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Association analysis of five SNP variants with gout in the Minnan population in China

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Backgrund/aim: There is a very high prevalence of gout in the Minnan population in China. We aimed to explore the genetic characteristics and genetic mechanisms of gout in the Minnan population by studying the association of 5 single nucleotide polymorphisms (SNPs) with gout.

Materials and methods: A total of 163 gout patients and 187 normal controls from Minnan were enrolled in this case-control study. SNPs (rs1165205, rs3733591, rs6855911, rs2231142, rs333049) were genotyped by allele-specific polymerase chain reaction (PCR) and analyzed with SPSS 16.0.

Results: Significant association with gout was found for rs2231142 ($P < 0.001$), consistent with our prior studies. An association between rs1333049 and gout was also found ($P = 0.03$) in the Minnan population. No association of SNPs rs6855911, rs3733591, and rs1165205 was found with gout in the Minnan population.

Conclusions: Rs1333049 is associated with gout in the Minnan population, although rs2231142 shows an even stronger association with gout. The C allele of rs1333049 and the A allele of rs2231142 might be crucial risk factors for gout.

Key words: Minnan population, gout, single nucleotide polymorphism, association analyses

1. Introduction

Minnan is located in the southeast of China. The prevalence of hyperuricemia in the middle- and old-aged Minnan population is 27.3% for males and 15.5% for females, which is among the highest nationwide (1–6). SLC17A3, SLC2A9, and ABCG2 have been confirmed to be important renal urate transporters; identified single nucleotide polymorphism (SNP) rs1165205 in SLC17A3 and SNP rs2231142 in ABCG2 have been associated with uric acid concentration and gout (7–9). SNP rs6855911, located within intron 7 of SLC2A9, showed the strongest signal with a protective effect of the minor allele in a German population ($P = 3.2 (10^{-7}$) (10). SNP rs3733591, an additional variant in SLC2A9, has been reported to be associated with gout in some populations of East Asia and Europe (11–14). It has been widely accepted that SNP rs1333049 in chromosome 9p21.3 is associated with coronary artery disease (15), first established by an association of rs1333049 with gout, whereas no association in New Zealand counterparts was found (16). Therefore, we examined the genotype and allele frequency distributions of these SNPs in the Minnan population with the aim of exploring their association with gout, which might help to further understand the genetic characteristics and possible underlying pathogenic mechanisms of gout in the Minnan population of China.

2. Materials and methods

2.1. Subjects and data collection

A total of 163 gout patients, diagnosed by a clinical endocrine physician according to the criteria of the American College of Rheumatology (17), were recruited. Patients with liver diseases, diabetes mellitus, kidney diseases, and other metabolic diseases were excluded to eliminate their influence on uric acid levels. Likewise, 187 normal controls without a personal or familial history of hyperuricemia, gout, or other serious illness were recruited. Total protein (TP), albumin, globulin, total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), blood urea nitrogen (BUN), creatinine, and uric acid of all participants were measured under the condition

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of no medical therapy. All patients and controls were selected from the same population residing in the Minnan area of China. The Ethics Committee of the Osteopathy Hospital of Quanzhou approved the study protocol, and all participants gave written informed consent at the time of inclusion and again at the time of follow-up investigations.

2.2. Genomic DNA extraction

Genomic DNA was isolated from 200 μL of peripheral venous blood according to the manufacturer's recommendations for the Pure Gene DNA Blood Kit (QIAGEN, Hilden, Germany).

2.3. Allele-specific PCR

All primers were designed using Primer 5.0 (Table 1). Two specific reverse primers, P1 and P2, were designed with complementary 3'-terminal nucleotides to the corresponding polymorphisms. To enhance specificity, a destabilizing mismatch was incorporated at the third or fourth nucleotide from the 3'-terminus in each specific primer (18,19).

