Relationship between rs1047763 polymorphism of the C1GALT1 gene and susceptibility to immunoglobulin A nephropathy in Xinjiang Uyghur people

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ABSTRACT. We explored the relationship between rs1047763, a single-nucleotide polymorphism (SNP) of the C1GALT1 gene, and genetic susceptibility to immunoglobulin A nephropathy (IgAN) in Xinjiang Uyghur people. The study comprised 90 patients with IgAN and 90 normal controls recruited from Uyghur people. The distribution of the rs1047763 polymorphism of C1GALT1 in each group was determined by direct sequencing analysis. The gene type, gene frequency, allele type, and allele frequency were calculated by direct counting and the genotype was investigated using the Hardy-Weinberg equilibrium test. The SPSS17.0 software was used for data processing, and genotype and allele frequencies were compared using the χ² test. In the IgAN group, the AA, AG, and GG
genotype frequencies in the rs1047763 polymorphism of the \textit{C1GALT1} gene were 21.10, 47.80, and 31.10\%, respectively, while AA, AG, and GG genotype frequencies in the control group were 17.8, 40.0, and 42.2\%, respectively. There was no statistically significant difference between the two groups (\(P > 0.05\)). The rs1047763 SNP of the \textit{C1GALT1} gene probably has no correlation with genetic susceptibility to IgAN in Xinjiang Uyghur people.

\textbf{Key words:} Immunoglobulin A nephropathy; \textit{C1GALT1} gene; Gene polymorphism; Uyghur people

\section*{INTRODUCTION}

Immunoglobulin A nephropathy (IgAN) is a primary glomerular disease with a high incidence on regional (Xinjiang), national (China), and international levels, and is a major cause of end-stage renal disease (ESRD). However, the etiology and pathogenesis of IgAN are still unclear. Researchers in Italy, Japan, the USA, and China studied IgAN patients of different races and ethnic backgrounds using enzyme-linked immunosorbent assay (ELISA), fluorophore-assisted carbohydrate electrophoresis (FACE), and mass spectrometry to provide the data for this study. The results suggest that there were some defects in O-glycan galactosylation in the hinge region of serum IgA1 molecules in IgAN patients (Moldoveanu et al., 2007; Gharavi et al., 2008; Shimozato et al., 2008; Lin et al., 2009). Xia and McEver (2006) found that \textit{C1GALT1} (core 1 synthase, glycoprotein-N-acetylgalactosamine 3-beta-galactosyltransferase), which is an enzyme encoded by the \textit{C1GALT1} gene, is a critical regulatory site in the protein glycosylation process. However, there is no consensus on whether single-nucleotide polymorphisms (SNPs) in the \textit{C1GALT1} gene are IgAN risk factors. Bertinetto et al. (2012) reported that there was a correlation between rs1008989 and rs7790522 polymorphisms in the \textit{C1GALT1} gene and IgAN pathogenesis. Wang et al. (2013) conducted a study on the Han population of southern China and reported that interactions between rs1008989 in \textit{C1GALT1} and rs340833 in \textit{ILRA31} were related to IgAN susceptibility, while there was no correlation between a single SNP and susceptibility to IgAN. In this paper, the authors investigated the distribution of the rs1047763 SNP of the \textit{C1GALT1} gene in Uyghur IgAN patients to determine its correlation with IgAN susceptibility.

\section*{MATERIAL AND METHODS}

\section*{Subjects}

The IgAN group were hospitalized in the Nephrology Department attached to the Xinjiang Uygur Autonomous Region People’s Hospital between May 2010 and August 2012, and were diagnosed with IgAN by biopsy pathology. There were 43 males and 47 females with a mean age of 38.81 ± 11.06 years. In the same period, 90 healthy cases including 42 males and 48 females with a mean age of 37.53 ± 11.68 years were recruited from the Xinjiang Uygur Autonomous Region People’s Hospital Medical Center. All the inductees were Uyghurs, were unrelated to each other, and were from families that had lived in Xinjiang for at least three generations. IgAN diagnostic criteria were in accordance with the 2001 pathological diagnosis
rs1047763 polymorphism of C1GALT1 and IgA nephropathy

standard guidelines (Zou, 2001), and patients with systemic lupus erythematosus (SLE), purpura, chronic liver disease, ankylosing spondylitis arthritis, psoriatic kidney damage, and other secondary IgA deposition diseases were excluded. Moreover, patients that also had other kidney diseases and had been treated with glucocorticoids or Tripterygium wilfordii Hook F. (a traditional Chinese herbal medicine), cyclophosphamide and azathioprine, or other immunosuppressive agents within 14 weeks were also excluded. All subjects were fully informed and participated voluntarily. The project was approved by the Medical Ethics Committee attached to Xinjiang Uygur Autonomous Region People’s Hospital.

