Original Article

JAGGED1 gene variations in Chinese twin sisters with Alagille syndrome

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Abstract: Variations in the JAGGED1 gene have been found to cause Alagille syndrome. Nevertheless, no particular hotspots in the gene have been found; any part of the entire coding regions for JAGGED1 may be involved. Twin sisters with jaundice visited our hospital and were diagnosed with Alagille syndrome. The gene variations in their JAGGED1 coding sequences were evaluated by complementary DNA sequencing. The 12-month-old twin sisters have broad foreheads, deep-set eyes, pointed chins, and triangular faces with jaundice. Clinical testing showed the presence of posterior embryotoxon, butterfly vertebrae, and atrial septal defect. Biochemical indexes showed cholestasis and liver damage. Three conserved variations were identified within exons 22 (c.2612C>G), 24 (c.2957T>A), and 26 (c.3417T>C) in the JAGGED1 coding sequence. The predicted consequences for c.2612C>G, c.2957T>A, and c.3417T>C were p.Pro871Arg, p.Leu986*, and p.Tyr1139=, respectively. The T to A change in the JAGGED1 coding sequence at 2957 will generate a stop codon and might lead to deletion of amino acid 233 at the C terminal of the JAGGED1 protein. Our data suggest that gene variations of c.2612C>G, c.2957T>A, and c.3417T>C, especially c.2957T>A, might have contributed to the pathogenesis of Alagille syndrome in these Chinese twin sisters and provided new gene evidences for Alagille syndrome.

Keywords: JAGGED1, gene variations, Alagille syndrome, bile duct paucity, posterior embryotoxon, butterfly vertebrae

Introduction

Alagille syndrome (ALGS) is a complex multisystem disorder that affects the liver, heart, eyes, kidneys, and skeletal system [1-3]. The typical pathophysiological characteristics of ALGS in liver, heart, eyes, kidneys, and skeletal system are bile duct paucity, peripheral pulmonary artery stenosis, posterior embryotoxon, renal dysplasia and renal tubular acidosis, and butterfly vertebrae, respectively [4-6]. Characteristic facial features mainly manifest as broad forehead, deep-set eyes, pointed chin, and always a triangular face [5]. ALGS is quite rare with a conservative estimate of its frequency reported at 1:70,000 to 1:100,000 among live births [7, 8]; ALGS was first described in 1973 by Watson and Miller and in 1975 by Daniel Alagille [9, 10]. Mortality is approximately 10%, with most deaths resulting from vascular accidents, cardiac disease, and/or liver disease [11]. The clinical features are highly variable, even within families, which complicates the diagnosis [12]. Clinical primary diagnosis is established with the identification of at least three of the above five main clinical characteristics in the liver, heart, eyes, kidneys, and skeletal system [13, 14].

ALGS is an autosomal dominant inherited disease with low penetrance and highly variable expression [15]. Genetic findings showed that up to 95% of ALGS cases are due to mutations in the JAGGED1 gene and <1% of ALGS cases are caused by mutations in the NOTCH2 gene [16, 17]. The JAGGED1 gene encodes the ligand for the NOTCH receptor, which, together with other NOTCH receptors, plays critical roles in development [7, 18]. The NOTCH signaling pathway activates genes that inhibit cellular differentiation along particular developmental pathways [19]. Hence, NOTCH signaling is critical for
Mutations in JAGGED1 disrupt the signaling pathway, causing errors in development, especially of the heart, bile ducts in the liver, spinal column, and certain facial features [21]. To date, at least five human NOTCH ligands and four receptors are reported and have been implicated in human diseases, including ALGS [22]. All NOTCH receptor ligands share a common structure that includes an amino-terminal extracellular conserved DSL domain, a variable number of EGF-like repeats, and a single transmembrane domain [22].