Reverse primer 1 or 2 generated a fragment with the common forward primer (Fw) in two different tubes. The procedure rendered a band both in two tubes in the heterozygote, but a distinct band just in one tube in the homozygote. The PCR reactions were carried out in a total volume of 15 μL containing 20–50 ng of DNA as a template, 0.5 U of Taq DNA polymerase, 0.125 mmol/L of each dNTP, 1.5 mmol/L $MgCl_2$, and 0.4 μmol/L of each primer and Taq buffer (Takara, Japan). PCR cycling was performed on a Master Cycler thermal cycler (Eppendorf, Germany) with an initial denaturation at 96 °C for 3 min, followed by 38 cycles of 94 °C for 20 s, 58 °C for 20 s, and 72 °C for 35 s, with a final extension at 72 °C for 10 min. The amplified products were resolved on 2.0% agarose gel (1X TAE buffer, pH 8.0) and stained with GelRed (Biotium, USA).

2.4. Statistical analyses

All statistical analyses were performed with SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Clinical parameters in case-control cohorts were compared by nonparametric tests. Chi-square analyses were used to determine whether the SNP genotypes of cases and controls deviated from Hardy–Weinberg equilibrium (HWE) and to compare actual genotype counts of both groups. Differences in allele frequencies between dichotomous traits were calculated by employing the same method. Prevalence odds ratios (ORs) with their 95% confidence intervals (CIs) were included. Logistic regression was used to adjust for covariates differentially distributed in case-control cohorts. An analysis of variance (ANOVA) was used to calculate the association between genotypes and clinical characteristics among gout patients. Two-tailed P-values of less than 0.05 were considered statistically significant.

3. Results

3.1. Characteristics of gout patients and controls

The difference in clinical characteristics between the study groups is shown in Table 2. The results showed that among the gout patients there was a significantly higher proportion of males than females ($P < 0.001$). Abnormal levels of albumin, TC, TG, HDL-C, LDL-C, and creatinine

Table 1. Primers used for genotyping by allele-specific PCR.

Polymorphism		Primers (5'-3')
rs1165205	Fw	CAATCCAAGCAGAGCCCCTAGTAG
	Rw_1	GCCTGGGAGATATTTTACCAAATGA***G***AA
	Rw_2	GCCTGGGAGATATTTTACCAAATGA***G***AT
rs2231142	Fw	TTGGCAAATCCTTGTATGAAGCAGT
	Rw_1	AGCCGAAGAGCTGCTGAGAA***T***TT
	Rw_2	AGCCGAAGAGCTGCTGAGAA***T***TG
rs3733591	Fw	TGGACACTCTAATCCCTGCTGAAAG
	Rw_1	CAGGCGGATGCTCCTCTGCA***A***GT
	Rw_2	CAGGCGGATGCTCCTCTGCA***A***GC
rs6855911	Fw	GGCAGTGGACATCTTTCAGGGTG
	Rw_1	GTCTTCATCTACTTGGCATCAT***C***TC
	Rw_2	GTCTTCATCTACTTGGCATCAT***C***TT
rs1333049	Fw	CTGCTGACTCTGAAGATCATACCCG
	Rw_1	TTACCTCTGCGAGTGGCTGCT***A***TTG
	Rw_2	TTACCTCTGCGAGTGGCTGCT***A***TTC

Fw: Forward primer; Rw: reverse primer. Specific nucleotides are underlined; specificity-enhancing mismatches are shown in bold italics.

Table 2. Clinical characteristics of the study population.

Parameter	Control (n = 187)	Case (n = 163)	P-value
Sex: male/female, n (%)	120 (64.2%), 67 (35.8%)	152 (93.3%), 11(6.7%)	<0.0001
Age, years	45.21 ± 13.44	42.62 ± 15.10	n.s.
TP, g/L	70.46 ± 10.84	69.73 ± 17.31	n.s.
Albumin, g/L	44.40 ± 4.98	47.26 ± 6.25	<0.0001
Globulin, g/L	27.56 ± 5.28	27.86 ± 7.95	n.s.
TC, mmol/L	5.20 ± 1.13	9.41 ± 40.31	0.03
TG, mmol/L	1.56 ± 1.82	3.18 ± 10.78	<0.0001
HDL-C, mmol/L	1.32 ± 0.55	2.07 ± 9.79	0.005
LDL-C, mmol/L	4.51 ± 15.84	3.63 ± 1.45	<0.0001
BUN, mmol/L	5.26 ± 1.77	5.80 ± 4.49	n.s.
Creatinine, μmol/L	104.33 ± 66.02	112.27 ± 32.32	<0.0001
C-reactive protein (CRP) (mg/L)	20.2 ± 5.45	3.54 ± 1.02	<0.001
Erythrocyte sedimentation rate (ESR) (mm/h)	30.31 ± 10.01	13.04 ± 2.04	<0.001

