Study of correlation between polymorphism of ST6GALNAC2 and susceptibility to IgA nephropathy

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Abstract. The aim of the present study was to explore the correlation between single nucleotide polymorphisms (SNPs) rs3840858 and rs2304921 in a specific α-2,6 sialyltransferase gene, ST6GALNAC2, and the susceptibility to immunoglobulin (IgA) nephropathy (IgAN). The distributions of genotypes of SNPs rs3840858 and rs2304921 in ST6GALNAC2 were detected by direct sequencing. The distributions of the genotype and allele frequencies of rs3840858 in patients with IgAN were significantly different from those in the control group (genotypes, P=0.001; alleles, P=0.001). The DI genotype ratio (17.8%) in the IgAN group was higher than that in the control group (5.6%) and the I allele frequency (8.9%) in the IgAN group was higher than that in the control group (2.8%). Univariate logistic regression analysis indicated that rs3840858 polymorphism is a risk factor of IgAN (P=0.001). The risk of developing IgAN in individuals who carried the DI genotype was 3-fold higher than that in individuals who carried the DD genotype [odds ratio (OR)=3.676, 95% confidence interval (CI)=1.284-10.519], and the risk of developing IgAN in individuals who carried the I allele was higher than that in individuals who carried the D allele (OR=3.415, 95% CI=1.223-9.531). The distributions of the genotype (AA, AG and GG) and allele (A and G) frequencies of rs2304921 did not have a statistically significant difference between patients with IgAN and those without (P>0.05). The SNP rs3840858 in the ST6GALNAC2 gene may be associated with the risk of developing IgAN in the population studied; however, polymorphism of rs2304921 appears to be irrelevant to the risk of developing IgAN in this population.

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Introduction

Immunoglobulin A (IgA) nephropathy (IgAN), as one of the most common primary glomerular diseases, accounts for 30%-40% of primary glomerular diseases, the incidence of which is increasing year by year (1-3). Relevant follow-up studies have shown that for 25-30% patients, IgAN will develop into end-stage renal disease after 20-25 years (4), which is the primary cause of maintenance hemodialysis in China at present (5). The clinical manifestations, pathological patterns and prognoses of IgAN show diversity, and the pathogenesis is not yet clear. Currently, it is considered that the pathogenesis is associated with infection, inflammation, immunological reactions and genetic factors (6,7). According to previous studies, the prevalence rate of IgAN shows certain geographical and ethnic differences, and genetic factors play a very important role in the onset of IgAN (8). Therefore, it is particularly important to search for genes associated with susceptibility to IgAN in order to provide a genetic target for therapeutic intervention in IgAN. There have been many Chinese studies on candidate genes associated with the severity and complications of IgAN (9-11), whereas there have been few studies looking into the candidate genes associated with its pathogenesis. A large-sample study on IgAN carried out by Li *et al* in China revealed that variation of the ST6GALNAC2 gene is associated with genetic susceptibility to IgAN (12). The present study analyzed the correlation of polymorphism of a specific α-2,6 sialyltransferase gene (ST6GALNAC2) and the susceptibility of the Uyghur population to IgAN, so as to get a better understanding of the pathogenesis and genetic background of IgAN in the Uyghur region.

Subjects and methods

Subjects of study. A total of 180 cases of hospital patients and outpatients of Uyghur ethnicity (86 males and 94 females, average age, 38.81±11.06 years), diagnosed with IgAN by renal biopsy in the Nephrology Department of the People's Hospital of Xinjiang Uyghur Autonomous Region (Urumqi, China) were collected. Renal biopsy pathological diagnostic criteria established by Zou in 2011 (13) were used as diagnostic criteria for IgAN. Patients with secondary IgA deposition diseases, such as systemic lupus erythematosus (SLE), allergic

Table I. Primers for rs3840858 and rs2304921.