Reagents

An EZUP pillar whole blood genomic extraction kit (Shanghai Biological Engineering Co., Ltd. Shanghai, China), a polymerase chain reaction (PCR) amplification kit (including Taq polymerase, 10X buffer, deoxyribonucleotide triphosphate (dNTP), MgCl₂, double-distilled H₂O) (Bei-jing Ding States Biological Technology Limited), a DNA marker, agarose, and ethidium bromide (BBI company) were used in the study.

Methods

Blood samples

Early in the morning, 5 mL venous blood was extracted from each fasting patient. Ethylenediaminetetraacetic acid was used as an anticoagulant and the samples were stored at -80°C until required.

Gene extraction

An EZUP pillar whole blood genomic DNA extraction kit was used. According to the manufacturer instructions, operations were performed step by step. DNA (50 μL) was extracted from leukocytes using an adsorption column and stored at -80°C until required. (Because the steps were complex and multi-step centrifugation was performed, we have not described the specific procedure in detail here.)

Amplification of C1GALT1 gene rs1047763 locus

The forward primer sequence was: 5’-CAAGCAAAACAAAATGAAGATAC-3’, and the reverse primer sequence was: 5’-CCTGTTTTTCTGACCATCTGTGT-3’. Related primers were synthesized by the Shanghai Biological Engineering Co., Ltd.

The PCR amplification system (total volume 50 μL) comprised: 26 μL double-distilled H₂O, 12 μL 10X buffer (including Mg²⁺), 4 μL dNTP, 1 μL upstream primer, 1 μL downstream primer, 3 U Taq enzyme, and 3 μL template DNA. The amplification conditions were: 94°C initial denaturation for 5 min; cycling at 94°C for 30 s, 50°C for 30 s, and 72°C for 40 s for a total of 36 cycles; extension at 72°C for 5 min; and storage at 4°C until required. The PCR amplification products were 352 bp long. The PCR amplification products (5 μL) were subjected to electro-
phoresis using 1.5% agaroseethidium bromide gel (voltage 120 V for 20 min). A UV transmission
gel imaging system was used to observe the distribution of bands, and digital photographs were
saved by category.

**Classification of C1GALT1 gene rs1047763**

*C1GALT1* gene rs1047763 amplification products were purified by the Beijing Dingguo
Company, and direct sequencing was used; homology sequence analysis of the gene sequenc-
ing results was performed using the BLAST gene service station. Chromas software was used for
sequencing analysis of the results and to obtain a classification for *C1GALT1* gene rs1047763.

**Statistical analysis**

The direct counting method was used for computing allele and genotype frequencies.
Hardy-Weinberg equilibrium (HWE) software was used to estimate the population genetics HWE.
The SPSS17.0 statistical software package was used for other statistical processing. The t-test
was used for comparison between groups. A 2 x 2 contingency table χ² test was used to obtain the
constituent ratio. A P value of < 0.05 was considered statistically significance. The odds ratio (OR)
and 95% confidence interval (95%CI) indicated relative risk.

**RESULTS**

**General information**

There were no statistical differences in gender and age between the IgAN and control
groups, as shown in Table 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cases</th>
<th>Gender (male/female)</th>
<th>Age (means ± SD, years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgAN group</td>
<td>90</td>
<td>43/47</td>
<td>38.81 ± 11.06</td>
</tr>
<tr>
<td>Control group</td>
<td>90</td>
<td>42/48</td>
<td>37.53 ± 11.68</td>
</tr>
<tr>
<td>t or χ²</td>
<td>0.022</td>
<td>0.754</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.881</td>
<td>0.452</td>
<td></td>
</tr>
</tbody>
</table>

**Sequence typing results for C1GALT1 gene rs1047763 locus**

The *C1GALT1* gene rs1047763 locus had A/G dimorphism. The PCR-amplified fragment
of the *C1GALT1* gene rs1047763 locus was 352 bp long. After purification and sequencing of the
PCR products, we found that the locus had three genotypes: AA, GG, and AG (Figure 1).

