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# Association between *C1GALT1* variants and genetic susceptibility to IgA nephropathy in Uygur

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**ABSTRACT.** Immunoglobulin A (IgA) nephropathy (IgAN) is a common form of primary glomerulonephritis characterized by diffuse glomerular mesangial IgA1 deposition leading to mesangial proliferation and chronic glomerular inflammation. Analyses of serum IgA1 from IgAN patients revealed abnormal galactosylation of the *O*-linked carbohydrate moieties of IgA that may result from altered activity in the core of 1 β1,3-galactosyltransferase (C1GalT1). To evaluate the association between *C1GalT1* single nucleotide polymorphisms (SNPs) and IgAN, we performed a case-control study on cohorts from the Uyghur population in China. A total of 180 IgAN patients and 180 healthy controls were recruited for the study. We sequenced 5 SNPs, including SNP1 (rs9639031), SNP2 (-527A/G), SNP3 (rs1008898), SNP4 (rs5882115), and SNP5 (rs1047763) in the *C1GalT1* gene in all eligible participants. The frequencies of the I allele and DI genotype of rs5882115 in IgAN patients were significantly higher than those in controls ($P < 0.05$). The frequency of haplotype GAGDA was significantly higher in patients than in controls (0.0719 *vs* 0.00, $P = 0.024$). Polymorphisms

 

in the *C1GALT1* gene were associated with genetic susceptibility to Uyghur IgAN.

**Key words:** *C1GALT1* gene; Gene polymorphism; IgA nephropathy; Uyghur

## INTRODUCTION

Immunoglobulin A (IgA) nephropathy (IgAN), the most common primary glomerulonephritis (Levy and Berger, 1988), is a complex-trait disease. Studies have shown that 25-30% IgAN patients will develop end-stage renal disease after 20-25 years (Barratt and Feehally, 2005), which is currently the primary cause of maintenance hemodialysis in China (Chen and Xie, 2004). The clinical manifestations, pathological patterns, and prognoses of IgAN are diverse, but the pathogenesis remains unclear. Increasing evidence suggests that genetic components are involved in IgAN pathogenesis and variation in clinical manifestation. Ethnic and geographic variations in prevalence and familial clustering have been observed (Hsu et al., 2000; Scolari, 2003). Therefore, identifying genes associated with susceptibility to IgAN may provide genetic targets for therapeutic intervention of IgAN.

Aberrant glycosylation of IgA1 molecules has recently been reported (Allen, 1995; Hiki et al., 1995; Tomana et al., 1997) in IgAN patients and considered to be the most important pathogenic mechanism of the disease, providing a candidate for studying genetic susceptibility to IgAN. β1,3-galactosyl transferase (C1GALT1) plays an important role in the galactosylation of IgA1 molecules, and the *C1GALT1* gene was shown to be associated with IgAN in subjects of Chinese Han nationality (Wang et al., 2013). We conducted a case-control association study by comparing single nucleotide polymorphism (SNP) frequencies and haplotypes of the *C1GAlT1* gene in IgAN patients and healthy controls to better understand the pathogenesis and genetic background of IgAN in Uyghur.

## MATERIAL AND METHODS

### Subjects

A total of 360 unrelated Uyghur subjects in China were enrolled in this study, including 180 patients diagnosed with IgAN by renal biopsy in the Nephrology Department of the People's Hospital of Xinjiang Uyghur Autonomous Region and 180 healthy controls (84 males, 96 females, average age, 37.53 ± 11.68 years), whose ages and genders were matched with IgAN patients and who attended the hospital for medical examination during the same time period. Renal biopsy pathological diagnostic criteria (Zou, 2001) were used as diagnostic criteria of IgAN in this study. Patients with Henoch-Schonlein purpura, systemic lupus erythematosus, and chronic hepatic diseases were excluded through detailed clinical and laboratory examinations. All participants were unrelated permanent Uyghur residents, 3 generations of whom all lived in Xinjiang. The average age of patients at the time of renal biopsy was 38.81 ± 11.06 years (range, 17-65 years), and the gender ratio (male to female) was 86 to 94.

All subjects provided informed consent and participated voluntarily in the study. This study was approved by the Medical Ethics Committee of The People's Hospital of Xinjiang Uyghur Autonomous Region.

## DNA extraction

Genomic DNA was isolated from whole peripheral blood using the Ezup pillar blood genomic DNA extraction kit (Bioteck, Beijing, China) according to the kit instructions. DNA concentration and quality [optical density $(OD)_{260}/OD_{280}$ and $OD_{260}/OD_{230}$] measurements were conducted in a Nanodrop ND1000 spectrophotometer (Nanodrop Technologies; Wilmington, DE, USA). Qualified DNA samples were stored at -20°C.