The JAGGED1 gene is located at 20p12.2, has 26 exons and 25 introns, and codifies for three different transcripts depending on alternative splicing [23]. The transcript JAG1-002 (Transcript: JAG1-002 ENST00000254958) expands 5.901 kb, and encodes a 1218-amino-acid protein with 11 different domains. To date, more than 400 JAG1 mutations have been identified, of which as many as 70% are protein-truncating and many are correlated with the pathogenesis of ALGS [4]. Nevertheless, there are no particular hotspots, and any part of the entire coding region may be involved [17, 24]. Thus, exploring new mutations or polymorphisms is still of great clinical importance. In this report, the gene variations in the JAGGED1 coding sequences were evaluated in Chinese twin sisters.

Materials and methods

Patients and ethical issues

12-month-old twin sisters, suspected of having infantile cholestatic hepatopathy, who visited our hospital in April 2014 met the standard criteria for the diagnosis of ALGS.

This study was approved by the Research Ethics Committee of Children’s Hospital of Fudan University and was conducted under the Declaration of Helsinki ethical principles for medical research involving human subjects (CHFU14-0032). Informed consent was obtained from the children's parents.

Clinical diagnosis

The clinical diagnosis of ALGS was established by the following pathophysiological features: butterfly vertebrae identified in the frontal and lateral X-ray examination; presence of posterior embryotoxon examined by ophthalmic testing; atrial septal defect identified by echocardiography; characteristic facial features (broad forehead, deep-set eyes, pointed chin, and a triangular face with jaundice) via observation.

Histologic examination of the liver, kidney, neurovasculature, and pancreas was waived considering ethical issues and clinical necessity. Instead of invasive biopsies, abdominal ultrasonography and biochemical tests were performed to elucidate the liver, spleen, and renal function and structure.

Differential diagnosis

Biliary atresia was excluded by color of the stool, biochemical tests, and isotope hepatobiliary scintigraphy. Metabolic disorders, Down syndrome, infectious diseases, immunologic disorders, and other conditions such as Zellweger syndrome and Ivemark syndrome were excluded by patient history, biochemical tests, and genetic testing. Rieger syndrome and Bannayan-Riley-Ruvalcaba syndrome were excluded by pathophysiological features in addition to posterior embryotoxon.

The deletion 22q11.2 syndromes, such as ventricular septal defect and tetralogy of Fallot, have also been reported as having butterfly vertebrae and poor growth; these diseases were excluded by the complication of liver disease and testing for this deletion.

Laboratory biochemical measurements

Liver function (including total protein, albumin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transpeptidase, total bilirubin, indirect bilirubin, direct bilirubin, and total bile acid) and renal function (including blood urea nitrogen, creatinine, albumin, and ions) were assessed in the hospital's laboratory according to routine procedures.

Gene variation analysis

Gene variations in the cDNAs of JAGGED1 were evaluated by RT-PCR and sequencing. Briefly, total RNA from peripheral blood lymphocytes was isolated with TRIzol® reagent (Life Technologies Corporation, Frederick, MD). The cDNA was synthesized using a two-step RT-PCR Clone Kit according to the manufacturer’s instructions (Biovisualab, Shanghai, China).
JAGGED1 mutants in ALGS

Table 1. Clinical and genetic features of patients

<table>
<thead>
<tr>
<th>Clinical and genetic indexes</th>
<th>Characteristics of the patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Female</td>
</tr>
<tr>
<td>Age, Months</td>
<td>12</td>
</tr>
<tr>
<td>Facial features</td>
<td>Yes</td>
</tr>
<tr>
<td>Jaundice</td>
<td>Yes</td>
</tr>
<tr>
<td>Disorder in kidney</td>
<td>Histopathological change unknown</td>
</tr>
<tr>
<td>Disorder in heart</td>
<td>Atrial septal defect</td>
</tr>
<tr>
<td>Disorder in liver</td>
<td>Histopathological change unknown</td>
</tr>
<tr>
<td>Posterior embryotoxon</td>
<td>Yes</td>
</tr>
<tr>
<td>Butterfly vertebrae</td>
<td>Yes</td>
</tr>
<tr>
<td>Total bilirubin</td>
<td>90+12 µmol/L</td>
</tr>
<tr>
<td>Direct bilirubin</td>
<td>76+23 µmol/L</td>
</tr>
<tr>
<td>Total bile acid</td>
<td>213+44 µmol/L</td>
</tr>
<tr>
<td>Gamma-glutamyl transpeptidase</td>
<td>892+75 IU/L</td>
</tr>
<tr>
<td>JAGGED1 gene variation</td>
<td>3 variations in JAGGED1 CDS</td>
</tr>
</tbody>
</table>

