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Relationship between -238A/G and -308A/G polymorphisms in the promoter region of TNF-α and susceptibility to gout in the Chinese Han male population

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Abstract

The study has investigated whether -238A/G and -308A/G polymorphisms in the promoter region of TNF-α are associated with susceptibility to gout in the Chinese Han male population. Two hundred and thirty eight gout patients and 263 gout-free controls were enrolled. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to detect the genotypes and allelic frequencies of TNF-α-238A/G and -308A/G. No statistically significant difference was found in the genotypes (for TNF-α-238A/G, $\chi^2 = 1.741$, DF = 1, p = 0.187, OR = 0.646, 95% CI: 0.336-1.241; for TNF-α-308A/G, $\chi^2 = 1.893$, DF = 2, p = 0.388) or allelic frequencies (for TNF-α-238A/G, $\chi^2 = = 1.669$, DF = 1, p = 0.196, OR = 1.520, 95% CI: 0.802-2.882; for TNF-α-308A/G, $\chi^2 = 0.032$, DF = 1, p = 0.857, OR = 0.957, 95% CI: 0.590-1.552) of TNF-α-238A/G and -308A/G polymorphisms between patients and controls. The -238A/G and -308A/G polymorphisms in the promoter region of TNF-α are not associated with susceptibility to gout and thus do not play a major role in the development of gout in the Chinese Han male population.

Key words: *TNF-α, polymorphism, gout, Han Chinese.*

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Introduction

Gout is an acute form of arthritis caused by the deposition of urate crystals, in which phagocytic cells and cytokines play a major pathogenic role. Gout can cause substantial inflammation. Acute gouty arthritis is a self-limiting inflammatory response to the intra-articular deposition of monosodium urate monohydrate (MSU) microcrystals. Monocytes, macrophages and synovial cells containing phagocytosed MSU could generate inflammatory molecules, such as interleukin 1β (IL-1β), tumor necrosis factor α (TNF-α) and IL-8, at the time of an acute attack of gout. These molecules could cause the infiltration of inflammatory cells, especially neutrophils [1-4]. Tumor necrosis factor α is a sensitive cytokine that is related to the inflammatory reaction. Therefore, we hypothesized that TNF-α might be related to the pathogenesis of gout, especially gouty arthritis.

Many studies have shown that TNF-α has polymorphic sites that correlate with different diseases such as eclampsia, ulcerative colitis and rheumatic immune diseases, including rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) [5, 6]. Two important functional variants, the -238A/G and -308A/G polymorphisms, in the promoter region of TNF-α could directly affect the expression level of TNF-α. Cytokine production in individuals largely depends on promoter genetic polymorphisms [7]. Wilson *et al.* [8] showed that TNF-α-308A is a much stronger transcriptional activator than TNF-α-308G in the human B-cell line; thus, polymorphism -308A/G has direct effects on TNF-α regulation. In the study of Abdallah *et al.* [9], polymorphism -308A was related to a lower plasma TNF-α level. A study on the relationship between the polymorphism of -308A/G and -863C/A in TNF-α and gout in male Taiwanese showed that the polymorphism

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TNF-α-863C/A was significantly associated with gout, and showed a significant association between the genotype AA at polymorphism -863C/A and the development of gout. However, no association was found for polymorphism -308A/G [10]. Therefore, in the present study, we investigated whether polymorphisms -238A/G and -308A/G in the promoter region of TNF-α are associated with susceptibility to gout in the Chinese Han male population.

Material and methods

Subjects

Two hundred and thirty eight gout cases and 263 healthy controls were recruited from the Department of Endocrinology and body checking at the Affiliated Hospital of Qingdao University Medical College. All patients fulfilled the criteria for the diagnosis of primary gout, which was established by the American Rheumatism Association in 1977 [11]. People with no personal or familial history of hyperuricemia or gout (or other serious illnesses) were recruited as control subjects. All subjects provided written informed consent and the Ethics Committee of the Affiliated Hospital of Qingdao University Medical College approved the study protocol. Body mass index (BMI) was measured by the body weight divided by the body height in meters (kg/m^2). An automated multichannel chemical analyzer was used to measure triglycerides (TG), total cholesterol (TC), blood urea nitrogen (BUN), creatinine (CR) and uric acid (UA) in the plasma.

Genotyping

Blood samples were taken from all participants and genomic DNA was isolated from peripheral blood leukocytes by conventional methods. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to detect the genotypes and allelic frequencies of TNF-α-238A/G and -308A/G. For TNF-α-238A/G, the primers used for PCR were: forward: 5'-AGAAGACCCCCCTCGGAACC-3' and reverse: 5'-ATCTGGAGGAAGCGGTAGTG-3'. For TNF-α-308A/G, the primers were: forward: 5'- AGGCAATAGGTTTTGAGGGCCAT-3' and reverse: 5'-CACAAGCATCAAGGATACCC-3'. In addition, ten subjects were selected to confirm the genotype by DNA sequencing. Statistical analysis was performed using the Statistical Package for Social Sciences version 17.0. Student's *t*-test was used to assess a significant difference in demographic and clinical characteristics between cases and controls. A goodness-of-fit χ^2 test was used to examine the Hardy-Weinberg equilibrium in cases and controls. Pearson's χ^2 test was used to compare the genotype and allele frequencies between patients and controls. The odds ratios (ORs) and 95% confidence intervals (95% CIs) were used as measures of the strength of the relationship of the genotype distribution and allele frequencies between patients and controls. *P* values less than 0.05 were considered statistically significant.