Locus	Forward primer (5'-3')	Reverse primer (5'-3')
rs3840858	GCTGACAGCCTTAGCTCCCCCACGA	CACTCCTGCCACTGCGCTCTCTCCA
rs2304921	AAAGCTTCCAAGGGGTAGGT	TCATCCTTCTCTGCTGTTGG

purpura, chronic liver diseases, ankylosing spondylitic renal damage and psoriatic renal damage were excluded. All the selected patients were unrelated, with permanent Uyghur residency, of three different generations and all lived in Xinjiang. The healthy controls were 180 healthy individuals (84 males and 96 females, average age, 37.53±11.68 years) who went to the aforementioned hospital for medical examination from July 2008 to January 2013.

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

Table II. Gender and age distribution in the IgAN and control groups.

Item	IgAN group	Control group	t- or χ^2 value	P-value
Age (years)	38.81±11.06	37.53±11.68	0.754	0.452
Gender (M/F)	86/94	84/96	0.045	0.833

IgAN, immunoglobulin A nephropathy; M, male; F, female.

Reagents. The reagents used included the whole blood genomic DNA extraction kit (Shanghai Sangon Co., Ltd., Shanghai, China), Taq polymerase, 10X buffer, dNTP (including $MgCl_2$), ddH_2O (Beijing Dingguo Biotechnology Co., Ltd., Beijing, China) and DNA marker (BBI, SeraCare Life Sciences, Inc., Milford, MA, USA). The other reagents were conventional molecular biology reagents.

Design and synthesis of primers. The primer sequences (shown in Table I) of ST6GALNAC2 gene rs3840858 and rs2304921 were as previously described (12) and were verified using primer5 software (Premier Biosoft, Palo Alto, CA, USA). The primers were synthesized by Shanghai Sangon Co., Ltd., following the requirements of the project group.

Research methods

Collection of blood samples. A 5 ml sample of venous blood was collected from each patient on an empty stomach in the morning. EDTA was used for anticoagulation. The samples were numbered and registered. Whole blood samples were placed at -80˚C for cryopreservation.

DNA extraction. DNA specimens were extracted with the Ezup pillar blood genomic DNA extraction kit according to the kit instructions and preserved at -20˚C.

Detection of gene polymorphism. i) PCR reaction conditions for SNP rs2304921. The total volume of the amplification stage of the PCR was 35 μl (containing 3 μl DNA, 20 μl ddH_2O, 5 μl buffer, 2 μl dNTP, and 1.2 and 2 μl Taq polymerase in the upstream and downstream directions, respectively). The amplification reaction conditions of PCR were: denaturation at 95˚C for 5 min; main cycling at 95˚C for 45 sec, 61.7˚C for 60 sec and 72˚C for 45 sec, 38 cycles in total; followed by 72˚C for 10 min and preservation at 4˚C. The PCR reaction was performed on the GeneAmp® PCR System 9700 Thermal cycler from Applied Biosystems®, Invitrogen Life Technologies (Foster City, CA, USA).

ii) PCR reaction conditions for SNP rs3840858. The total volume of the amplification reaction of PCR was 35 μl (containing 3 μl DNA, 20.6 μl ddH_2O, 5 μl buffer, 2 μl dNTP, 1.5 and 2 μl Taq polymerase in the upstream and downstream directions, respectively). The amplification reaction conditions of PCR were: denaturation at 95˚C for 5 min; main cycling conditions at 95˚C for 45 sec, 61.0˚C for 60 sec and 72˚C for 45 sec, 37 cycles in total; followed by 72˚C for 10 min and preservation at 4˚C.

iii) Genotyping. Every PCR amplification product was electrophoresed at a voltage of 120 V for 20 min using 1.5% agarose gel to which ethidium bromide nucleic acid dye had been added in advance. Imaging was conducted using a UV transmission automatic image analyzer (Bio-Rad Laboratories, Inc., Hercules, CA, USA). All PCR products for each SNP locus were genotyped by direct sequencing (conducted by Beijing Dingguo Biotechnology Co., Ltd.).

Statistical analysis. Whether the genotypes of the population were in Hardy-Weinberg equilibrium was estimated using the χ^2 test. Other statistical analyses were performed with SPSS software, version 17.0 (SPSS, Inc., Chicago, IL, USA). The allele and genotype frequencies were calculated by χ^2 test. A t-test was applied for comparison of measurement data between groups. Logistic regression analysis was applied to analyze the correlation between polymorphism and IgAN. All statistics are two-sided with a test level a=0.05. P<0.05 was considered to indicate that a difference was statistically significant.

