

Novel intronic *JAG1* variant associated with Alagille syndrome in a three-generation Lebanese family with variable features

Johnny Awwad^a, Tony Yammine^b, Layal Hamdar^a, Mirna Souaid^b and Chantal Farra^{b,c}

Clinical Dysmorphology 2023, 32:80–83

^aObGyn Department, Fertility Unit, American University of Beirut Medical Center,
^bGenetics Department, Medical Genetics Unit, Saint Joseph University, Beirut and
^cMedical Genetics Department, Hotel-Dieu de France University Hospital, Beirut, Lebanon

Correspondence to Chantal Farra, MD, Genetics Department, Medical Genetics Unit, Saint Joseph, University Beirut, B.P.17-5208 Mar Mikhael, Beirut 11042020, Lebanon
 Tel: +961 1 421 632; fax: +961 1421 632; e-mail: cfarra@hotmail.com

Received 1 February 2022 Accepted 28 November 2022.

List of key features

Alagille syndrome
JAG1
 Whole exome sequencing
 Novel variation
 Cardiac abnormalities
 Diabetes

Introduction

Alagille syndrome (ALGS), otherwise known as arteriohepatic dysplasia, is a complex multisystem autosomal dominant disorder first described in 1969 by Daniel Alagille. ALGS presents itself with variable phenotypes and mostly affects the liver, heart, face, eyes and skeletal system (Mitchell *et al.*, 2018). The estimated prevalence of this syndrome was reported to be 1: 70 000, however, molecular diagnosis has shown that this frequency may reach up to 1: 30 000–1: 50 000 births due to the wide phenotypical range and variability in disease manifestation (Leonard *et al.*, 2014). Primarily, ALGS was diagnosed in the event of absence or scarcity of intrahepatic bile duct, along with a minimum of three of the following five clinical symptoms: chronic cholestasis, cardiac dysfunction or pulmonary artery stenosis, ocular defects, skeletal deformities such as the vertebral arch defects and disease-specific facial features. Characteristic facial features in ALGS include a prominent forehead, protruding ears, a wide nasal bridge and a trilateral face with a pointed chin. Cholestasis characterized by biliary flow obstruction and conjugated hyperbilirubinemia is the most commonly present symptom among ALGS infants (Bresnahan *et al.*, 2016). Growth impairment or delay is present in 50–80% of ALGS patients and is sometimes accompanied by mental retardation (Vajro *et al.*, 2012). Patients of ALGS may also sometimes present with vascular disease and renal failure (Kamath and Piccoli, 2003). Cardiac abnormalities remain the leading symptom in almost 97% of ALGS patients.

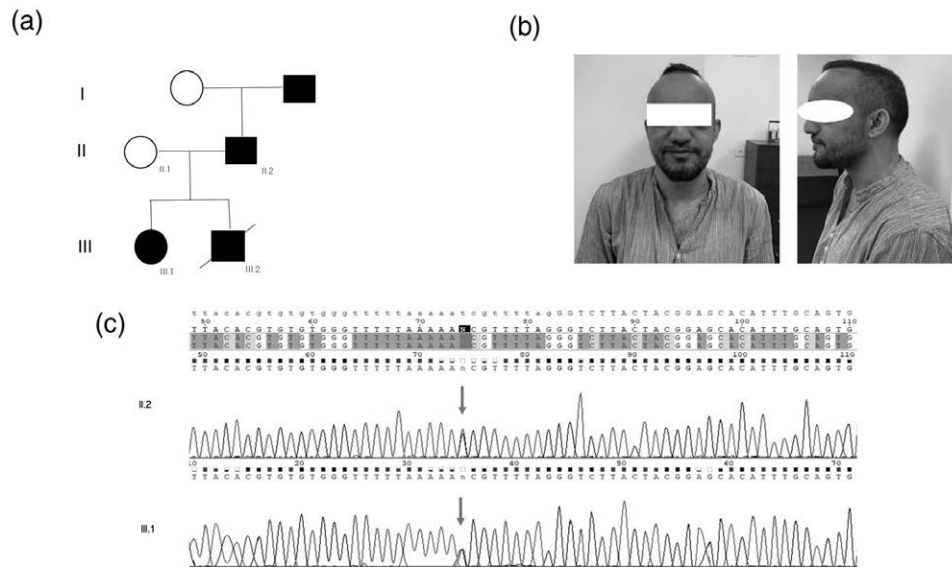
ALGS is caused by intragenic mutations/deletions of one of two genes involved in the Notch signaling pathway which has a significant role in early embryonic development and adult tumor development. These genes are *JAGGED-1* (#601920) located on chromosome 20p12, also known as *JAG1*, and the *NOTCH2* (#600275) located on chromosome 1p12 (Leonard *et al.*, 2014). *JAG1* gene mutations have been identified in around 95% of ALGS patients, whereas only a minority of less than 2% carried *NOTCH 2* mutations (Yokoyama *et al.*, 2019). *JAG1* codes for a cell surface ligand for the Notch receptor and is expressed in the mesocardium, major arteries such as the pulmonary artery, the portal vein, the neural tube and optic vesicle and precursors of the inner ear. There exists a staggering 60% frequency of de novo mutations in ALGS.

