+ MODEL

Genes & Diseases (2024) 11, 100998

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.keaipublishing.com/en/journals/genes-diseases

RAPID COMMUNICATION

Two unrelated Alagille syndrome cases of South Indian origin: Showing multi-exonic deletion and a novel mutation in *JAG1* gene

Alagille syndrome (ALGS; OMIM #118450) is a highly variable, multi-systemic, autosomal dominant disorder, caused by mutations in the Jagged Canonical Notch Ligand 1 (*JAG1)* gene (20p12.2) or in the Neurogenic locus notch homolog protein 2 (*NOTCH2)* gene (1p13).[1] It primarily affects the liver, heart, eyes, face, kidney, skin, vertebrae, and skeleton.[1,2] The *JAG1* gene has 26 exons and about 90% of the ALGS cases result from pathogenic variants in the *JAG1* and around 7% of cases have deletions in chromosome 20 that include this gene.[1,3] Latest reports reveal that the spectrum of mutations includes 75% protein-truncating mutations and the rest 25% are non-protein-truncating mutations. Few cases (<1%) have also been reported with pathogenic variants in a second gene called *NOTCH 2* gene.[1,3,4] Various studies have elucidated that ALGS clinical phenotype is caused by different pathogenic mutations in *JAG1* and *NOTCH2* genes suggesting the haploinsufficiency of these two genes as the primary mechanism for disease pathobiology rather than a dominant negative mechanism.[1,2] Here we present a case report of two protein-truncating *JAG1* mutations detected in two unrelated cases of South Indian origin.

In case I, we observed a boy aged 4 years and born at the 39th week of gestation through normal delivery (weight 2.6 kg) to non-consanguineous normal parents. The child had global developmental delay, mild intellectual disability, and failure to thrive. Dysmorphic features included triangular facies with a high prominent forehead, pointed chin, icterus, and xanthomas (Fig. 1). He was short-statured with a height of 83 cm (<3rd%ile) and a weight of 11 kg (<3rd% ile). Abdomen ultrasound showed a normal liver in size, echotexture, and normal bilateral kidneys. He had non-visualization of gall bladder (history of previous surgery) and no features of intrahepatic biliary radical dilatation (IHBRD), and his spleen was enlarged (13.4 cm). Laboratory workup showed evidence of cholestatic liver disease and mild anemia. At 35 days of birth, the child had presented with complaints of passing pale-colored stool and was diagnosed with cholestatic jaundice elsewhere. He was evaluated and laboratory workup revealed conjugated hyperbilirubinemia (T/D = 11.9/5.6), raised alkaline phosphatase (ALP)/gamma-glutamyl transferase (GGT), and low hemoglobin (10.2 gm %) (Table S1). The child had neonatal hepatitis/biliary atresia and conjugated hyperbilirubinemia. Histopathology studies showed features of cholestatic liver disease, minimal ductular reaction, and inconspicuous native duct possibility of pediatric-onset inflammatory bowel disease (PIBD). Hepatobiliary iminodiacetic acid (HIDA) scan also suggested biliary atresia and he had undergone Kasai portoenterostomy at 40 days of birth. There was no family history. The ophthalmologic evaluation revealed posterior embryotoxon and there was no evidence of congenital heart disease (CHD). Based on the above clinical features, a probable diagnosis of ALGS was made and therefore the parents were counseled about the need for targeted gene testing.

In case II, we observed a boy aged 4 years and born at the 39th week of gestation followed by normal delivery (weight 2.8 kg) to non-consanguineous normal parents, having dysmorphic facies and features of Alagille syndrome. The child had the following clinical features: deep-set eyes, microcornea, embryotoxon, pointed chin, microstomia, and failure to thrive, with normal developmental milestones. Laboratory findings were suggestive of cholestatic jaundice in the newborn period. The cardiac evaluation revealed mild pulmonary stenosis. He had undergone liver transplantation surgery because of hepatocellular carcinoma at 2.5 years of age. Based on the above clinical features and laboratory findings (Table S2), a diagnosis of Alagille syndrome was made, and targeted gene testing was offered. There was no family history of the same, however,

Peer review under responsibility of Chongqing Medical University.

