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Original Article

De novo JAG1 gene deletion causes atypical severe Alagille syndrome in a Chinese child

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Abstract: Alagille syndrome (ALGS) is a multisystem autosomal dominant disorder caused by defects in the Notch signaling pathway. The classical criteria for ALGS diagnosis include bile duct paucity on liver biopsy in association with three of the following: Cholestasis, congenital heart disease, vertebral abnormalities, characteristic facial features, and posterior embryotoxon. However, the diagnosis is very difficult in an atypical case. Molecular confirmation of ALGS diagnosis is valuable. Here we report an atypical severe ALGS case with de novo JAG1 gene deletion. A 2-month-old Chinese boy presented with cholestasis and was initially referred to our hospital. Biliary atresia (BA), progressive familial intrahepatic cholestasis, ALGS, and other conditions with cholestasis were suspected. BA was ruled out by Omnipaque imaging through peritoneoscopy. The etiology of the child's cholestasis was unclear after liver biopsy, Sanger sequencing of the JAG1 gene, and next-generation sequencing of a panel of 41 genes known to cause genetic cholestasis disorders in children. The child's ALGS was eventually diagnosed through multiplex-ligation-dependent probe amplification dosage analysis. Heterozygosis JAG1 gene deletion was found in the child's JAG1 gene. The child died because of liver failure when he was 9 months old, although he underwent adequate treatment. We concluded that even though the child had no other typical ALGS manifestation, an infant with cholestasis should always prompt pediatricians to exclude ALGS with different genetic testing methods. And the heterozygosis De Novo JAG1 gene deletion can lead to severe liver disease and cause liver failure and death at an early age.

Keywords: Alagille syndrome, JAG1 gene, gene variations, atypical, liver failure

Introduction

Alagille syndrome (ALGS; OMIM 118450) is a multisystem autosomal dominant disorder caused by defects in the Notch signaling pathway, which can affect the liver, heart, skeleton, eyes, kidneys, and central nervous system, and there may be characteristic facial features [1-3]. The prevalence of ALGS is reported to be 1:70,000, based on the presence of neonatal liver disease, but this is almost certainly a significant underestimate because it does not account for the variability and reduced penetrance of the condition, which became clear through family studies and the advent of genetic testing [4].

The phenotypic findings in ALGS are highly variable with regard to severity and clinical significance [5]. Previously, ALGS was thought to have a relatively good long-term prognosis in terms of liver disease [1, 6-8]. But it is now well recognized that some patients with ALGS can present with severe liver complications [9-12].

Laboratory findings most commonly include elevations of serum bile acids, conjugated bilirubin, alkaline phosphatase, cholesterol, and g-glutamyl transpeptidase (GGT), which indicate a defect in biliary excretion. Few patients have a normal GGT level [13, 14].

In 1997, the association of ALGS with mutations in the human Jagged 1 gene (JAG1), which encodes a ligand in the Notch signaling pathway, was published [15, 16]. Recently, using improved detection methods, up to 94% of phenotypic patients with ALGS have been shown to have JAG1 mutations, with a small sub-

set showing mutations in the gene for its receptor Notch2 [17, 18].

The classical criteria for ALGS diagnosis include bile duct paucity on liver biopsy in association with three of the followings: cholestasis, congenital heart disease, vertebral abnormalities, characteristic facial features, and posterior embryotoxon [1]. In an atypical or mild case, molecular confirmation of ALGS diagnosis is valuable [19]. And diagnosis of ALGS can be made from a compatible genetic abnormality with either one syndromic feature or family history of ALGS [20-22]. Thus, exploring new mutations or polymorphisms is still of great clinical importance.

Among the reported patients in whom mutations have been identified, total gene deletions, protein truncating mutations (frameshift and nonsense), splicing, and missense mutations have all been reported. The mutations are distributed across the entire coding region. Fifty percent to 81.1% of mutations are de novo [18, 23].

Total gene deletions are observed in 5-7% of patients [13]. Here we report an atypical severe ALGS case in a Chinese child with de novo heterozygosis JAG1 gene deletion.