## SNP identification

Five SNPs, including SNP1 (rs9639031), SNP2 (-527A/G), SNP3 (rs1008898), SNP4 (rs5882115), and SNP5 (rs1047763), in *C1GALT1* screened according to previous research (Li et al., 2007) were examined in our study. Reference sequences of the *C1GALT1* gene and SNPs were obtained from the National Center for Biotechnology Information Gene database (http://www.ncbi.nlm.nih.gov/entrez). Primer sequences (shown in Table 1) for all SNPs were designed based on a previous study (Li et al., 2007) and were verified using the Primer5 software. The primers were synthesized by Shanghai Sengon Co., Ltd. (Shanghai, China), conforming to the requirements of the project group.

**Table 1.** Primer endonucleases for genotyping SNPs of the *C1GALT1* gene.

| SNP | Forward primer (5'-3') | Reverse primer (5'-3') |
|---|---|---|
| SNP1 | CCTGACTTCAAGTGATCCACCGAC | GAACACACCCATGCCCATTCATTTAT |
| SNP2 | GGCTAGGTACGGTTTTGTGA | TTGGGTCTCATGTGGTTTCT |
| SNP3 | GGTTTGCTAACTTTTGGGTTGGAGGA | CTTCCCACCAGGATCCTTTGTGGAT |
| SNP4 | AGAAGAAGATGCAACAGAAACCACA | CTTTCTTTGCTGTTAACTCTGAGGAC |
| SNP5 | CAAGCAAACAAAAATGAAGATAC | AACACATGGTCAGAAAACAGG |

## Polymerase chain reaction (PCR) conditions

The total volume for PCR amplification was 35 μL, containing 3 μL DNA, 20 μL $ddH_2O$, 5 μL buffer, 2 μL dNTPs, and 1.2 μL and 2 μL *Taq* polymerase in the upstream and downstream reactions, respectively. The following PCR protocol was used: modified 5 min at 95°C, the main loop condition modified to 45 s at 95°C, 5 SNP annealing steps for 60 s at 58°, 64.8°, 60°, 61°, and 63°C, respectively in order, and then extension for 45 s at 72°C, for 38 cycles, following by extension for 10 min at 72°C and preservation at 4°C. All PCR amplification products were electrophoresed at a voltage of 120 V for 20 min on a 1.5% agarose gel containing ethidium bromide. Images were obtained using an ultraviolet transmission automatic image analyzer.

## Genotyping

All PCR products for each SNP locus were genotyped by direct sequencing by Beijing Ding Guo Biotechnology Co., Ltd. (Beijing, China). Next, the results of sequencing were analyzed using the Chromas software (Version 1.45, Technelysium, South Brisbane, Australia) for genotyping.

## Statistical analysis

The population genetics Hardy Weinberg equilibrium of genotypes were estimated

with the HWE software. Other statistical analyses were performed using the SPSS17.0 software (SPSS, Inc., Chicago, IL, USA). The allele and genotype frequencies were calculated using the chi-squared test. Pairwise linkage disequilibrium between each SNP was quantified as D', measured by the online SHEsis software package (http://analysis.bio-x.cn/SHEsisMain.htm). SHEsis was used to test the association between statistically inferred haplotypes and IgAN. All statistical analyses were 2-sided, at a test level of $\alpha = 0.05$; $P < 0.05$ indicated that the difference was statistically significant.

## RESULTS

There was no statistically significant difference in terms of gender and age between the IgAN group and the control group ($P > 0.05$) (Table 2).

**Table 2.** Gender and age distribution features of the IgAN group and control group.

| Item | IgAN group | Control group | *t* or $\chi^2$ value | P value |
|---|---|---|---|---|
| Age (Year) | 38.81 ± 11.06 | 37.53 ± 11.68 | 0.754 | 0.452 |
| Gender (Male/Female) | 86/94 | 84/96 | 0.045 | 0.833 |

### Linkage disequilibrium analysis of 5 SNPs in the *C1GALT1* gene

Pairwise linkage disequilibrium coefficients (D') of the 5 SNPs in the *C1GALT1* gene are displayed in Table 3. Shaded areas indicate that significant linkage disequilibrium was found within the *C1GALT1* gene. Five polymorphic loci, including SNP1 (rs9639031), SNP2 (-527A/G), SNP3 (rs1008898), SNP4 (rs5882115), and SNP5 (rs1047763), showed significant linkage disequilibrium, which is also shown in Figure 1.