Values denote mean ± standard deviation; n.s.: not significant.

were observed in gout patients compared to the controls (Table 2, $P < 0.05$).

3.2. Genetic analyses

Genotype distributions and allele frequencies in the control cohort are shown in Table 3. No deviation from HWE was observed for the 5 SNPs in the gout-free controls ($P > 0.05$). However, rs3733591 showed deviation from HWE in gout cases ($P = 0.024$), whereas the other SNPs exhibited P-values of >0.13. Significant association with gout was found for rs2231142 ($P < 0.0001$). The frequencies of AA, AC, and CC genotypes were 28.4%, 49.7%, and 21.9%, respectively, among gout patients, and 13.6%, 44.3%, and 59%, respectively, among the controls. In accordance with the genotypic associations, the allele distributions differed significantly between these 2 groups ($P < 0.0001$). The frequency of the A allele in gout cases was significantly higher than in the controls (53.2% and 35.7%, respectively). SNPs, A/C polymorphism, and an increased risk of gout were associated with the A allele (OR 2.048 [95% CI 1.471–2.852]) (Table 3).

There was a weak association between SNP rs1333049 and gout ($P = 0.03$). The frequencies of the GG, GC, and CC genotypes were 19.6%, 54.0%, and 26.4%, respectively, among gout patients, and 32.1%, 44.9%, and 23%, respectively, among the controls. The frequency of the C allele in gout cases was higher than in the controls (53.4% and 45.5%, respectively). There was a significantly increased risk of gout in carriers of the CC genotype associated with the C allele (OR 1.374 [95% CI 1.020–1.850) (Table 3).

3.3. Associations of clinical characteristics with genotypes

To explore the cause of gout, the association of different genotypes with clinical characteristics was further investigated. Based on the significant association of rs2231142 with gout, one test showed a detailed genotype–clinical characteristics correlation. In all recruits, globulin levels showed a statistically significant difference among various genotypes ($P = 0.011$). Carriers of genotype AA showed lower globulin levels compared to noncarriers (25.23 ± 4.78 g/L vs. 27.66 ± 6.00 g/L vs. 27.67 ± 5.21 g/L). Moreover, carriers with the AA genotype were associated with elevated uric acid levels compared with the CC genotype and the heterozygous genotype (476.89 ± 150.28 μmol/L vs. 420.32 ± 159.19 μmol/L vs. 374.47 ± 145.40 μmol/L; $P < 0.001$) (Table 4). The uric acid levels again suggested that the A allele might be the risk allele for gout.

As for SNP rs1333049, no difference in clinical characteristics between different genotypes was observed (Table 5). This indicated that rs1333049 might not be involved in the development of gout.

4. Discussion

In the present study, some clinical characteristics showed a statistically significant difference in the case-control study ($P < 0.05$) (Table 2), but no difference in their genotypes (Tables 4 and 5). This directly supports the view that gout is highly associated with metabolic syndrome and that its mechanism is complex (19,20).

We were able to confirm a significant association between gout and rs2231142 in ABCG2 among the Minnan population. The A allele of SNP rs2231142 might play a significant role in controlling the level of uric acid in the Minnan population.