https://doi.org/10.1016/j.gendis.2023.04.029

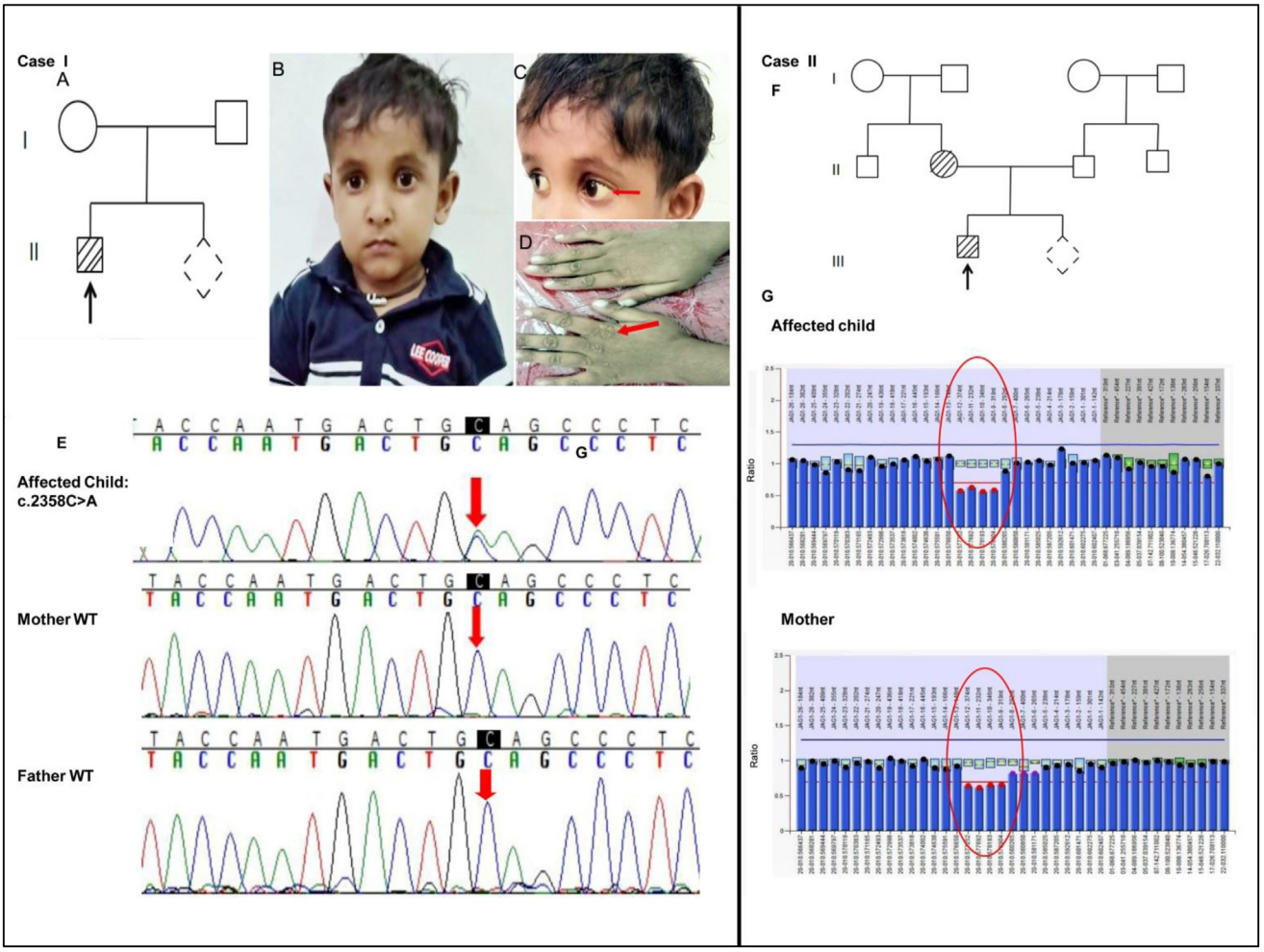

Figure 1 Pedigree, clinical photos, and molecular results of Alagille syndrome. **(A)** Pedigree **(B)** dysmorphic facies, **(C)** scleral icterus, and **(D)** xanthomas in both hands of case I. **(E)** Sanger sequencing results of *JAG1* gene in exon 19 in case I; case I patient carries the heterozygous variant c.2358C >A, and parents are normal. The red arrow indicates the mutation site. **(F)** Pedigree of case II. **(G)** MLPA images of *JAG1* gene of the case II patient and mother showing a multi-exonic deletion 9—12 (red circle).

the mother had mild facial dysmorphism with a pointed chin, prominent broad forehead, and squeaky voice. No embryotoxon, xanthomas, or cardiac disease was noted. Genetic counseling was offered and explained about the conditions of genetic etiology and genetic testing.

DNA was isolated for both cases from EDTA blood using a commercial QI Aamp—DNA mini kit (QIAGENE) and was subjected to targeted gene testing for *JAG1* and *NOTCH 2*. The sequence files in the form of variant call format (VCF) files were collected from sequencing and were subjected to *in silico* analysis as per the American College of Medical Genetics(ACMG) Guidelines and the variants were then analyzed by genetic variant interpretation tool by the University of Maryland for the final interpretation and pathogenecity[5] (Table S3).

