correspondence:
Diana Avila-Jaque
dianaavilajaque@gmail.com

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Macarena Gajardo Zurita

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Introduction

Alagille syndrome (ALGS) (OMIM #118450) is an autosomal dominant, multisystem disease with a variable phenotype among those affected. It has an estimated prevalence of 1 in 30,000 persons in the general population[1]. It is characterized primarily by bile duct paucity, chronic cholestasis, cardiac, ocular, skeletal, vascular, and renal anomalies, and specific facial features (table 1)[2].

Approximately 95% of ALGS cases are due to pathogenic variants in the *JAG1* gene and 2.5% are related to variants in the *NOTCH2* gene, which are ligand and receptor, respectively, of the NOTCH signaling pathway. Among patients with variants in the *JAG1* gene, only 13% correspond to complete deletions of the gene.

Besides, in approximately 3% of individuals who meet the clinical diagnostic criteria for ALGS, the cause is still unknown[3].

We present the clinical case of a patient diagnosed with ALGS, caused by the arr[GRCh37] 20p12.2(10414643_10792802)x1 deletion, not previously reported in the literature, which includes the deletion of the entire *JAG1* gene.

The objective of this report is to contribute to the phenotypic delineation and interpretation of a microdeletion on chromosome 20 not previously described in the literature.

Table 1. Clinical characteristics of Alagille Syndrome[2,9,12,13]

Characteristic	Frequency
Heart Malformations	75-97%
- Branch pulmonary artery stenosis (35%)	
- Tetralogy of Fallot (12%)	
- Pulmonic stenosis (8%)	
Thoracic butterfly vertebrae	33-93%
Distinctive facial features	80-90%
Ocular anomalies	13- 90%
- Posterior embryotoxon (56-90%)	
- Optic disc anomalies (76%)	
- Axenfeld anomaly (13%)	
Hepatic involvement	75 - 89%
- Chronic cholestasis (89%)	
- Bile duct paucity (75%)	
Pruritus	45% to 88%
Kidney anomalies	39%
Intracranial hemorrhage and cerebrovascular accidents	25%

Clinical Case

A 15-month-old female patient, first child of healthy, non-consanguineous parents. The pregnancy had normal prenatal care checkups and had no complications. She was born vaginally at 40 weeks of gestational age, weighing 2,635 grams (2nd percentile), had a length of 48 cm (8th percentile), and a head circumference of 33 cm (5th percentile), according to the Alarcón and Pittaluga curves[4] with an Apgar score of 9-10.

She evolved with normal psychomotor development and had no intercurrent diseases. At 4 months-old, a heart murmur was identified on auscultation in a pediatric check-up, so she was referred to cardiology. An echocardiogram was performed and revealed branch pulmonary artery stenosis, which, added to the finding of a prominent forehead in the physical examination, led to her referral to clinical genetics.

In the genetic evaluation, characteristics such as high anterior hairline, straight eyebrows, deep-set eyes, prominent ears, low nasal bridge, convex nasal profile with a bulbous tip, short columella, and pointed chin were observed. In addition, a systolic murmur was detected in the pulmonic area during auscultation. No abnormalities were evident in the abdomen, genitalia or extremities.

Suspecting ALGS, complementary examinations were performed. Spine X-rays showed D4 and D6 with butterfly morphology and incomplete ossification of the posterior arch of L5. X-rays of the extremities and pelvis, as well as abdominal and renal ultrasound, showed no abnormalities. In the analysis of hormonal, biochemical, hepatic, and renal function parameters, only a slight increase in LDH was observed.

Because of the main diagnostic hypothesis, a commercial panel for congenital heart defects and heterotaxia was performed, which included the two ALGS-associated genes (*JAG1* and *NOTCH2*), allowing the study of point variants and copy number variations (CNVs) of the genes analyzed, a relevant aspect for the pathology under study. This analysis identified a heterozygous pathogenic microdeletion encompassing the entire coding sequence of the *JAG1* gene, in addition to three heterozygous variants of uncertain significance in the *DNAH11*, *NME8*, and *NEK8* genes (associated with primary ciliary dyskinesia and nephronophthisis, both of autosomal recessive inheritance).

Since a complete deletion of the *JAG1* gene was identified and considering reports of contiguous genes associated with pathologies that might require additional interventions in the patient, it was decided to perform a more exhaustive study by molecular karyo-

typing (Affymetrix CytoScan 750K array). The array confirmed the presence of a pathogenic microdeletion in arr[GRCh37] 20p12.2(10414643_10792802)x1, of approximately 378 kb, which included 5 genes: *MKKS*, *SLX4IP*, *JAG1*, *MIR6870*, and *LINC01752*. This molecularly confirmed ALGS due to the complete deletion of the *JAG1* gene.