Results

Clinical and biochemical characteristics of patients and controls

T-tests showed that for TNF-α-238A/G and -308A/G, there were no significant differences in age and BUN; however, there were significant differences in BMI, TG, TC, CR and UA between patients and controls ($p < 0.001$) (Table 1).

Genotype and allelic frequencies of TNF-α-238A/G and -308A/G

The genotype frequencies of TNF-α-238A/G and -308A/G were in accordance with the Hardy-Weinberg genetic equilibrium in patients (for -238A/G, $\chi^2 = 0.614$ and $p = 0.433$; for -308A/G, $\chi^2 = 1.321$ and $p = 0.250$) and controls (for -238A/G, $\chi^2 = 0.293$ and $p = 0.588$; for -308A/G, $\chi^2 = 0.333$ and $p = 0.564$). No statistical difference was found in the genotypes and allelic frequencies of the TNF-α-238A/G and -308A/G polymorphisms between patients and controls. For TNF-α-238A/G, the gen-

Table 1. Clinical and biochemical characteristics of TNF-α-238A/G and -308A/G gout patients and controls

Parameter	TNF-α-238A/G and TNF-α-308A/G		
	gout patients (238)	controls (263)	*P*-value
age (years)	48.98 ±9.37	50.16 ±13.27	0.255
body mass index (BMI) (kg/m^2)	27.45 ±3.40	23.50 ±3.15	< 0.001
triglyceride (TG) (mmol/l)	2.39 ±1.71	1.03 ±0.47	< 0.001
cholesterol (TC) (mmol/l)	5.29 ±1.33	4.66 ±0.78	< 0.001
blood urea nitrogen (BUN) (mmol/l)	5.76 ±2.60	5.60 ±1.41	0.408
creatinine (CR) (μmol/l)	89.12 ±26.62	97.20 ±10.73	< 0.001
uric acid (UA) (μmol/l)	501.33 ±133.42	314.23 ±57.88	< 0.001

Table 2. Genotypes and allelic frequencies of TNF-α-238A/G and -308A/G polymorphisms in gout patients and controls

Loci	Group	No.	Genotype frequency (%)			Allele frequency (%)	
			AA	GG	AG	A	G
TNF-α-238A/G	gout patients	238	0 (0%)	215 (90.3%)	23 (9.7%)	23 (4.8%)	453 (95.2%)
	controls	263	0 (0%)	246 (93.5%)	17 (6.5%)	17 (3.2%)	509 (96.8%)
			χ^2 = 1.741, DF = 1, p = 0.187 OR = 0.646, 95% CI: 0.336-1.241			χ^2 = 1.669, DF = 1, p = 0.196, OR = 1.520, 95% CI: 0.802-2.882	
TNF-α-308A/G	gout patients	238	0 (0%)	205 (86.1%)	33 (13.9%)	33 (6.9%)	443 (93.1%)
	controls	263	2 (0.8%)	227 (86.3%)	34 (12.9%)	38 (7.2%)	488 (92.8%)
			χ^2 = 1.893, DF = 2, p = 0.388			χ^2 = 0.032, DF = 1, p = 0.857, OR = 0.957, 95% CI: 0.590-1.552	

otype frequencies of AA GG, and AG were 0%, 90.3% and 9.7%, respectively, in patients and 0%, 93.5% and 6.5%, respectively, in controls. For TNF-α-308A/G, the genotype frequencies of AA, GG and AG were 0%, 86.1% and 13.9%, respectively, in patients and 0.8%, 86.3% and 12.9%, respectively, in controls. For TNF-α-238A/G, the allele frequencies of A and G were 4.8% and 95.2%, respectively, in patients and 3.2% and 96.8%, respectively, in controls. For TNF-α-308A/G, the allele frequencies of A and G were 6.9% and 93.1%, respectively, in patients and 7.2% and 92.8%, respectively, in controls (Table 2).

Clinical and biochemical characteristics of TNF-α-238A/G and -308A/G of the GG genotype and AA+AG genotype in gout patients

There were no statistical differences of age, BMI, TG, TC, BUN, CR or UA between GG genotype versus AA and AG genotypes of TNF-α-238A/G and -308A/G ($p > 0.05$) (Table 3).