Meanwhile, this study studied the association of rs2231142 with the clinical characteristics of the Minnan population (21,22). Globulin levels showed a statistically

Table 3. Genotype distributions and allele frequencies in the cases and controls.

rs1165205	Genotype frequency, n (%)				Allele frequency, n (%)	
Group	n	T/T	T/A	A/A	T	A
Control	187	127 (67.9)	57 (30.5)	3 (1.6)	311 (83.2)	63 (16.8)
Case	163	108 (66.1)	49 (30.2)	6 (3.7)	258 (79.1)	68 (20.9)
	χ^2 = 1.530, df = 2, P = 0.465				χ^2 =0.47, df = 1, P = 0.494 OR = 0.873, 95% CI 0.592, 1.288	
rs3733591	Genotype frequency, n (%)				Allele frequency, n (%)	
Group	n	A/A	A/G	G/G	A	G
Control	187	61 (32.6)	99 (53.0)	27 (14.4)	221 (59.1)	153 (40.9)
Case	163	42 (25.8)	95 (58.2)	26 (16.0)	179 (54.9)	147 (45.1)
	χ^2 = 1.970, df = 2, P = 0.373				χ^2 = 1.24, df = 1, P = 0.265 OR = 0.843, 95% CI 0.624, 1.138	
rs6855911	Genotype frequency, n (%)				Allele frequency, n (%)	
Group	n	G/G	G/A	A/A	G	A
Control	187	9 (4.8)	43 (23.0)	135 (72.2)	61 (16.3)	313 (83.7)
Case	163	5 (3.01)	33 (20.2)	125 (76.7)	43 (13.2)	283 (86.8)
	χ^2 = 1.203, df = 2, P = 0.548				χ^2 = 1.34, df = 1, P = 0.247 OR = 1.283, 95% CI 0.841, 1.956	
rs1333049	Genotype frequency, n (%)				Allele frequency, n (%)	
Group	n	G/G	G/C	C/C	G	C
Control	187	60 (32.1)	84 (44.9)	43 (23.0)	204 (54.5)	170 (45.5)
Case	163	32 (19.6)	88 (54.0)	43 (26.4)	152 (46.6)	174 (53.4)
	χ^2 = 7.002, df = 2, P = 0.030				χ^2 = 4.37, df = 1, P = 0.037 OR = 1.374, 95% CI 1.020–1.850	
rs2231142	Genotype frequency, n (%)				Allele frequency, n (%)	
Group	n	A/A	A/C	C/C	A	C
Control	140	19 (13.6)	62 (44.3)	59 (42.1)	100 (35.7)	180 (64.3)
Case	155	44 (28.4)	77 (49.7)	34 (21.9)	165 (53.2)	145 (46.7)
	χ^2 = 17.542, df = 2, P < 0.0001				χ^2 = 18.23, df = 1, P < 0.0001 OR = 2.048, 95% CI 1.471, 2.852	

significant difference between different genotypes. As is known, globulin is involved in the inflammatory response, and rs2231142 is a loss-of-function mutation that causes hyperuricemia and gout (23,24). Thus, the potential correlation between rs2231142 and globulin level in the development of gout needs further investigation.

It was recently reported that the C allele of rs1333049 conferred risk for gout in the Chinese population of the Qingdao region (25), while no significant association between rs1333049 and gout in New Zealand patients was noted (16). Hence, it is unclear whether or not SNP rs1333049 is closely linked with gout in other ethnicities. In the present study, we also found an association between rs1333049 and gout ($P < 0.05$). In addition, the C allele of SNP rs1333049 might play a significant role in the elevated levels of uric acid.

SNP rs1165205 in SLC17A3 was associated with uric acid levels, as previously described (9). However, we could not find any association between SNP rs1165205 and gout in the present study. This suggested that differences in genetic background could affect susceptibility to gout.