The patient met three of the seven clinical criteria for the diagnosis of ALGS, presenting with branch pulmonary artery stenosis, butterfly-shaped thoracic vertebrae, and typical facial features including deep-set eyes, bulbous nasal tip, and pointed chin. In subsequent check-ups, no alterations in liver or renal function tests, ophthalmologic abnormalities, or the presence of xanthomas were observed. Her neurodevelopment was according to her age.

A molecular karyotyping study was suggested to the parents to determine the possibility of being carriers of CNVs, a condition that would confer a 50% risk of recurrence in another pregnancy. However, due to economic reasons, this test was not performed.

Discussion

Traditionally, the diagnosis of ALGS was established by the finding of bile duct paucity on liver biopsy and at least three other of the following clinical features: chronic cholestasis, cardiac, ocular, and skeletal abnormalities, and typical facial features[2]. However, bile duct paucity is seen in only 75% of cases and may be absent in young children or develop during the first 12 months of life[5]. Therefore, liver biopsy is no longer a mandatory requirement for clinical diagnosis, but the presence of at least three of the following features is required: cholestasis, cardiac anomalies (usually peripheral pulmonary artery stenosis), ocular anomalies (mainly posterior embryotoxon), skeletal, vascular, and renal anomalies, or characteristic facial features. Alternatively, the diagnosis is established if two criteria are present along with a first-degree relative with confirmed ALGS[1].

Liver involvement in ALGS varies widely, from mild biochemical alterations to severe cholestasis, portal hypertension, and liver failure requiring transplantation[6]. Up to half of children with severe cholestasis require transplantation before adulthood, while in the rest of children, the liver condition improves or stabilizes[7].

Cardiac malformations occur in 75-94% of patients with ALGS, mainly branch pulmonary artery stenosis (35%), followed by tetralogy of Fallot (12%), and pulmonary valve stenosis (8%)[8].

In almost 90% of ALGS cases, posterior embryotoxon, a prominent Schwalbe's line, is observed. In addition, other ocular anomalies such as Axenfeld anomalies, microcornea, keratoconus, congenital macular dystrophy, shallow anterior chamber, exotropia, and cataracts have been identified. Retinal changes have also been observed, although the visual prognosis is usually favorable[9].

Between 33% and 93% of patients with ALGS have butterfly shaped thoracic vertebrae. Other skeletal phenotypes include tapered distal phalanges and additional digital flexion creases[1].

Renal anomalies affect 39% of ALGS patients, with renal dysplasia being the most common (58.9%). Renal tubular acidosis, vesicoureteral reflux, and urinary obstruction have also been reported[10].

Intracranial hemorrhages and strokes have been documented in about 15% of patients, being fatal in 25-50% of cases. In some of them, the cause could be preexisting vascular malformations such as aneurysms of the basilar artery and middle cerebral artery, moyamoya disease, and internal carotid anomalies. There are also systemic vascular manifestations such as aortic aneurysms and coarctation, renal artery stenosis, and anomalies of large arteries (aorta, celiac, superior mesenteric, subclavian)[11].

The initial approach to individuals diagnosed with ALGS should include an evaluation by gastroenterology to determine the liver function and coagulation; cardiology for physical examination and echocardiography; ophthalmology to rule out anterior chamber alterations; traumatology to determine skeletal involvement, especially butterfly vertebrae; nephrology to study renal function and anatomy; and by clinical genetics to guide molecular diagnostic confirmation and perform pre- and post-test counseling for the family. In addition, children should maintain their usual pediatric check-ups, with anthropometric measurements and neurodevelopmental monitoring[12].

Regarding follow-up, liver disease is one of the most important determinants of morbidity and mortality in ALGS. A model has been developed that combines serum total bilirubin levels in the first two years of life, identification of fibrosis on liver biopsy, and the presence of xanthomas before age 5 years to predict progression of severe liver disease and the need for liver transplantation[7].

Vascular defects are dynamic and can manifest at any age and, because of their high prevalence, routine screening with MRI or angiography is recommended at age 8 years (the approximate age at which general anesthesia is not required for an MRI) and before any major surgery. In addition, these examinations should be performed whenever there are symptoms suggestive of neurological deficit, persistent headache, and hypertension, among others[11].

Given that renal disease manifests in almost 40%

of patients, function tests after the initial nephrological evaluation are essential, especially in children presenting with failure to thrive. This is important because problems such as tubular acidosis are associated with growth difficulties, which can be treated. In addition, in patients undergoing liver transplantation, special attention is needed to monitor the potential nephrotoxicity of immunosuppressants[10].