Results

General patient characteristics. There was no statistically significant difference in terms of gender and age between the IgAN group and the control group (P>0.05; Table II).

Distributions of genotype and allele frequencies of rs3840858 in the ST6GALNAC2 gene. Two genotypes, a DD genotype and a DI genotype, were detected by direct forward sequencing (Fig. 1), and the II genotype was absent. The distributions of

Table III. Distribution of genotype and allele frequencies of rs3840858 polymorphism of the ST6GALNAC2 gene in the IgAN and control groups.

		Genotype frequency, n (%)		Allele frequency, n (%)	
Group	No. of cases	DD	DI	D	I
IgAN	180	148 (82.2)	32 (17.8)	328 (91.1)	32 (8.9)
Control	180	170 (94.4)	10 (5.6)	350 (97.2)	10 (2.8)
χ^2-value		13.046		12.238	
P-value		0.001		0.001	

IgAN, immunoglobulin A nephropathy.

Table IV. Correlation between polymorphism of the ST6GALNAC2 gene and the susceptibility to IgAN.

Genotype or allele	Control group, n (%)	IgAN group, n (%)	P-value	OR	95% CI
rs3840858					
Genotype					
DD	170 (94.4)	148 (82.2)		1	
DI	10 (5.6)	32 (17.8)	0.001	3.676	1.284-10.519
Allele					
D	350 (97.2)	328 (91.1)		1	
I	10 (2.8)	32 (8.9)	0.001	3.415	1.223-9.531
rs23840858					
Genotype					
AA	6 (3.3)	4 (2.2)		1	
GG	54 (30.0)	72 (40.0)	0.778	1.3	0.209-8.082
AG	120 (66.7)	104 (57.8)	0.176	0.65	0.349-1.211
Allele					
G	294 (81.7)	280 (77.8)		1	
A	66 (19.3)	80 (22.2)	0.228	1.273	0.760-2.132

IgAN, immunoglobulin A nephropathy., OR, odds ratio; CI, confidence interval.

the DD and DI genotypes in the IgAN and control groups were in accordance with Hardy-Weinberg equilibrium (P>0.05). As shown in Table III, the DI genotype ratio (17.8%) in the IgAN group was higher than that in the control group (5.6%), while the DD genotype ratio (82.2%) in the IgAN group was lower than that in the control group (94.4%). A statistically significant difference was observed in the distributions of each genotype between the IgAN and control groups (χ^2=13.046, P=0.001).

In the IgAN group, the I allele frequency (8.9%) was higher than that in the control group (2.8%), while the D allele frequency (91.1%) was lower than that in control group (97.2%). There was a statistically significant difference in the I and D allele distributions between the IgAN group and the control group (χ^2=12.238, P=0.001).

Univariate logistic regression analysis indicated that rs3840858 polymorphism may be a risk factor of IgAN (P=0.001). The risk of developing IgAN in individuals who carried the DI genotype was 3-fold higher than that in those who carried the DD genotype [odds ratio (OR)=3.676, 95% confidence interval (CI)=1.284-10.519], and the risk of developing IgAN in individuals who carried the I allele was higher than that in those who carried D allele (OR=3.415, 95% CI=1.223-9.531; Table IV).

Distributions of genotype and allele frequencies of rs2304921 in the ST6GALNAC2 gene. Three genotypes, GG, AG and AA genotypes, were detected by direct reverse sequencing (Fig. 2). The distributions of the GG, AG and AA genotypes in the IgAN and control groups were in accordance with Hardy-Weinberg equilibrium (P>0.05). As demonstrated in Table IV, the AG genotype rate (40.0%) in the IgAN group was higher than that in control group (30.0%), while the AA and GG genotype rates (2.2% and

Table V. Distribution of genotype and allele frequencies of rs2304921 polymorphism of the ST6GALNAC2 gene in the IgAN and control groups.