Here, we report a unique case of a father and daughter with ALGS who were found to have a never before described likely pathogenic variant in the *JAG1* gene.

Case

A couple presented to our fertility unit with concerns about future pregnancies because of a low ovarian function and the advanced age of the wife (Fig. 1a). However, she showed normal phenotypic features, her 39-year-old husband, presented with syndromic dysmorphic facial features such as a prominent forehead, deep-set eyes and prominent chin suggestive of ALGS (Fig. 1b). He was diagnosed with coarctation of the aorta, an onset of diabetes type 1 at age 9 years, and had undergone a recent splenectomy and kidney transplant due to cholestasis. The patient also suffered from liver disease and hypertension. Blood tests revealed notably elevated Gamma-glutamyl transferase. Renal artery stenosis, although mild, was also noted. Eye examination showed posterior bilateral embryotoxon, however, vertebral defects were identified. The patient reported that his father showed similar facial features and had passed away at the age of 42 years in a similar clinical picture of ALGS with cardiac

Fig. 1



(a) Pedigree of the family studied here (b) Individual II-2 with heterozygous variation, c.2917-9T>G in *JAG1* (c) Sanger sequencing electropherograms for individuals II.1 and III.1 showing the heterozygous c.2917-9T>G variation in *JAG1*.

malformation. No family history of diabetes was otherwise reported in the family. The couple had previously given birth to two children. One son was delivered at 7 months of gestation and died at birth due to tetralogy of Fallot, which is the most common complex cardiac feature in ALGS, and a daughter who was conceived through an intracytoplasmic sperm injection cycle. The latter was born with intrauterine growth restriction and showed dysmorphic facies with mild frontal bossing, wide deep set eyes, depressed nasal cartilage, pointed chin and thin sparse hair. At 4 months of age, she presented with heart murmur, liver cholestasis, failure to thrive and branch pulmonary stenosis with hypo-plastic pulmonary arteries. She, otherwise, showed no signs of hepatomegaly, had normal neurological development, no symptoms of diabetes type 1 and no vertebral or ocular changes at the time of presentation at the age of 7 years.

Investigations

Exome sequencing

After obtaining written informed consent, DNA was extracted from peripheral blood using the QIAamp DNA Blood Mini kit according to the manufacturer's instructions (Qiagen, Germany). Upon fragmentation of the genomic DNA, library preparation was performed using the Twist Human Core Exome Plus Kit (Twist Bioscience, USA) following manufacturers' instructions. The generated library was sequenced on an Illumina HiSeq 2500 (Illumina, USA). An in-house bioinformatics pipeline, including genomic alignment against the hg19/GRCh37 assembly and variant calling was performed with Burrows-Wheeler Aligner- Maximal Exact Matches v.0.7.12 and

genome analysis toolkit Haplotype Caller v.3.4 (Broad Institute, Boston, Massachusetts, USA). An in-house script filter was used to filter the identified variants according to the hypothesized possible inheritance patterns (de novo, autosomal recessive; autosomal dominant and X-linked inheritance). This was also performed for protein-altering variants which include: truncating variants, canonical splice-site variants, and missense variants based on their frequency in the gnomAD database. Variants with minor allele frequency, more than 1% in the gnomAD database, were excluded to only retain rare exonic variants.

