A Chinese girl molecularly diagnosed with Alagille syndrome

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Background: **Alagille syndrome (AGS) is a rare or fatal disease affecting multiple systems including the liver, heart, eyes, skeleton and face. It has been considered a genetically heterogeneous disorder of the Notch signaling pathway.**

Methods: **A 28-month-old Chinese girl with congenital heart disease and jaundice was diagnosed with Alagille syndrome by liver biopsy showing a paucity of the intrahepatic bile ducts. Variants of the *JAG1* gene were detected by DNA sequencing in the patient and her unaffected father.**

Results: **A heterozygous missense mutation was identified in exon 2 of the *JAG1* gene in the proband but not in exon 2, 4, 6, 9, 17, 23, 24 by DNA sequencing in her father. The mutation G→T change was seen at position 133 in the cDNA sequence (c.133 G→T), causing a substitution of a leucine for a valine (V45L) residue in the N terminus between signal peptide and DSL domain of the Notch ligand. This mutation, however, was absent in her father.**

Conclusion: **Genes in the Notch signaling pathway should be further studied in AGS, and used to confirm clinical or prenatal diagnosis and facilitate genetic counseling.**

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Introduction

Alagille syndrome (AGS) was first described by David Alagille as an autosomal dominant disease affecting multiple systems including the liver, heart, eyes, skeleton and face.[1-3] It is characterized by a paucity of the intrahepatic bile duct with cholestasis and phenotypic manifestations. AGS is mostly caused by mutations or deletions of gene encoding Jagged1 (*JAG1*), a ligand involved in the Notch signaling pathway.[4] Recently a small subset of other gene mutations for the Notch2 receptor (*NOTCH2*) involved this pathway have been reported.[5] The Notch signals may be directly transmitted from the cell surface to the nucleus and control cell fate during the course of development, especially when precursor cells progress to a more differentiated state.[6,7]

This report presents a 28-month-old Chinese girl with congenital heart disease and jaundice who was diagnosed with Alagille syndrome by liver biopsy.

Case report

A 28-month-old Chinese girl was admitted for the treatment of congenital cardiac disease. She was born at 36th week gestation via cesarean section because of hypertension-induced complication during pregnancy of her mother. Her birth weight was 2400 g, and heart murmur was heard. Her parents were non-consanguineous with no family history of hereditary diseases. At 1 month of age, she had been referred to our hospital because of congenital heart defect and persistent jaundice. Clinical examination showed repeated respiratory infection, jaundice, acholic stools, dark urine, and intense pruritus.

On admission, weight 10.0 kg and height 80 cm indicated growth retardation of the patient. Her head circumference was 44 cm and the anterior fontanelle was not closed (2.0×2.0 cm). Facial abnormalities were present with a prominent forehead and deep-set eyes. Ophthalmologic slit lamp examination for posterior embryotoxon failed because of non-cooperation of the patient. A harsh III grade systolic ejection murmur was heard favorably at the left sternal border. Her abdomen

was soft with marked hepatomegaly. Liver edge was firm and palpable approximately 4 cm below the costal margin, and the spleen was normal. Liver function test showed increased levels of total bilirubin 125.6 μmol/L (reference value 0-19 μmol/L), direct bilirubin 102 μmmol/L (0-6.8 μmmol/L), alanine aminotransferase 63 U/L (5-50 U/L), aspartate aminotransferase 109 U/L (5-55 U/L), g-glutamyltransferase 347 U/L (5-50 U/L), and alkaline phosphatase 926 U/L (<500 U/L). Lipid evaluation revealed total cholesterol 8.08 mmol/L (0-4.4 mmol/L), and triglycerides 5.63 mmol/L (0.36-1.50 mmol/L). The levels of total cholesterol and triglycerides were 10.81 mmol/L and 10.28 mmol/L respectively. Total fatty acid level was not measured. Echocardiography revealed atrial and ventricular septal defects, and pulmonary hypertension. Radiography demonstrated scaphocephaly, but normal vertebrae. A liver biopsy showed portal inflammation with a few lymphocytes infiltrated and a paucity of the interlobular bile ducts (Fig. 1). Alagille syndrome was diagnosed based on clinical manifestations as well as findings of liver biopsy.

With screening of *JAG1* gene mutations by genomic DNA sequencing in exon 2, 4, 6, 9, 17, 23, and 24 in the proband and her father, a heterozygous missense mutation, G→T change at position 133 (c.133 G→T) in exon 2 was identified, which caused a substitution of a leucine for a valine residue in the N terminus of the Notch ligand (V45L). Missense mutation was absent in her father (Fig. 2), while her mother was not detected for unknown reasons.

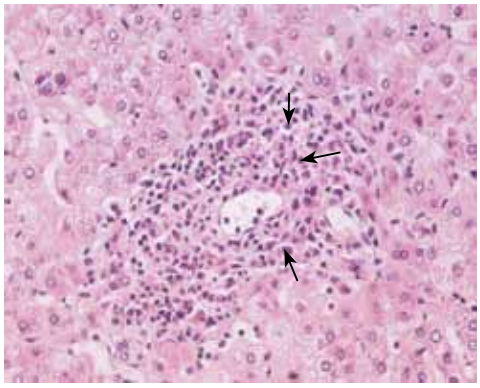

Fig. 1. Liver biopsy showing a paucity of interlobular bile ducts and portal inflammation with a few lymphocytes infiltrated (arrow).