Patient history

A 2-month-old male infant with normal facial features was initially referred to our hospital for jaundice and lightly yellowed stool. The infant had been full-term at birth (spontaneous delivery via parturient canal) with a weight of 2500 g. He was formula-fed and showed delayed growth and development. Laboratory tests conducted at that time showed elevated liver enzymes and cholestasis. Gamma glutamyl transpeptidase (GGT) level was elevated slightly, but serum albumin level and the test of blood clotting were normal. Renal function test was also normal. Blood gas analysis showed slightly metabolic acidosis. Blood glucose was slightly lower than normal, but cholesterol and triglyceride were normal after fasting for 4 hours. Tandem mass spectrometry analysis of blood and gas chromatography mass spectrometry analysis of urine were normal. Markers for active hepatitis A, B, C, and E infection were negative, as well as for IgM antibodies to toxoplasma, cytomegalovirus, herpes virus, and Epstein-Barr virus. Cytomegalovirus DNA in blood and urine was negative. The test of anti-HIV, toluidine red unheated serum test, and treponema pallidum particle assay were negative. The child showed no excretion of the isotope 24 hours after undergoing a hepatobiliary iminodiacetic scan. Biliary atresia (BA) was suspected. Omnipaque imaging through peritoneoscopy was done 10 days after hospital admission. Gallbladder, cystic duct, and choledochus were normal, but intrahepatic bile ducts phanerosized ambiguously and were very thin; Intrahepatic biliary hypoplasia was founded. Liver biopsy was done, and cholestasis in liver cells and small bile duct proliferation in the portal area were found. Echocardiography showed patent ductus arteriosus and open foramen ovale. An ophthalmologist examined the patient, and posterior embryotoxon was negative. “Butterfly” vertebrae were found in some thoracic vertebrae by spine radiography. Abdominal ultrasound found slight hepatomegaly and renal ambiguity. Ursodeoxycholic acid, cholestyramine, and lipid-soluble vitamins were given to the child. However, total bilirubin and direct bilirubin elevated gradually. Lower albumin, prolonged prothrombin time, and highly international normalized ratio were noted when he was 7 months old. The child died because of liver failure when he was 9 months old.

Mutation detection

With the approval of the ethics committee of Children’s Hospital of Fudan University and written informed consent from parents, gene tests of the child and his parents were done. All 26 coding exons of JAG1 (RefSeqNM_000214.2) of the child were sequenced. Homozygosis mutation c.133G>T (p.V45L) located within exon 2 in the JAG1 coding sequence were found in the child. The mutation was associated with BA. Heterozygosis mutation c.133G>T was found in the mother but not in the father. Multiplex-ligation-dependent probe amplification (MLPA) dosage analysis was conducted to detect partial or whole gene deletions in the JAG1 gene. MLPA analysis was performed according to the manufacturer’s instructions using the P184 MLPA kit available from MRC-Holland (Amsterdam, Netherlands). Heterozygosis JAG1 gene deletion was found in the child, but his mother and father were not found to have the deletion (**Figure 1**).