CDS, coding sequence.

cDNA of JAGGED1 (5988 base pairs) was then amplified by high-fidelity PCR using a HiFiFast DNA polymerase (Biovisualab, Shanghai, China) and primers (sense: 5’ctgcccggcg tgctgggtag aggtggccag ccccggccgc t3’; antisense: 5’tggc catt aatccagtgg tgtttattca agcagtatt3’; NCBI Reference Sequence: NM_000214.2). The PCR products were sequenced in an ABI PRISM® 310 Genetic Analyzer (Life Technologies Corporation, CA) using a BigDye® Terminator v3.1 Cycle Sequencing Kit (Life Technologies Corporation, CA). All sequences were read on both strands.

Bioinformatics analysis

The JAGGED1 sequences were spliced and analyzed using Sequencer DNA Sequence Analysis Software (version 4.9, Gene Codes Corporation, Ann Arbor, MI). NM_000214.2 in NCBI was used as reference sequences for JAGGED1. Detected variations were localized using Homo sapiens chromosome 20, GRCh37. p13 primary assembly (NCBI Reference Sequence: NC_000020.10). Gene variations were defined according to “Recommendations for the description of protein sequence variants (v2.0)” and “Recommendations for the description of DNA sequence variants-v2.0” released by the Human Genome Variation Society. Any reported variations were positioned using the NCBI SNP database (dbSNP Short Genetic Variations). Minor allele counts were referenced from the database of 1000 Genomes.

Results

Clinical features

Twin sisters, age twelve months, visited our hospital due to jaundice. Observation showed that the girls have typical facial features of ALGS with jaundice: broad forehead, deep-set eyes, pointed chin, and a triangular face. Ophthalmic testing showed the presence of posterior embryotoxon (Table 1). Frontal and lateral X-ray examination showed the presence of butterfly vertebrae (Table 1). Echocardiography detected atrial septal defect (Table 1). Abdominal ultrasound showed no swelling in the liver or spleen. Biochemical tests showed that the average total bilirubin, direct bilirubin, total bile acid, and gamma-glutamyl transpeptidase of the twins were 90+12 µmol/L, 76+23 µmol/L, 213+44 µmol/L, and 892+75 IU/L, respectively (Table 1). All of these biochemical indexes exceeded the range of normal values, suggesting cholestasis and liver damage. Biochemical and ultrasonic detection showed no obvious abnormalities in renal function and structure.

Genetic findings

Three gene variations were found and conserved in the twins in the JAGGED1 coding sequence. The first is located within exon 22. Sequencing showed a G/C heterozygote. The G at the site of position 3128 of the mRNA (NCBI Reference Sequence: NM_000214.2) will lead amino acid 871 of the JAGGED1 protein to change from Pro to Arg (Table 2). The minor allele count of C in the 1000 Genomes database is 0.0529 out of 265 subjects (Table 2). The variation at this site was documented in the NCBI SNP database and defined as rs35761929 (Table 2). Although this variation was considered a benign allele, no reliable data to date have focused on the correlations between c.2612C>G and ALGS. The second is located within exon 24. Sequencing showed an A/T heterozygote. An A at position 3473 of the mRNA (NCBI Reference Sequence: NM_000214.2) will result in a stop codon and might lead to deletion of amino acid 233 at the C terminal of the JAGGED1 protein including the
transmembrane domain (Table 2). The third one is located within exon 26, which showed a T/C heterozygote. A C at this site will not alter amino acid 1139 of the JAGGED1 protein. The minor allele count of A in the 1000 Genomes database is 0.2873 of 1439 subjects. The variation at this site was documented in the NCBI SNP database and defined as rs1051419 (Table 2). Similar to c.2612C>G, although this variation was considered a benign allele, no reliable data to date have focused on the correlations between c.3417C>G and ALGS.