**Distribution of genotype and allele frequencies of C1GALT1 gene rs1047763 locus**

**Genetic equilibrium test of the locus**

The HWE law was used to test the genetic equilibrium of the population; the distribution
of the AA, AG, and GG genotypes in the IgAN and control groups was consistent with genetic
rs1047763 polymorphism of C1GALT1 and IgA nephropathy

equilibrium (P > 0.05), showing that the genotype frequencies were normal and the sample was representative.

Figure 1. Sequence-based typing of the C1GALT1 gene rs1047763 polymorphism.

Distribution of genotypes in the two groups

In the IgAN group, the frequencies of the AA and AG genotypes were higher (21.10 and 47.80%, respectively), and the GG genotype frequency was lower (31.10%) than those of the control group (17.80, 40.00, and 42.20%, respectively); no statistically significant difference was found among the three genotypes ($\chi^2 = 2.393, P = 0.302$) (Table 2).

Table 2. Distribution and frequency of C1GALT1 gene rs1047763 genotypes and alleles in the immunoglobulin A nephropathy (IgAN) and control groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>Genotype [cases, N (%)]</th>
<th>Allele [N (%)]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AA</td>
<td>AG</td>
</tr>
<tr>
<td>IgAN group</td>
<td>90</td>
<td>19 (21.1)</td>
<td>43 (47.8)</td>
</tr>
<tr>
<td>Control group</td>
<td>90</td>
<td>16 (17.8)</td>
<td>36 (40.0)</td>
</tr>
<tr>
<td>$\chi^2$</td>
<td></td>
<td>2.393</td>
<td></td>
</tr>
<tr>
<td>$P$</td>
<td></td>
<td>0.302</td>
<td></td>
</tr>
</tbody>
</table>

Distribution of alleles in the two groups

The IgAN group had a higher A allele frequency (45.0%) and a lower G allele frequency (55.0%) than the control group (37.8 and 62.2%, respectively); no statistically significant difference was found between the two alleles ($\chi^2 = 1.935, P = 0.164$) (Table 2).

Further stratified analysis showed that G allele frequency in the IgAN group was higher than in the control group; compared with the A allele, the G allele was not found to be associated with the onset of Uyghur IgAN (OR = 0.742, 95%CI = 0.487-1.130), and the difference was not statistically significant ($P = 0.164$) (Table 3).
Table 3. Association between $C1GALT1$ gene rs1047763 genotypes and alleles and immunoglobulin A nephropathy (IgAN) susceptibility in Uyghur subjects.

<table>
<thead>
<tr>
<th>Genes and alleles</th>
<th>Control group (N = 90)</th>
<th>IgAN group (N = 90)</th>
<th>P</th>
<th>OR</th>
<th>95%CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotypes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>16 (17.8)</td>
<td>19 (21.1)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AG</td>
<td>36 (40.0)</td>
<td>43 (47.8)</td>
<td>0.989</td>
<td>1.006</td>
<td>0.452-2.236</td>
</tr>
<tr>
<td>GG</td>
<td>38 (42.2)</td>
<td>28 (31.1)</td>
<td>0.255</td>
<td>0.620</td>
<td>0.272-1.416</td>
</tr>
<tr>
<td>Alleles</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>68 (37.8)</td>
<td>81 (45.0)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>112 (62.2)</td>
<td>99 (55.0)</td>
<td>0.164</td>
<td>0.742</td>
<td>0.487-1.130</td>
</tr>
</tbody>
</table>

The AG genotype frequency in the IgAN group was higher than in the control group; compared with the AA genotype, the AG genotype frequency was not found to be related to IgAN pathogenesis (OR = 1.006, 95%CI = 0.452-2.236), and the difference was not statistically significant (P = 0.989). The GG type frequency in IgAN patients was lower than in the controls; compared with the AA genotype, statistical analysis showed that the GG genotype may not be related to IgAN pathogenesis (OR = 0.620, 95%CI = 0.272-1.416), and the difference was not statistically significant (P = 0.255).