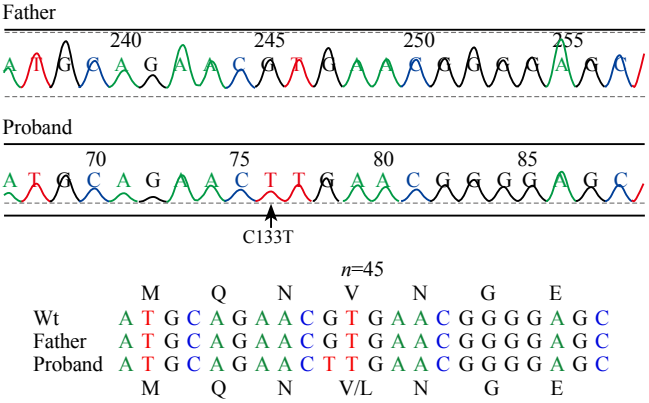

Fig. 2. *JAG1* gene mutations by genomic DNA sequencing: A heterozygous missense mutation, G→T change at position 133 (c.133 G→T) in exon 2, which causes a substitution of a leucine for a valine residue in the N terminus of Notch ligand (V45L), whereas this mutation was absent in her father.

Discussion

The estimated incidence of AGS is between 1/70 000 and 1/100 000 live births.[8,9] The diagnostic criteria of AGS included a histologic paucity in the bile duct shown by liver biopsy in association with 3 of the following 5 major clinical features: (1) chronic cholestasis; (2) congenital cardiac disease; (3) skeletal malformation (butterfly-like vertebrae); (4) ocular posterior embryotoxon; and (5) characterized face. Other features include mental and growth retardation, renal disease, intracranial bleeding, endocrine abnormalities, and dermatologic manifestations.

This phenotypic variability causes difficulties in clinical diagnosis of atypical or mild cases. Liver biopsy is therefore valuable in confirming the diagnosis. The timing of liver biopsy may be critical to demonstrating the paucity of interlobular ducts. Liver biopsies performed after 6 months of age may typically show the characteristic paucity of bile ducts; however, the biopsies performed before 6 months of age may show non-specific intrahepatic cholestasis and portal inflammation.[10]

The present case presented with 3 of the 5 major features of AGS (chronic cholestasis, congenital heart disease and characteristic face) associated with a paucity of interlobular bile duct in liver biopsy specimen. Cutaneous anomalies including excoriations and lichenification were apparent because intense pruritus caused by cholestasis can be an important indication for clinical evaluation of AGS. Moreover, the patient failed to thrive since infancy because of growth retardation, an important clinical feature in some patients, which can be identified in more than 80% of patients.

AGS is thought to be a genetically heterogeneous disorder of the Notch signaling pathway. Most cases are caused by point mutations but a few (3%-7%) have 20p micro-deletion of variable size.[4] Using an aggressive and sequential screening approach combined with mutation detection techniques and rigorous clinical phenotyping, Warthen et al[11] identified *JAG1* mutations

in 94% of individuals. Most of the *JAG1* mutations detected in AGS patients mapped to the extracellular and trans-membrane domains of the protein and led to a premature termination codon, inhibiting the Notch signaling pathway in 70% of the individuals.[11,12] The underlying pathogenic mechanism of AGS remains unclear. Haploinsufficiency for the *JAG1* gene has been proposed as the primary mechanism responsible for AGS since patients with large deletions including the entire *JAG1* gene have phenotypes similar to those with intragenic mutations.[4]

The correlation between mutations of the *JAG1* gene and AGS phenotype is still uncertain because variable manifestations in affected members of a AGS family result from the same mutation. In a Japanese study,[13] two fraternal twin children inherited the same mutation from their father and underwent the same intrauterine conditions of development but had different phenotypes. The only hypothesis is that the pathogenesis of AGS involves the regulation of other genes except *JAG1* or that expression of the mutant *JAG1* gene is affected by different maternal genes.

A heterozygous missense mutation was identified in exon 2 of the *JAG1* gene in the proband but not in his unaffected father. The mutation G→T change at position 133 in the cDNA sequence (c.133 G→T) causes a substitution of a leucine for a valine (V45L) residue in the N terminus between signal peptide and DSL domain of the Notch ligand. This mutation has been detected in a Japanese patient with extrahepatic biliary atresia (EHBA).[14] Thus *JAG1* missense mutations in AGS suggested that the mutation results in failure of the *JAG1* mutant protein to reach the cell surface and is probably associated with loss of Nocth signaling activity relative to wild type *JAG1*, which may impair either differentiation or cell migration during the development of multiple systems.[15,16]

The genes in the Notch signaling pathway should be further studied in AGS and used to confirm clinical or prenatal diagnosis and facilitate genetic counseling.

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