Discussion

Mutations in JAGGED1 are known to cause ~94-96% of cases of ALGS. Mutations in NOTCH2 are known to cause ALGS in 1-2% of individuals [25]. Nevertheless, no particular hotspots in these genes have been found; any part of the entire coding regions for JAGGED1 and NOTCH2 may be involved [17, 24]. In this study, the gene variations in the coding sequences of JAGGED1 and NOTCH2 were evaluated in 12-month-old twin sisters with ALGS.

Three conserved variations in the complementary DNA were identified within exons 22, 24, and 26. The c.3417T>C within exon 26 is a silent change, which did not change the coding sequence of Leu at 1139. The c.2612C>G within exon 22 is a missense change, which results in a change of Pro to Arg at 871. Amino acid 871 is located within a von Willebrand factor (vWF) type C domain. The vWF type C domain was thought to be involved in functions such as transcription, DNA repair, ribosomal and membrane transport, and the proteasome [26, 27]. Although no functional assessment was performed regarding p.Pro871Arg in the vWF type C domain of the JAGGED1 protein, we could not exclude the possibility that p.Pro871Arg in the C terminal of JAGGED1 might contribute to the pathogenesis of ALGS. The c.2957T>A within exon 24 is a nonsense variation, which introduces an immediate translation stop codon at 986. The predicted consequence for p.Leu986* is a deletion of the whole transmembrane region of JAGGED1. The synthesis, post-translational modification, and proper folding of the protein will be hindered greatly if the transmembrane region is lost [28]. Thus, although we have not performed an assessment on the adverse impact of c.2957T>A of JAGGED1 complementary DNA in the somatic cells of the twin sisters, c.2957T>A is suspected to be the main cause of their ALGS.

The major clinical manifestation of ALGS is cholestasis, characterized by bile duct paucity on liver biopsy [29]. It is impossible to perform liver biopsy in patients as young as ours. Instead of biopsy, abdominal ultrasound examination was performed, and it showed no swelling in the liver and spleen. Liver function examination by biochemical approaches showed abnormal bile metabolism. The echocardiogram showed congenital cardiac defects in the form of an atrial septal defect. Further assessment of the echocardiogram excluded the presence of tetralogy of Fallot, so the cardiac phenotype of the twin sisters is atrial septal defect, not peripheral pulmonary artery stenosis. We are unsure if this is due to c.2957T>A in their JAGGED1 coding sequence.

This is a timely and primary report on JAGGED1 gene variations in Chinese twin sisters with
JAGGED1 mutants in ALGS

Alagille syndrome. We have not confirmed whether the sisters are identical twins or dizygotic twins, and we have not confirmed the genetic background of the JAGGED1 gene of their parents. In our department, we have assembled 50 ALGS children, and an in-depth study is underway. The above issues will be addressed in our upcoming studies. We did not include control subjects without ALGS in this study for the following reasons: so far, ALGS is only associated with polymorphism or mutation in either the JAGGED1 or NOTCH2 genes, and the latter is very rare. Also, this is not a population-based association study: our goal in this study was to determine the genotype of the twin sisters with ALGS, not to compare the frequency of a certain genotype between healthy controls and patients. In addition, the minor allele frequency from the 1000 Genomes database was referred to, although we could not find details for c.2957T>A in the 1000 Genomes database.

In conclusion, our data suggest that gene variations of c.2612C>G and c.3417T>C and especially c.2957T>A might contribute to the pathogenesis of Alagille syndrome in the Chinese population.

Disclosure of conflict of interest

None.

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