Case I showed a heterozygous nonsense variation in the *JAG1* gene (NM_000214.3:*JAG1* gene, exon 19, c.2358C >A, chr20:10644371G>T) (Fig. 1). This nonsense mutation (c.2358C > A) occurred at position 2358 with a C>A transition leading to a stop codon at amino acid position 786 (p.cys786*). This nonsense mutation causes premature truncation of the protein at position 786 leading to a loss of 433 amino acids which affects the protein structure and function that will lead to deleterious consequences and hence is a pathogenic variant (Ia). The parents were not carriers of the same mutation suggesting it to be a *de novo* mutation. This variant has not been reported in any known databases such as 1000 genomes, Exome Aggregation Consortium (ExAC), and Genome Aggregation Database (gnomAD), suggesting it to be a novel mutation to the best of our knowledge.

In case II, the blood sample was subjected to NGS-based targeted screening and the results showed multi-exon deletion (c.(755 + 1_756-1)_(1120 + 1_1121-1)del) at position 755. Since it was an out-of-frame deletion, the same DNA sample was analyzed using the multiplex ligation-dependent probe amplification (MLPA) technique and it showed a heterozygous deletion of exon 9—12 in *JAG1* gene (Fig. 1) which may result in loss-of-function. *In silico* predictions using standard tools such as SIFT, Polyphen-v2.3, and CADD were not possible due to large deletions. The child's mother had a mild phenotype and DNA analysis

showed the same mutation (multi-exon deletion of exon 9—12) (Fig. 1, case II). As the child's mother showed the same mutation with mild facial features only, it makes us understand the autosomal dominant (AD) mode of inheritance with variable expression. Discordance in phenotype is a known phenomenon in patients belonging to the same family and with the same mutation in ALGS.[1,3] As seen in case II, where the child had severe clinical features and liver carcinoma and died (not photographed), whereas the mother had mild features and had reached the second decade without many health issues suggesting variable expression. A single case of similar deletion of exon 9—12 has been reported in a Vietnamese Cohort study.[4] Since the mother is a carrier of the mutation in *JAG1* gene, she is at high risk (50%) of passing the defective mutation to her offspring in every pregnancy.[3] Therefore, genetic counseling becomes important for this family. Options of prenatal diagnosis by chorionic villus sampling (CVS) and amniocentesis were discussed. The pre-implantation genetic testing-monogenic (PGT-M) option also has to be discussed as it eliminates the fear and anxiety of affected fetus/child in such high-risk couples. A comprehensive genetic study using next-generation sequencing (NGS) helped diagnose and identify the mutations, providing genetic counseling and recurrence risk prediction. Both mutations are rare and have not been reported in the Indian population. This study elucidates the importance of genetic workup, future management, and reproductive options like prenatal diagnosis/pre-implantation genetic testing.

Conflict of interests

The authors declare that they have no conflict of interests.

Acknowledgements

The authors are thankful to Prof Dr. Jayasree. P, HOD, Department of Pediatrics Lifeline Superspeciality Hospital for her valuable and expert opinion in the clinical assessment of case I. Thanks are duly acknowledged to Medgenome Labs Private Limited, Bangalore, for helping us in targeted sequencing. We are also thankful to Blessy Mariam Thomas, trainee genetic technologist, for helping us in editing, drafting, and critical review.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2023.04.029.

References

1. Turnpenny PD, Ellard S. Alagille syndrome: pathogenesis, diagnosis and management. *Eur J Hum Genet*. 2012;20(3):251—257.
2. Ayoub MD, Kamath BM. Alagille syndrome: diagnostic challenges and advances in management. *Diagnostics*. 2020;10(11):907.
3. Gilbert MA, Bauer RC, Rajagopalan R, et al. Alagille syndrome mutation update: comprehensive overview of JAG1 and NOTCH2 mutation frequencies and insight into missense variant classification. *Hum Mutat*. 2019;40(12):2197—2220.
4. Lin HC, Le Hoang P, Hutchinson A, et al. Alagille syndrome in a Vietnamese cohort: mutation analysis and assessment of facial features. *Am J Med Genet*. 2012;158A(5):1005—1013.
5. Richards S, Aziz N, Bale S, et al. 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*. 2015;17(5):405—424.

Aswathi N[a], Jincy M. Jose[a], Sreelata B. Nair[a,*], Prabha S. N[a], Pappachan S[b]

[a] *Department of Medical Genetics, Lifeline Super Specialty Hospital, Pathanamthitta, Kerala 691523, India*
[b] *Lifeline Super Specialty Hospital, Pathanamthitta, Kerala 691523, India*

*Corresponding author.
E-mail address: sreelata.nair@gmail.com (S.B. Nair)

29 November 2022
Available online 24 June 2023