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 χ^2 test. In the IgAN group, the AA, AG, and GG

genotype frequencies in the rs1047763 polymorphism of the *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 *C1GALT1* gene probably has no correlation with genetic susceptibility to IgAN in Xinjiang Uyghur people.

Key words: Immunoglobulin A nephropathy; *C1GALT1* gene; Gene polymorphism; Uyghur people

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 C1GALT1 (core 1 synthase, glycoprotein-N-acetylgalactosamine 3-beta-galactosyltransferase), which is an enzyme encoded by the *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 *C1GALT1* gene are IgAN risk factors. Bertinetto et al. (2012) reported that there was a correlation between rs1008898 and rs7790522 polymorphisms in the *C1GALT1* gene and IgAN pathogenesis. Wang et al. (2013) conducted a study on the Han population of southern China and reported that interactions betweenrs1008898 in *C1GALT1* and rs340833 in *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 *C1GALT1* gene in Uyghur IgAN patients to determine its correlation with IgAN susceptibility.

MATERIAL AND METHODS

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

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), $MgCl_2$, double-distilled H_2O) (Beijing 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'-CAAGCAAACAAAAATGAAGATAC-3', and the reverse primer sequence was: 5'-CCTGTTTTCTGACCATCTGTGTT-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 H_2O, 12 µL 10X buffer (including Mg^{2+}), 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 sequencing 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 χ^2 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 1. Distribution of gender and age in the immunoglobulin A nephropathy (IgAN) and control groups.

Groups	Cases	Gender (male/female)	Age (means ± SD, years)
IgAN group	90	43/47	38.81 ± 11.06
Control group	90	42/48	37.53 ± 11.68
t or χ^2	0.022	0.754	
P	0.881	0.452	

Sequence typing results for*C1GALT1* gene rs1047763 locus

The *C1GALT1* gene rs1047763locus 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

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

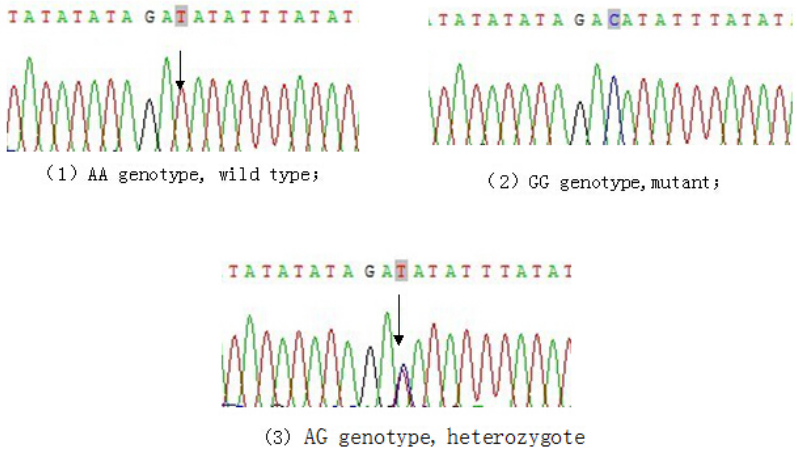

Figure 1. Sequence-based typing of the *C1GALT1* gene rs1047763 polymorphism.

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 (χ^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.

Group	Cases	Genotype [cases, N (%)]			Allele [N (%)]	
	AA	AG	GG	A	G	A
IgAN group	90	19 (21.1)	43 (47.8)	28 (31.1)	81 (45.0)	99 (55.0)
Control group	90	16 (17.8)	36 (40.0)	38 (42.2)	68 (37.8)	112 (62.2)
χ^2		2.393			1.935	
P		0.302			0.164	

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 (χ^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.

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

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 C1GALT-1by 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

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 Uygur 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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REFERENCES

Bai YW (2011). The role of low sugar levels in IgA nephropathy. *J. Clinical Nephrol.* 711: 334-336.

Bertinetto FE, Calafell F, Roggero S, Chidichimo R, et al. (2012). Search for genetic association between IgA nephropathy and candidate genes selected by function or by gene mapping at loci IGAN2 and IGAN3. *Nephrol. Dial. Transplant.* 27: 2328-2337.

Chen MX and Xie YS (2006). The incidence and treatment of IgA nephropathy. *ZhongGuoZhong Xi Yi Jie He ShenZang Bing ZaZhi* 7: 683-686.

Gharavi AG, Moldoveanu Z, Wyatt RJ, Barker CV, et al. (2008). Aberrant IgA1 glycosylation is inherited in familial and sporadic IgA nephropathy. *J. Am. Soc. Nephrol.* 19: 1008-1014.

He X, Zhao P, Kang S, Ding Y, et al. (2012). C1GALT1 polymorphisms are associated with Henoch-Schönleinpurpura nephritis. *Pediatr. Nephrol.* 27: 1505-1509.

Jia YL (2012). The research progress of kidney disease susceptibility genes. *Xian Dai Yu Fang Yi Xue* 39: 1062-1064.

Li GS, Zhang H, Lv JC, Shen Y, et al. (2007). Variants of C1GALT1 gene are associated with the genetic susceptibility to IgA nephropathy. *Kidney Int.* 71: 448-453.

Lin X, Ding J, Zhu L, Shi S, et al. (2009). Aberrant galactosylation of IgA1 is involved in the genetic susceptibility of Chinese patients with IgA nephropathy. *Nephrol. Dial. Transplant.* 24: 3372-3375.

Moldoveanu Z, Wyatt RJ, Lee JY, Tomana M, et al. (2007). Patients with IgA nephropathy have increased serum galactose-deficient IgA1 levels. *Kidney Int.* 71: 1148-1154.

Pirulli D, Crovella S, Ulivi S, Zadro C, et al. (2009). Genetic variant of C1GalT1 contributes to the susceptibility to IgA nephropathy. *J. Nephrol.* 22: 152-159.

Shimozato S, Hiki Y, Odani H, Takahashi K, et al. (2008). Serum under-galactosylated IgA1 is increased in Japanese patients with IgA nephropathy. *Nephrol. Dial. Transplant.* 23: 1931-1939.

Vuong MT, Lundberg S, Gunnarsson I, Wramner L, et al. (2009). Genetic variation in the transforming growth factor-beta1 gene is associated with susceptibility to IgA nephropathy. *Nephrol. Dial. Transplant.* 24: 3061-3067.

Wang W, Sun Y, Fu Y, Yu X, et al. (2013). Interaction of C1GALT1-IL5RA on the susceptibility to IgA nephropathy in Southern Han Chinese. *J. Hum. Genet.* 58: 40-46.

Xia L and McEver RP (2006). Targeted disruption of the gene encoding core 1 beta1-3-galactosyltransferase (T-synthase) causes embryonic lethality and defective angiogenesis in mice. *Methods Enzymol.* 416: 314-331.

Zou WZ (2001). Renal biopsy pathology diagnosis standard guidance.*Chin. J. Nephrol.* 17: 270-275.