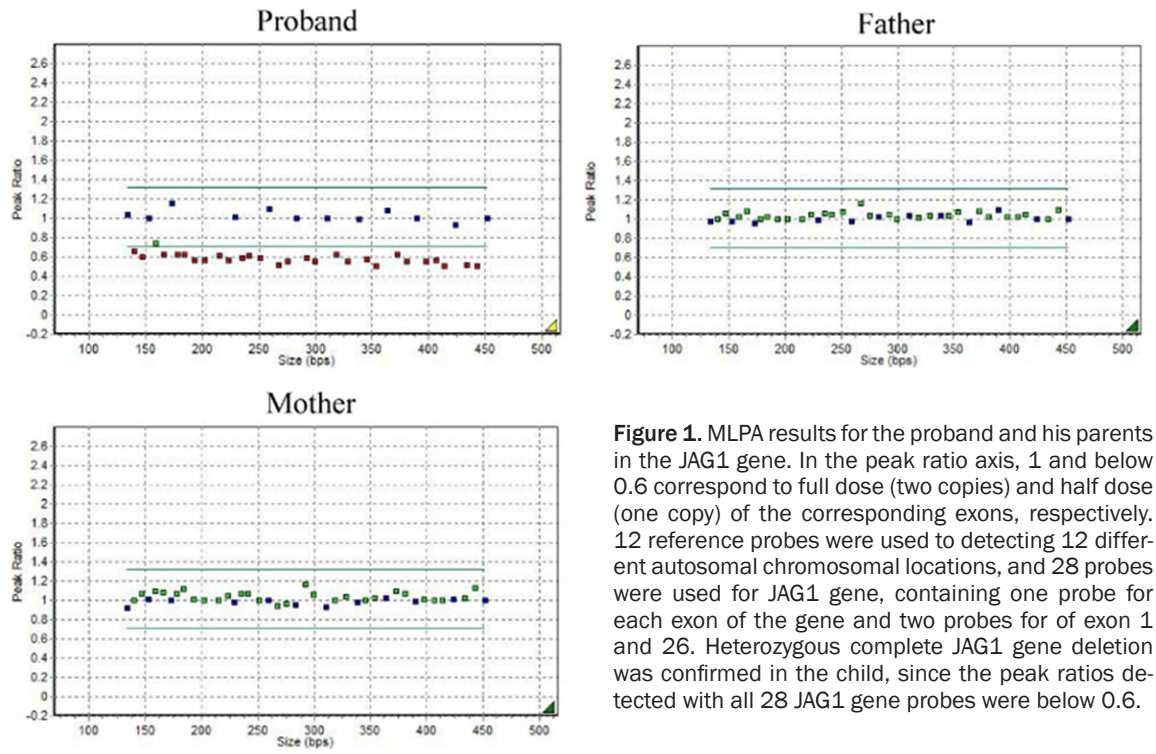

Figure 1. MLPA results for the proband and his parents in the JAG1 gene. In the peak ratio axis, 1 and below 0.6 correspond to full dose (two copies) and half dose (one copy) of the corresponding exons, respectively. 12 reference probes were used to detecting 12 different autosomal chromosomal locations, and 28 probes were used for JAG1 gene, containing one probe for each exon of the gene and two probes for of exon 1 and 26. Heterozygous complete JAG1 gene deletion was confirmed in the child, since the peak ratios detected with all 28 JAG1 gene probes were below 0.6.

ALGS caused by Notch2 gene mutation, progressive familial intrahepatic cholestasis (1, 2, 3, and 4), citrin deficiency, bile acid synthetic defects, alpha-1-antitrypsin deficiency, Wolman disease, neonatal ichthyosis-sclerosing cholangitis syndrome, cerebrotendinous xanthomatosis, mitochondrial DNA Depletion syndrome, hereditary tyrosinemia type I, galactosemia, fructose intolerance, cystic fibrosis, and ARC syndrome were ruled out by the next generation sequencing (NGS) of a panel of 41 genes known to cause genetic cholestasis disorders in children.

Discussion

Diagnostic criteria of ALGS have been defined as the presence of intrahepatic bile duct paucity on liver biopsy in association with at least three of the five major clinical features: Chronic cholestasis, cardiac disease (most often peripheral pulmonary stenosis), skeletal abnormalities (typically butterfly vertebrae), ocular abnormalities (primarily posterior embryotoxon), and characteristic facial features [1, 24, 25]. The revised diagnostic criteria state that the presence of a disease-causing mutation in addition to at least one major clinical feature is sufficient for a diagnosis of ALGS [1, 2, 26].

The child's ALGS was eventually diagnosed through the MLPA gene test. However, the case was very complicated. Distinguishing ALGS from other causes of infantile cholestasis, especially BA, is important but can be difficult. In ALGS, the presence of other nonspecific liver biopsy findings such as bile duct proliferation early in life causes diagnostic confusion with BA [11]. The child had homozygosis mutation c.133G>T (p.V45L) located within exon 2 in the JAGGED1 coding sequence by direct sequence. Homozygosis mutation c.133G>T (p.V45L) is a causative mutation of BA. However, heterozygosis mutation c.133G>T was found in the mother but not in the father. Furthermore, BA was ruled out by Omnipaque imaging through peritoneoscopy. We believe that it is the heterozygosis JAG1 gene deletion that causes the illusion of homozygosis mutation c.133G>T (p.V45L) in the child when detected by the Sanger direct sequence. NGS technologies have revolutionized genomic and clinical genetic research [11, 27-29]. NGS offers comprehensive sequencing of multiple known causative or associated genes in highly heterogeneous diseases [29]. For this child, we distinguished ALGS from progressive familial intrahepatic cholestasis, neonatal intrahepatic cholestasis

caused by Citrin deficiency, and other conditions caused infant cholestasis by NGS.

The ALGS phenotype varies from subclinical to life-threatening, and can include heart or liver failure. ALGS has long been considered to have a relatively good long-term prognosis in terms of liver disease, and liver complications were the cause of death in only 5% of patients in a previous study. It is now recognized, however, that some patients with ALGS can present with severe liver complications such as liver failure and/or hepatocellular carcinoma [9, 30]. The child died because of liver failure when he was nine months old even though he underwent adequate treatment.

In conclusion, an infant with cholestasis should always prompt pediatricians to exclude ALGS with genetic testing even though he had no other typical ALGS manifestation except cholestasis. Also, we sometimes need several different gene testing methods to make the eventual diagnosis of ALGS. Sometimes, Sanger sequencing of the JAG1 gene is not enough to confirm the etiology of cholestasis; We need further gene testing, like MLPA. Furthermore, the heterozygosis JAG1 gene deletion can lead to severe liver disease and cause liver failure at an early age (early-onset liver failure), but it has little impact on the other systems or organs. It was unfortunate that we did not examine the JAG1 protein level and its function of the heterozygosis JAG1 gene deletion in our patient. Further research should be done in this area.

Disclosure of conflict of interest

None.

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