In both the father and daughter, whole exome sequencing data revealed a heterozygous variant in intron 23 of the *JAG1* gene *JAG1* (NM_000214.2): c.2917-9T>G, r.2916_2917ins2917-9_2917-1, p.(G1y9773_Val11219delinsArgPheArgValLeuLeuArgSerThrPheAlaValAsnTer) [Chr20(GRCh38): g. 10641253A>C]. No clinically relevant variant was detected in the mother. The finding was confirmed by Sanger sequencing in the proband and her father, who were both found to be heterozygous for the variant (Fig. 1c). Primers were designed using Primer3 for amplifying and sequencing exon 24: 24F (5'-AGCCAGCCTCAAAGAGAACA-3') and 24R (5'-TAACCGAACTGCCTTGCCAT-3'). The identified variant is absent in the gnomAD database and has never been described in association with ALGS.

According to the database of Functional Predictions and annotations for human Nonsynonymous and Splice-Site SNVs database, the variant disrupts the highly conserved acceptor splice site of exon 24 of the *JAG1* gene, resulting

in loss of function. *JAG1* gene has an extreme intolerance to loss of function with a pLI score of 1.

Other splicing variants affecting the acceptor site of exon 24 have been described as disease causing for autosomal dominant Alagille (Liu *et al.*, 2020).

RNA extraction and cDNA sequencing

To assess the hypothetical functional consequences of the identified variant in the patient, a reverse transcription PCR (RT-PCR) study was performed in the affected individuals. RNA was extracted from whole blood using TRIzol reagent (Invitrogen Life Technologies, USA). Reverse transcription was realized using 1500ng RNA with the superscript II reverse transcriptase (Invitrogen Life Technologies, USA). To rule out contamination of RNA samples by genomic DNA, PCR of the housekeeping gene β -globin was performed using the following primers: Globin-F (5'-AAG TTG GTG GTG AGG CCC TG-3') and Globin-R (5'-TTG CCA AAG TGA TGG GCC AG-3'). An amplicon spanning exons 23 and 25 cDNA was analyzed by RT-PCR using the following primers: *JAG1*-23F (5'-ACCTTGCCTGCTCCACAAAG-3') and *JAG1*-25R (5'-CAGCGAGCTGTTTCCATCAC-3'). Sanger sequencing confirmed the modification of the acceptor splice site of exon 24 (Fig. 2a), and the insertion of eight nucleotides into the patient's cDNA resulting in a frameshift mutation, and the creation of a premature stop codon (Fig. 2b). The low expression level of the mutant allele in comparison to the wild type allele may suggest a potential involvement of the nonsense-mediated mRNA decay mechanism (Chen *et al.*, 2020). The variant is classified as likely pathogenic according to the

American College of Medical Genetics (Richards *et al.*, 2015).

Computational tools such as combined annotation-dependent depletion and deep annotating neural network predict pathogenicity for this variant. Ada score (0.9999) and Rf score (0.9500) indicate high deleterious potential, with a splice artificial intelligence score of 0.701.

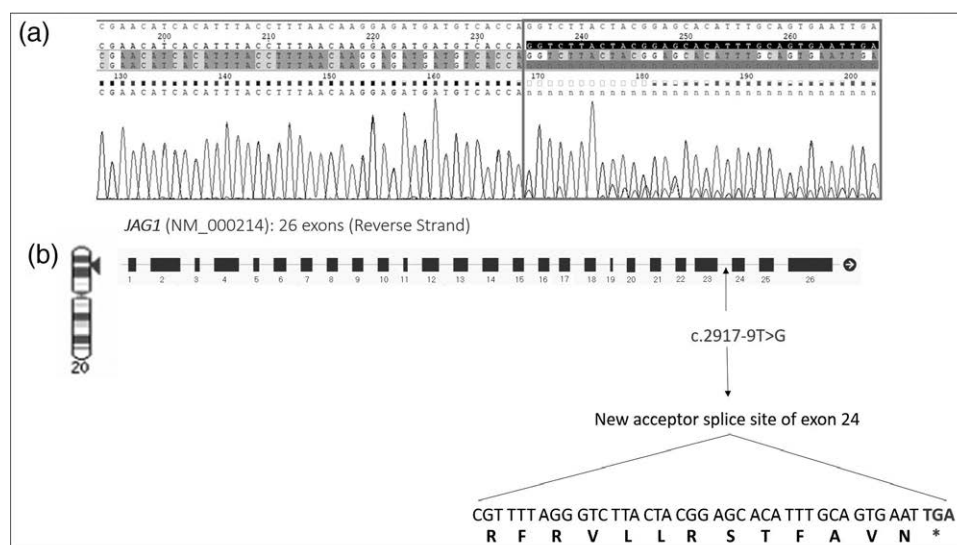
According to Liu *et al.* (2020), single nucleotide variants within splicing consensus regions score 1; meaning that they would likely be more deleterious than all potential non-synonymous single-nucleotide variations predicted by in silico studies.