**Table 3.** Pair-wise linkage disequilibrium coefficients for 5 polymorphisms of the *C1GALT1* gene in 102 individuals.

| | SNP2 | SNP3 | SNP4 | SNP5 |
|---|---|---|---|---|
| SNP1 | **0.997** | 0.943 | 0.368 | 0.681 |
| SNP2 | - | **0.999** | 0.999 | 0.882 |
| SNP3 | - | - | **1.000** | 0.810 |
| SNP4 | - | - | - | **0.999** |

D' is shown in the table; a value higher than 0.7 indicates that the linkage disequilibrium was statistically significant.

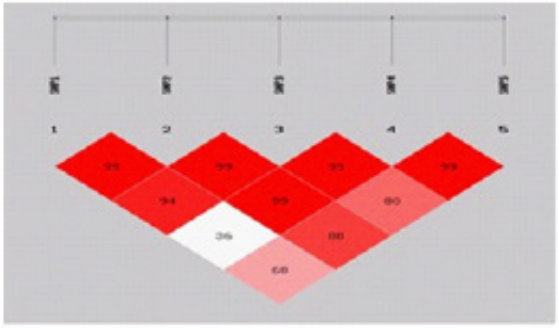

**Figure 1.** Pairwise linkage disequilibrium coefficients in 5 polymorphisms of the *C1GALT1* gene in 102 individuals.

## Comparison of polymorphisms and haplotypes between patients with IgAN and normal controls

Association analyses were performed separately for each SNP followed by haplotype-based analysis. We first compared the frequency of alleles and genotypes between patients with IgAN and normal controls for each SNP. The genotype and allele distributions of the 5 SNPs are listed in Table 4. SNP analysis indicated that the frequency of the I allele in SNP4 was significantly higher in patients with IgAN than in controls ($\chi^2 = 15.577$, P = 0.001). Moreover, the SNP4 II/ID genotype was significantly more frequent in patients ($\chi^2 = 17.139$, P = 0.001). Neither alleles nor genotypes of the other 4 SNPs differed significantly between the 2 groups.

**Table 4.** Allele and genotype distribution in IgAN patients and controls.

| Variants | | IgAN patients | Controls | $\chi^2$ | P |
|---|---|---|---|---|---|
| SNP1 | Genotype | | | | |
| | CC | 72 (40.0) | 58 (32.2) | | |
| | CT | 76 (42.2) | 84 (46.7) | | |
| | TT | 32 (17.8) | 38 (21.2) | 2.422 | 0.298 |
| | Alleles | | | | |
| | C | 220 (61.1) | 200 (55.6) | | |
| | T | 140 (38.9) | 160 (44.4) | 2.286 | 0.151 |
| SNP2 | Genotype | | | | |
| | AA | 126 (70.0) | 128 (70.5) | | |
| | AG | 52 (28.9) | 48 (27.8) | | |
| | GG | 2 (1.1) | 4 (1.7) | 0.842 | 0.652 |
| | Alleles | | | | |
| | A | 304 (84.4) | 304 (84.4) | | |
| | G | 56 (15.6) | 56 (15.6) | 0.00 | 1.00 |
| SNP3 | Genotype | | | | |
| | TT | 22 (12.2) | 18 (10.0) | | |
| | GT | 64 (35.6) | 56 (31.1) | | |
| | GG | 94 (52.2) | 106 (58.9) | 1.653 | 0.438 |
| | Alleles | | | | |
| | T | 108 (30.0) | 92 (25.6) | | |
| | G | 252 (70.0) | 268 (74.4) | 1.772 | 0.106 |
| SNP4 | Genotype | | | | |
| | DD | 118 (65.6) | 152 (84.4) | | |
| | DI | 58 (32.2) | 26 (14.4) | | |
| | II | 4 (2.2) | 2 (1.1) | 17.139 | 0.001* |
| | Alleles | | | | |
| | D | 294 (81.7) | 330 (91.7) | | |
| | I | 66 (18.3) | 30 (8.3) | 15.577 | 0.001* |
| SNP5 | Genotype | | | | |
| | AA | 38 (21.1) | 32 (17.8) | | |
| | AG | 86 (47.8) | 72 (40.0) | | |
| | GG | 56 (31.1) | 76 (42.2) | 4.785 | 0.091 |
| | Alleles | | | | |
| | A | 162 (45.0) | 136 (37.8) | | |
| | G | 198 (55.0) | 224 (62.2) | 3.870 | 0.058 |

The SHEsis software was used to calculate the significance of the association between susceptibility to IgAN and the haplotypes consisting of the 5 SNPs. Global differences in haplotype frequency profiles between the IgAN patients and control group were statistically significant ($\chi^2 = 14.57$, P = 0.037). Omnibus analysis revealed that the frequency of haplotype TAGDG was highest in both IgAN patients and controls, but the difference was not significant. The frequency of haplotypes CAGDA in IgAN patients was 6.5%, but was not observed in

controls. There were significant differences in haplotypes CAGDA between groups. The frequencies of other haplotypes showed no significant differences (Table 5).