Pruritus is a common symptom affecting 45% to 88% of children with ALGS. It can be severe and is associated with skin lesions, sleep problems, and mood disturbances[13]. For its management, skin hydration, keeping nails short, and taking short showers to reduce skin dryness are recommended. Improvement of pruritus has also been observed with the use of ursodeoxycholic acid[14].

Although current medical management of individuals with ALGS focuses on symptomatic management, liver transplantation is indicated in cases of severe pruritus, liver failure, portal hypertension, bone fractures, or failure to thrive[15]. The development of new therapies that inhibit the ileal bile acid transporter is under investigation[16].

Facial features described in patients with ALGS include a triangular-shaped face, prominent forehead, deep-set eyes, moderate hypertelorism, bulbous nasal tip, and pointed chin[2]. It is important to note that, in different ethnic groups, this phenotype may not be as evident or manifest with age, especially in young children[17].

Molecularly, 94.3% of ALGS cases are caused by variants in the *JAG1* gene. The most common variants (~75%) are those associated with truncated proteins (frameshift and nonsense mutations; exonic and splice-site deletions). Variants with missense mutation (13%) and whole gene deletions (13%) have also been reported[2,3], as in our case.

Variants that generate truncated proteins and complete gene deletions cause similar phenotypes, supporting the idea that the disease mechanism could be haploinsufficiency[3,18]. The patient has the characteristic phenotype associated with complete deletions of the *JAG1* gene[19].

Amonglarge deletions of the *JAG1* gene, there is variability both in their extent and in the location of their endpoints, suggesting that there are no specific points of rearrangement in this region[3]. In patients with deletions that exceed the critical 5.4 Mb region associated with the *JAG1* gene, other phenotypes not characteristic of the syndrome have been observed, such as developmental delay, hearing loss, autism, and obesity, among others[3,19]. The patient had adequate psychomotor development for her age, which is consistent with the size and location of the identified deletion.

The alteration detected in the patient's chromosome 20 has not been previously reported in the literature but meets the criteria to be classified as pathogenic according to the recommendations of the American College of Medical Genetics (ACMG)[20]. This deletion contains 5 genes: *MKKS*, *SLX4IP*, *JAG1*, *MIR6870*, *LINC01752*. Of these, only *MKKS* and *JAG1* are described in OMIM. The *MKKS* is associated with Bardet-Biedl and McKusick-Kaufman syndromes, both with autosomal recessive inheritance[21,22].

In the congenital heart defects and heterotaxia panel, three variants of uncertain significance were also identified: *DNAH11* c.9884G > A, *NEK8* c.2077del, and *NME8* c.461T > A, all in heterozygosis. The variant in *DNAH11* is associated with autosomal recessive primary ciliary dyskinesia type 7 (OMIM #611884) and is classified in the ClinVar database as of uncertain significance. The *NEK8* gene causes nephronophthisis type 9 (OMIM #613824), an autosomal recessive disease leading to the formation of renal cysts with subsequent progressive renal failure. The variant found in this gene corresponds to a deletion, which is also published in ClinVar and remains of uncertain significance. Finally, *NME8* has been linked to primary ciliary dyskinesia type 6 (OMIM #610852), which is also autosomal recessive. The specific variant was not previously reported and does not meet sufficient criteria to be classified as pathogenic[23].

This case highlights the importance that a genetic diagnostic test should always be accompanied by adequate pre- and post-test genetic counseling. Such counseling helps patients understand and adapt to the medical, psychological, and familial implications of genetic contributions to disease.[24] In addition, it should be considered that, following internationally accepted recommendations, this process should be performed by individuals trained in cytogenetic and molecular concepts, as well as being familiar with the ethical and moral implications[25].

Conclusions

The use of gene sequencing, through a panel including ALGS-associated genes, followed by confirmation with molecular karyotyping, enabled the identification and delineation of a previously unreported pathogenic microdeletion, encompassing the entire *JAG1* gene, in a Chilean patient whose phenotype is consistent with ALGS.

The follow-up of individuals with this condition should be carried out by a multidisciplinary team, focusing especially on hepatic, renal, and vascular involvement, systems that present a higher incidence of morbidity and mortality in this disease.

Ethical Responsibilities

Human Beings and animals protection: Disclosure the authors state that the procedures were followed according to the Declaration of Helsinki and the World Medical Association regarding human experimentation developed for the medical community.

Data confidentiality: The authors state that they have followed the protocols of their Center and Local regulations on the publication of patient data.

Rights to privacy and informed consent: The authors have obtained the informed consent of the patients and/or subjects referred to in the article. This document is in the possession of the correspondence author.

Conflicts of Interest

Authors declare no conflict of interest regarding the present study.

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