Discussion and conclusion

ALGS is inherited in an autosomal dominant manner with high penetrance, but variable expressivity results in a wide range of clinical features which involve multiple organ systems.

JAG1 involves 26 exons and codes for the Jagged-1 cell surface protein that plays a role as a ligand in the Notch pathway (Turnpenney and Ellard, 2012). The broad scale of *JAG1* mutations that are linked to ALGS encompasses full gene deletions and nonsense, frameshift and missense mutations. A reason to suggest that the clinically penetrant and variable phenotype of ALGS is caused by the haploinsufficiency of *JAG1* (Grochowski *et al.*, 2016). The location and type of *JAG1* mutation have not yet been proven to suggest the severity of the syndrome. ALGS reports in the Lebanese population, especially adults, are scarce. In the case presented here, we identified a novel heterozygous intronic variant that modifies the acceptor splice site of

Fig. 2



(a) Sequencing of *JAG1* cDNA showing the insertion of eight nucleotides at exon 24 in the patient (b) Figure showing the effect of the variation.

exon 24, creates a premature stop codon and produces a truncated protein. ALGS has been rarely reported in Arab populations and seldom reported in adult patients. A review of the literature revealed a single case of a Lebanese family with ALGS caused by a frameshift variant in exon 11 of the *JAG1* gene (El-Rassy *et al.*, 2008). Our patient and his daughter both, present with common ALGS clinical signs. The father, however, developed type 1 diabetes which has not been previously described in ALGS.

In conclusion, this is a report on a familial ALGS caused by a novel heterozygous *JAG1* likely pathogenic variant. Further reports will contribute to a more thorough understanding of the syndrome and an ultimate genotype-phenotype correlation.

Acknowledgements

The patient's parents signed an informed consent according to the university ethics committee regulations.

Informed consent has been obtained from patients that grants permission for the publication of images as part of this work.

Conflict of interest

There are no conflicts of interest.

References

- Bresnahan JJ, Winthrop ZA, Salman R, Majeed S (2016). Alagille syndrome: a case report highlighting dysmorphic facies, chronic illness, and depression. *Case Rep Psychiatry* **2016**:1657691.
- Chen Y, Liu X, Chen S, Zhang J, Xu C (2020). Targeted sequencing and RNA Assay reveal a noncanonical *JAG1* splicing variant causing alagille syndrome. *Front Genet* **10**:1363.
- El-Rassy I, Bou-Abdallah J, Al-Ghadban S, Bitar F, Nemer G (2008). Absence of NOTCH2 and Hey2 mutations in a familial Alagille syndrome case with a novel frameshift mutation in *JAG1*. *Am J Med Genet A* **146A**:937–939.
- Grochowski CM, Loomes KM, Spinner NB (2016). Jagged1 (*JAG1*): structure, expression, and disease associations. *Gene* **576**:381–384.
- Kamath BM, Piccoli DA (2003). Heritable disorders of the bile ducts. *Gastroenterol Clin North Am* **32**:857–875.
- Leonard LD, Chao G, Baker A, Loomes K, Spinner NB (2014). Clinical utility gene card for: Alagille Syndrome (ALGS). *Eur J Hum Genet* **22**:435.
- Liu X, Li C, Mou C, Dong Y, Tu Y (2020). dbNSFP v4: a comprehensive database of transcript-specific functional predictions and annotations for human nonsynonymous and splice-site SNVs. *Genome Med* **12**:103.
- Mitchell E, Gilbert M, Loomes KM (2018). Alagille Syndrome. *Clin Liver Dis* **22**:625–641.
- Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, *et al.*; ACMG Laboratory Quality Assurance Committee (2015). Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of medical genetics and genomics and the association for molecular pathology. *Genet Med* **17**:405–424.
- Turnpenny PD, Ellard S (2012). Alagille syndrome: pathogenesis, diagnosis and management. *Eur J Hum Genet* **20**:251–257.
- Vajro P, Ferrante L, Paoletta G (2012). Alagille syndrome: an overview. *Clin Res Hepatol Gastroenterol* **36**:275–277.
- Yokoyama K, Minami T, Seki M, Okada Y, Kumagai H, Yamagata T (2019). A boy with Alagille syndrome coexisting with mid-aortic syndrome and renovascular hypertension. *J Cardiol Cases* **21**:28–31.