Discussion

The main characteristic of gout is hyperuricemia, which is caused by an increased uric acid and/or reduction of its excretion. Patients are mainly middle and old-aged males and the sex ratio of the incidence is 20 to 1 (male to female). Gouty inflammation is caused by MSU crystal-induced release of proinflammatory cytokines from leukocytes. An acute attack of gout may be triggered by any event that stimulates the fresh recruitment from the circulation of neutrophils or monocytes capable of secreting TNF-α and other proinflammatory factors in response to available MSU crystals, thereby leading to amplification of the inflammatory response. Many reports have demonstrated that certain cytokines, in particular TNF-α and IL-1,

Table 3. Clinical and biochemical characteristics of TNF-α-238A/G and TNF-α-308A/G GG genotype and AA+AG genotype gout patients

Parameter	TNF-α-238A/G			TNF-α-308A/G		
	GG 215 (90.3%)	AA+AG 23 (9.7%)	p-value	GG 205 (86.1%)	AA+AG 33 (13.9%)	p-value
age (years)	48.87 ±9.52	50.00 ±7.97	0.585	48.85 ±9.40	49.82 ±9.28	0.582
body mass index (BMI) (kg/m^2)	27.42 ±3.46	27.77 ±2.70	0.636	27.46 ±3.48	27.40 ±2.84	0.924
triglyceride (TG) (mmol/l)	2.46 ±1.77	1.78 ±0.84	0.074	2.38 ±1.72	2.48 ±1.69	0.746
cholesterol (TC) (mmol/l)	5.32 ±1.30	5.04 ±1.60	0.334	5.26 ±1.30	5.49 ±1.55	0.351
blood urea nitrogen (BUN) (mmol/l)	5.80 ±2.71	5.36 ±1.17	0.440	5.80 ±2.76	5.52 ±1.30	0.567
creatinine (CR) (mmol/l)	90.04 ±27.22	80.50 ±18.46	0.102	89.33 ±27.29	87.81 ±22.36	0.761
uric acid (UA) (mmol/l)	504.08 ±136.74	475.57 ±95.48	0.331	502.38 ±137.10	494.81 ±109.39	0.763

IL-6 and IL-8, may have major roles in the pathogenesis of joint diseases. Tumor necrosis factor α is a cytokine released by mast cells and contributes to the promotion of the inflammatory process [12-14]. The important biological effects of TNF-α and the location of TNF-α (within a region of chromosome 6) have prompted studies on the relationship between TNF-α polymorphisms and diseases. Studies have found that the TNF-α-238A/G polymorphism is closely associated with many autoimmune diseases, such as Hepatitis B [15], SLE [16], coronary heart disease complicated with diabetes mellitus [17], and aplastic anemia [18]. TNF-α-238A is the disease susceptibility allele and -238G is the protective allele. The G allele may be associated with a lower expression of TNF-α [19, 20]. In addition, polymorphism -308A/G is associated with SLE, primary Sjögren's syndrome and tuberculosis [21]. Yen *et al.* [22] performed a study of the Taiwanese population, which showed that polymorphism -308A/G had a protective association with RA patients, but only in those who were HLA-DR4-negative.

In the present study, no statistically significant difference was found in the genotypes and allelic frequencies of polymorphisms TNF-α-238A/G and -308A/G between 238 patients and 263 controls in the Chinese Han male population. Therefore, we could not conclude that polymorphisms TNF-α-238A/G and -308A/G are associated with gout. Our results are consistent with the study of Chang *et al.* [10] on the relationship between the polymorphism of -308A/G and -863C/A in TNF-α and gout in male Taiwanese. They showed that polymorphism -863C/A in TNF-α was significantly associated with gout, and showed a significant association between the genotype AA of polymorphism -863C/A and the development of gout; however, no association was found for polymorphism -308A/G. As far as we know, this is the first investigation of SNPs in TNF-α in relation to gout in the Chinese Han male population.

In summary, the present study has demonstrated that polymorphisms -238A/G and -308A/G in the promoter region of TNF-α are not associated with susceptibility to gout and thus do not play a major role in the development of gout in the Chinese Han male population. Gout is a polygenic inherited disease; therefore, many other genes might be associated with susceptibility to gout, and we should not just analyze a single gene when studying the effect of gene mutation on the onset of the disease. However, the present study has several limitations related to the following factors: firstly, gout is a polygenic disease and the associated gene in our research is not the only susceptibility gene of gout. It may be a genetic marker in linkage disequilibrium, which is associated with susceptibility genes. Transcriptional regulation of a gene promoter is not determined by a single polymorphism, but is affected by the interaction of regulatory sequences in the transcriptional regulation regions. Individuals with different genotypes may also show different regulatory functions. Secondly, the sample size is small, and the test may have inadequate power for detecting a particular effect. Thus, further study is necessary to clarify the relationship between TNF-α polymorphisms and susceptibility to gout, such as analyzing more races and populations and using larger research samples. These future studies will provide important information for early screening, prevention, gene therapy and gene chip development for gout.

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