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Original Article

rs3806268 of *NLRP3* gene polymorphism is associated with the development of primary gout

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Abstract: The aim of the present study was to investigate the association between seven functional SNPs in *NALP3* gene and the susceptibility to primary gout. A total of 247 patients with primary gout and 247 controls were selected in this study. Genotyping of *NALP3* rs4612666, rs3806268, rs12239046, rs10754558, rs7512998, rs12137901 and rs12565738 was performed using the Sequenom MassARRAY platform. Comparison analysis showed that primary gout patients were more likely to have a higher body mass index, DBP, SBP, TG, urea nitrogen and uric acid ($P < 0.05$). According to logistic regression analysis, individuals carrying with the *GG* genotype of rs3806268 were associated with increased risk of primary gout when compared with the *AA* genotype (OR=1.83, 95% CI=1.03-3.26). However, no significant associations were identified for the remaining SNPs. In conclusion, we found a significant association between rs3806268 in NLRP3 gene and the risk of primary gout in a Chinese population. Further clinical and genetic studies are required to investigate the mechanisms underlying the association between NALP3 polymorphisms and the development of primary gout.

Keywords: *NLRP3*, polymorphism, primary gout

Introduction

Gout is the most common form of autoinflammatory arthritis among men, and this disease is characterized by higher urate in serum and monosodium urate crystal deposition in tissues, and thus to cause acute arthritis in and around the joints, nephrolithiasis and urate nephropathy [1, 2]. It is reported that the morbidity is showing an increasing trend recently worldwide, and gout has affected about 6% of adults [3]. A recent epidemiology study has reported that the prevalence of gout in eastern China is about 2% in men and 0.5% in women, while it is less than 0.1% about 10 years ago [4]. It is well known that gout is a long-term chronic disease, and the pathogenesis of this disease is involved in a complex and multifactorial process. Many environmental factors and immune dysregulation may play an important role in the development of gout, such as hyperuricemia. However, not all the individuals who expose to the similar risk factors would develop gout, which suggest that genetic factors could contribute to the underlying pathogenesis of this disease.

The accumulation of monosodium urate in tissues plays a critical role in the attack of gouty arthritis [5]. Blood monocytes and monocytes and macrophages in synovial fluid could release a mass of *IL-1β* stimulated by monosodium urate, and mediate inflammatory response. Caspase-1 catalyzes inactive *proIL-1β*, and thus causes the production of active interleukin IL-1β. The activation of caspase-1 depends on active *NALP3* inflammasome [6]. NLRP3 is a member of the nucleotide-binding domain and one of best-described members of nod like receptor (NLR) family [7]. *NLRP3* could active caspase-1 and subsequent secretion of proinflammatory cytokines such as *IL-1β* and IL-18 [8, 9]. The variants of *NLRP3* could influence the mRNA stability and expression of *NLRP3*. Recent studies have reported the association of *NLRP3* polymorphisms with the pathogenesis of autoinflammatory diseases, such as type-2 diabetes and inflammatory bowel disease [10, 11]. Currently, only one study reported the role of *NLRP3* polymorphisms in the development of gout [12]. Therefore, we hypothesize that *NLRP3* polymorphisms could contribute to the development of gout, and we conducted a

case-control study to investigate the association between seven functional SNPs in *NALP3* and the susceptibility to primary gout.

Material and methods

Patients

Between March 2013 and January 2015, a total of 247 patients with primary gout were collected from the Love & Health Hospital of Huangshi. The diagnosis of primary gout was based on preliminary criteria for the classification of gout of the American Rheumatism Association published by the American College of Rheumatology in 1997 (Wallace et al., 1977). Additionally, a total of 247 adult healthy subjects without primary gout were collected from individuals who received health check-up examinations in the Love & Health Hospital of Huangshi during the same period of collecting the patients with primary gout. The exclusion criteria for control subjects were those who had no history of primary gout, coronary atherosclerosis heart disease, diabetes, hyperlipidemia, cancer, hepatic disease, or renal disease.

The demographic characteristics of patients with primary gout and control subjects were investigated by trained nurses with a self-designed questionnaire, such as BMI index, tobacco smoking and alcohol drinking. The clinical characteristics were collected from medical records, such as blood pressure, urea nitrogen, creatinine, uric acid and etc. All individuals voluntarily participated in the study and gave their informed consent. The protocol of this study was approved by the ethics committee of the Love & Health Hospital of Huangshi.