		Genotype frequency, n (%)			Allele frequency, n (%)	
Group	No. of cases	GG	AG	AA	G	A
IgAN	180	104 (57.8)	72 (40.0)	4 (2.2)	280 (77.8)	80 (22.2)
Control	180	120 (66.7)	54 (30.0)	6 (3.3)	294 (81.7)	66 (19.3)
χ^2-value			4.114		1.684	
P-value			0.128		0.228	

IgAN, immunoglobulin A nephropathy.

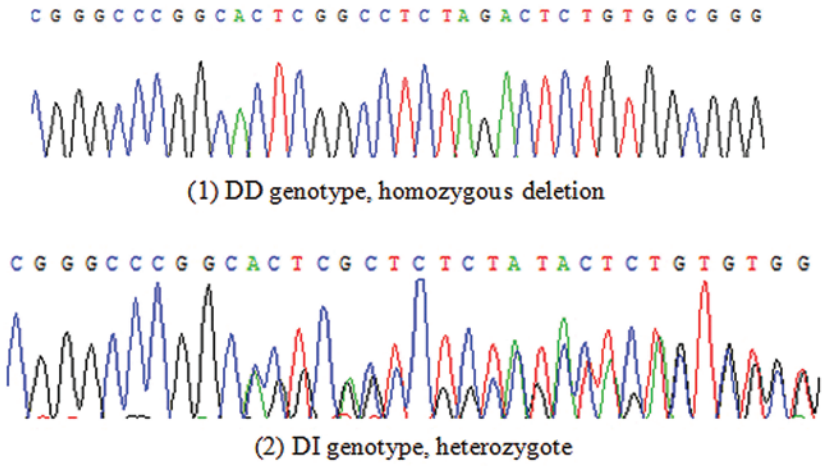

Figure 1. Genotypes of rs3840858 polymorphism in the ST6GALNAC2 gene.

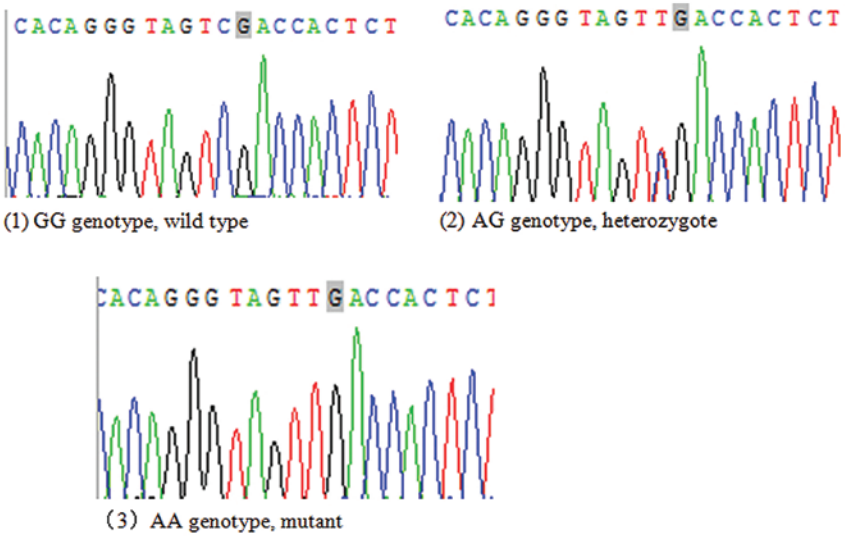

Figure 2. Genotypes of rs2304921 polymorphism in the ST6GALNAC2 gene.

57.8%, respectively) in the IgAN group were lower than those in the control group (3.3% and 66.7%, respectively). The differences in the distributions of each genotype of the ST6GALNAC2 gene rs2304921 polymorphism between the IgAN and control groups were not statistically significant (χ^2=4.114, P=0.128).

The A allele frequency (22.2%) in the IgAN group was higher than that in the control group (19.3%), while the G allele frequency (77.8%) in the IgAN group was lower than that in the control group (81.7%). The difference in A and G allele distributions between the IgAN group and the control group was not statistically significant (χ^2=1.684, P=0.228).