**Table 5.** Haplotypes of the *C1GALT1* gene and association with IgAN.

| Haplotype | Frequency | | | Odds ratio (95% confidence interval) |
|---|---|---|---|---|
| | Case | Control | P value | |
| CATDA | 24.38 (0.239) | 17.86 (0.235) | 0.972 | 1.013 (0.501-2.045) |
| CATIG | 4.57 (0.045) | 2.19 (0.029) | 0.588 | 1.567 (0.304-8.074) |
| CAGDG | 6.10 (0.060) | 4.10 (0.054) | 0.878 | 1.106 (0.305-4.017) |
| CAGIG | 7.43 (0.073) | 5.90 (0.078) | 0.893 | 0.926 (0.300-2.855) |
| TAGDA | 2.21 (0.022) | 3.23 (0.043) | 0.417 | 0.494 (0.087-2.809) |
| TAGDG | 34.04 (0.334) | 32.76 (0.431) | 0.167 | 0.646 (0.348-1.202) |
| CGGDA | 13.47 (0.132) | 7.00 (0.092) | 0.417 | 1.490 (0.566-3.924) |
| CAGDA | 6.58 (0.065) | 0.00 (0.00) | 0.024 | - |

## DISCUSSION

IgAN is considered to be a polygenic and multifactorial disorder. Various genetic association studies have revealed that several genes may be related to the predisposition and progression of IgAN (Gharavi et al., 2000; Hsu et al., 2000; Scolari, 2003; Chow et al., 2005), most of which were focused on the progression rather than the pathogenesis of IgAN. The incidence of IgAN varies in different species, and studies have confirmed the genetic and environmental factors that can lead to development of the disease (Hsu et al., 2000; Barratt and Feehally, 2005). For example, Uyghur residents live in a different habitat and environment than the Han population in China.

Aberrant glycosylation of IgA1 molecules is considered to be the most important pathogenic mechanism of the disease. The *C1GALT1* gene, which encodes β1,3-galactosyl transferase, a key enzyme in the process of galactosylation of IgA1 molecules, has recently gained attention. Variations in the *C1GALT1* gene were reportedly associated with the susceptibility to IgAN in Italian patients (Pirulli et al., 2004). In their study, 1365G/G of *C1GALT1* was associated with the susceptibility to IgAN. In addition, the promoter CGATW haplotype was significantly less frequent in IgAN patients than in healthy controls. Another larger case-control population study showed that the D allele and DD genotype of IgAN patients were significantly lower than those in controls, the frequency of haplotype YATIG was significantly lower, and the frequency of YAGDA was significantly higher in patients than in controls.

Based on this information, we conducted a genetic association study based on haplotype analysis of *C1GALT1* in a Uyghur population sample of IgAN patients and healthy controls. Correlation analysis between SNPs in *C1GALT1* and IgAN showed that the frequencies of the I allele and DI genotype of rs5882115 in IgAN patients were significantly higher than those in controls, which differed from the results of Li et al. (2007). Our results showed that rs5882115 was associated with the pathogenesis of IgAN, further illustrating the variable effect of the same genotype on phenotypes in different races or environments. Linkage disequilibrium analysis illustrated that the 5 SNPs, SNP1 (rs9639031), SNP2 (-527A/G), SNP3 (rs1008898), SNP4 (rs5882115), and SNP5 (rs1047763), in *C1GALT1* were in significant linkage disequilibrium. The correlation analysis between haplotypes in *C1GALT1* and IgAN showed that the frequency of haplotype GAGDA was significantly higher in patients

than in controls, which was similar to the results of Li et al. (2007). However, gene segments in *C1GALT1* were associated with the pathogenesis of IgAN.

In conclusion, this study showed that polymorphisms in the *C1GALT1* gene may be associated with susceptibility to IgAN in the Uyghur population. Because our sample size was limited, the association between the *C1GALT1* gene and susceptibility to IgAN in Uyghur should be examined in a multi-zone and multi-site study with a larger sample size to determine risk prediction as well as prevention and target spots for treatment.

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