Table 4. Associations between rs2231142 and characteristics in all recruits.

rs2231142	AA (n = 63)	AC (n = 139)	CC (n = 93)	P-value
TP, g/L	69.27 ± 12.95	69.26 ± 14.43	70.09 ± 13.73	0.895
Albumin, g/L	46.71 ± 6.68	45.95 ± 5.87	45.60 ± 5.54	0.528
Globulin, g/L	25.23 ± 4.78	27.66 ± 6.00	27.67 ± 5.21	0.011
TC, mmol/L	5.67 ± 1.32	5.55 ± 2.49	10.83 ± 49.78	0.433
TG, mmol/L	2.02 ± 1.21	2.76 ± 11.27	1.76 ± 1.61	0.669
HDL-C, mmol/L	3.57 ± 15.77	1.25 ± 0.36	1.30 ± 0.65	0.142
LDL-C, mmol/L	8.27 ± 29.41	3.40 ± 1.08	3.48 ± 3.52	0. 089
BUN, mmol/L	6.06 ± 6.78	5.46 ± 2.07	5.52 ± 1.86	0.539
Creatinine, μmol/L	108.58 ± 21.13	109.10 ± 34.40	111.61 ± 91.36	0.934
Uric acid, μmol/L	476.89 ± 150.28	420.32 ± 159.19	374.47 ± 145.40	<0.001
ESR, mm/h	19.32 ± 2.23	20.14 ± 4.59	24.54 ± 7.67	0.134
CRP, mg/L	8.1831 ± 1.23	14.49 ± 3.21	14.95 ± 4.54	0.022

Table 5. Associations of clinical characteristics with the genotypes of rs1333049.

rs1333049	GG (n = 92)	GC (n = 172)	CC (n = 86)	P-value
TP, g/L	69.53 ± 13.65	70.54 ± 15.14	69.99 ± 12.47	0.856
Albumin, g/L	44.88 ± 6.77	46.31 ± 4.80	45.32 ± 6.23	0.133
Globulin, g/L	27.57 ± 5.38	27.66 ± 6.51	27.88 ± 7.96	0.947
TC, mmol/L	5.18 ± 1.15	5.58 ± 2.32	11.64 ± 52.61	0.234
TG, mmol/L	1.54 ± 0.99	2.88 ± 10.18	1.76 ± 1.50	0.349
HDL-C, mmol/L	1.34 ± 0.71	1.23 ± 0.33	2.80 ± 12.93	0.228
LDL-C, mmol/L	3.19 ± 1.00	4.87 ± 17.11	3.75 ± 3.80	0.595
BUN, mmol/L	5.80 ± 5.60	5.41 ± 1.68	5.38 ± 1.69	0.608
Creatinine, μmol/L	113.97 ± 93.08	104.81 ± 27.84	107.41 ± 26.77	0.424
Uric acid, μmol/L	376.00 ± 148.42	416.20 ± 157.99	419.80 ± 159.21	0.157

SNP rs3733591 has been reported to significantly contribute to the elevated urate concentrations in Solomon Island and Japanese patients, but not in their Caucasian counterparts (13). SNP rs3733591 has also been reported to contribute significantly to the development of gout in Han Chinese in Taiwan (12), although no association with gout was found in our study.

It has been reported that rs6855911 has a sex-specific effect on uric acid concentrations, resulting in a greater reduction in women (26). However, a case-control study of Shanghai Chinese showed that rs6855911 was not significantly different between gout and control subjects (P = 0.04) (27). Similar results were found in our study.

Our study has several limitations that should be taken into account. First, the sample size and number of SNPs studied is relatively small, which limits the study of sex and the understanding of the genetic background of gout in the Minnan population. Second, our study concentrated only on the association of SNP variants with gout; further functional studies on the effect of SNPs on transcription factor-binding activity and changes at the protein level are necessary. Thirdly, our genotype–clinical characteristics study results are too premature to explain the underlying pathological mechanisms of gout. More detailed clinical investigations are necessary.

In conclusion, this study showed that rs1333049 is associated with gout in the Minnan population, and rs2231142 shows an even stronger association with gout. SNPs rs1165205, rs3733591, and rs6855911 are population-specific variants of gout. The C allele of rs1333049 and the A allele of rs2231142 might be crucial risk factors for gout. Furthermore, these results show the simple genotype–clinical characteristics correlation. However, our findings need to be explored with other SNPs with a much larger sample size, well-designed clinical investigations, and functional analyses, so as to shed light on the genetic characteristics of gout in the Minnan population and the potential mechanisms underlying the genetic associations.

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