Univariate logistic regression analysis demonstrated that the rs2304921 polymorphism is not likely to impact the risk of developing IgAN (P>0.05; Table V).

Discussion

The serum IgA levels of the majority of patients with IgAN increase (14). However, due to lack of specificity, this cannot be regarded as a criterion for assisting clinical diagnosis. In recent years, with several kinds of technologies such as ELISA and mass spectrometry, scholars from research institutions in Japan, the United States of America and China all observed that aberrant glycosylation of IgA1 molecules in IgAN patients may be the most important mechanism of pathogenesis (15-17). It has been widely accepted that glycosylation defects lead to the formation of immune complexes in the pathogenesis of IgAN. The study conducted by Gharavi *et al* (18) suggests that glycosylation defects of IgA1 are a genetic characteristic and a genetic risk factor of IgAN. Thus, aberrant glycosylation provided a new candidate gene for the study of the genetic susceptibility of IgAN.

The ST6GLNAC2 gene encodes a specific α-2,6 sialyltransferase enzyme that participates in galactosylation in the IgA formation process. The study conducted by Patsos *et al* (19) indicates that defective expression of the ST6GLNAC2 gene decreases the activity of α-2,6 sialyltransferase. According to the literature, α-2,6 sialyltransferase activity on serum IgA1 in patients with focal proliferative type and sclerotic type IgAN (Haas stage III-IV) is reduced, which delays the sialylation of GalNAc in the molecular hinge area of serum IgA1 and affects the galactosylation of O-linked glycosyl in this area, eventually causing changes to the molecular charge and spatial structure of IgA1 (20). An increase in the activity of α-2,6 sialyltransferase may cause premature sialylation of GalNAc in the molecular hinge area of serum IgA1 and then affect the galactosylation of O-linked glycosyl in this area (21).

Li *et al* (12) conducted a large-sample study with sporadic Han IgAN patients as subjects. The results demonstrated that the frequency of the ADG haplotype in the promoter region of the ST6GALNAC2 gene was increased in patients with IgAN, and the ADG haplotype was associated with a deficiency of α-2,6 sialylation of IgA1. The authors also analyzed the correlation between variants of C1GALT1 and ST6GALNAC2 genes and the predisposition and severity of IgAN. The results revealed that the IgA1 O-glycosylation-related genes, C1GALT1 and ST6GALNAC2, were associated with the disease predisposition and severity of IgAN.

The analysis of SNP rs3840858 in the ST6GALNAC2 gene in this study revealed the existence of two genotypes (DD and DI) as detected by sequencing; the DI genotype rate in the IgAN group was higher than that in the control group, and the I allele frequency in the IgAN group was higher than that in the control group. The differences between the two groups were significant. Moreover, univariate logistic regression analysis showed that the risk of developing IgAN in individuals who carried the DI genotype was 3-fold higher than that in those who carried DD genotype. This suggests that the rs3840858 polymorphism is associated with the susceptibility to IgAN. In the study by Li *et al* (12), three genotypes (DD, DI and II) of SNP rs3840858 were detected by a restriction enzyme digestion technique and the differences in the genotype and allele frequencies between IgAN and control groups was not found to be statistically significant. The lack of conformity of the results may be due to geographic and ethnic differences in the gene polymorphism.

The analysis of SNP rs2304921 in the ST6GALNAC2 gene in this study revealed the existence of three genotypes (GG, AG and AA) as detected by sequencing. The differences in genotype and allele frequencies between the IgAN group and control group were not statistically significant, which suggests that the rs2304921 polymorphism is irrelevant to the susceptibility to IgAN. This result was in accordance with the study by Li *et al* (12).

To conclude, this study indicated that the rs3840858 polymorphism of the ST6GALNAC2 gene might be associated with the susceptibility to IgAN in an Uyghur population, whereas rs2304921 polymorphism appears to be irrelevant to IgAN in Uyghur. Due to the limited sample size, the correlation of the ST6GALNAC2 gene with susceptibility to IgAN requires investigation in a multi-zone and multi-site study with a larger sample size so as to provide new evidence for risk prediction as well as prevention and treatment.

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