DISCUSSION

Currently, the etiology and pathogenesis of IgAN are not entirely clear, although genetic and environmental factors are thought to play an important role in IgAN pathogenesis; susceptibility to IgAN and its disease progression may be associated with certain genetic polymorphisms. Research has shown that genetic variation of the Megsin gene may be related to the occurrence of IgAN in the Chinese population (Chen and Xie, 2006). Vuong et al. (2009) suggested that genetic variation in the $TGFB1$ gene has an important relationship with IgAN susceptibility. In addition, a study by Jia (2012) found that the interleukin gene, the angiotensin-converting enzyme gene, the mannose-binding protein, the transferrin receptor gene, the plasminogen activator inhibitor-1 gene, the P-selectin gene, the vascular endothelial growth factor gene, and the apolipoprotein E gene are associated with the pathogenesis of IgAN.

In many related studies, the role of abnormal O-glycosylated IgA1 molecules in the incidence and progression of IgAN has received increasing attention from researchers. Studies have shown that serum β-1,3galactosyltransferase activity in patients with IgAN is significantly reduced, so that the process of sialylation is increased and overactive, leading to low galactosylation of IgA1 molecules (Bai, 2011), which plays an important role in the pathogenesis of IgAN. Li et al. (2007) selected a large sample of sporadic IgAN Han patients as subjects to conduct a case-control study, and showed that the $C1GALT1$ gene polymorphism was associated with IgAN susceptibility in the Han. A number of studies have shown that $C1GALT1$ plays an important role in the pathogenesis of IgAN; the generation of β-1,3-galactosyltransferase has been determined by its encoding gene $C1GALT1$. Therefore, the $C1GALT1$ gene may be a candidate gene for susceptibility to IgAN.

Some $C1GALT1$ gene loci polymorphisms regulate and affect the synthesis of $C1GALT1$ by a transcriptional pathway; studies have shown that the rs5882115 SNP of the $C1GALT1$ gene is associated with Han IgAN pathogenesis (Li et al., 2007). Moreover, the rs1047763 (1365G/A) polymorphism of the $C1GALT1$ gene can promote Henoch-Schönleinpurpura nephritis (HSPN) development (He et al., 2012). Pirulli et al. (2009) studied rs1047763 (1365G/A) and found that the 1365G allele and the 1365G/G genotype were correlated with IgAN susceptibility.

To date, there has been no report on the correlation between the rs1047763 polymorphism
rs1047763 polymorphism of C1GALT1 and IgA nephropathy

and Uyghur IgAN susceptibility. In this paper, a case-control study was conducted to examine the association between the C1GALT1 gene rs1047763 SNP and susceptibility to IgAN in Uyghur people.

We found no statistically significant differences in rs1047763 genotype and allele frequencies between the Uyghur IgAN and control groups; stratified analysis showed that in the IgAN group, A allele frequency and AG genotype frequency were significantly higher than in the control group, and they were not associated with IgAN susceptibility in Uyghurs. G allele and GG genotype frequencies in the control group were higher than in the IgAN group, which was inconsistent with the results reported of Li et al. (2007), and suggests that the rs1047763 SNP of the C1GALT1 gene may be subject to regional and/or ethnic differences.

In summary, we found no correlation between the rs1047763 polymorphism and Uyghur IgAN pathogenesis in this study, possibly for the following reasons. Firstly, the C1GALT1 gene rs1047763 polymorphism is weakly associated with IgAN, and the small sample size may have affected the statistical analysis. Secondly, the different genetic backgrounds of the subjects and the different gene-environment interactions may have led to different findings.

This study was the first exploration of the relationships between the C1GALT1 gene rs1047763 polymorphism and Uyghur IgAN, but found no correlation between the rs1047763 polymorphism and IgAN pathogenesis. Owing to the small sample size, the genes were limited; we look forward to a study with a larger sample in combination with other susceptibility genes and environmental factors for further verification.

Conflicts of interest

The authors declare no conflict of interest.

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