DNA extraction and genotyping

2 ml peripheral blood sample was drawn from each patient with primary gout and control subject, which was stored at -20°C until use. DNA was extracted from peripheral blood by using a commercially available Qiagen kit (Hilden, Germany). All patients and health controls were asked to provide 5 mL venous blood, which was stored at -20°C until use and included 0.5 mg/mL EDTA as an anticoagulant. DNA was extracted from peripheral blood leukocytes using a commercially available Qiagen kit (Hilden, Germany). Genotyping of *NALP3* rs4612666, rs3806268, rs12239046, rs10754558, rs7512998, rs12137901 and rs12565738 was performed using the Sequenom MassARRAY platform (San Diego, CA, USA). Multiplex PCR mixture contained 1× HotStar Taq buffer, 2.8 mM $MgCl_2$, 0.1 U of HotStar Taq polymerase, 2 ng of genomic DNA, 0.5 pmol of each primer, and 0.5 mmol of dNTPs. Reaction was performed at 94°C for 15 min, followed by 45 cycles at 94°C for 20 s, 56°C for 30 s, and 72°C for 1 min, with a final incubation at 72°C for 3 min. Unincorporated dNTPs were deactivated using 0.3 U of shrimp alkaline phosphatase (SAP) followed by primer extension using 5.4 pmol of each extension probe, 50 mmol of iPlex Termination Mix, and 0.5 U of iPLEX enzyme (Sequenom; San Diego, CA). The extension reactions were carried out with an initial denaturation step of 8 min at 94°C, followed by 30 cycles at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 1 min. Purified extension reaction products with cation exchange resin were spotted onto SpectroCHIPs and measured by MALDI-TOF mass spectrometry.

Statistical analysis

Frequencies were used to describe the distribution of categorical variables and median and interquartile range was used for continuous variables. Deviations from Hardy-Weinberg equilibrium of *NALP3* rs4612666, rs3806268, rs12239046, rs10754558, rs7512998, rs12137901 and rs12565738 were assessed by χ^2-test with one degree of freedom. Multiple conditional logistic regression models were established to estimate the association between *NALP3* rs4612666, rs3806268, rs12239046, rs10754558, rs7512998, and rs12137901 and risk of primary gout, and the results were evaluated by the odds ratio (OR) and 95% confidence intervals (CIs). Additional stratification analysis was designed to analyze the gene-environmental interaction on the risk of primary gout. These values were adjusted for potential confounding factors, and the wild-type genotype was used as the reference group. Differences for 2-sided P values < 0.05 were considered statistically significant.

Results

The baseline information of patients with primary gout and control subjects were shown in **Table 1**. The mean ages of the primary gout

Table 1. Characteristics of patients with primary gout and control subjects

Variables	Patients N=247	%	Controls N=247	%	*t* or χ^2 value	*P* value
Age, years						
Mean age, years	51.60±9.15		51.95±8.52		0.44	0.33
< 50	144	58.30	147	59.51		
≥ 50	103	41.70	129	52.23	1.34	0.25
Gender						
Female	31	12.55	31	12.55		
Male	216	87.45	216	87.45	0.00	1.00
Tobacco smoking						
Never	159	64.37	146	59.11		
Ever	88	35.63	101	40.89	1.45	0.23
Alcohol drinking						
Never	132	53.44	124	50.20		
Ever	115	46.56	123	49.80	0.52	0.47
BMI, kg/m^2	25.13±1.15		24.20±2.05		6.22	< 0.001
DBP, mmHg	139.40±13.75		131.55±15.60		5.93	< 0.001
SBP, mm Hg	89.42±11.30		85.43±10.53		4.06	< 0.001
TG, mmol/L	2.47±2.10		1.53±0.93		6.43	< 0.001
TC, mmol/L	5.16±1.26		5.22±2.10		0.39	0.35
Urea nitrogen, mmol/L	6.15±2.40		5.81±1.55		1.87	0.03
Creatinine, μmol/L	92.50±35.35		95.65±16.73		1.27	0.10
Uric acid, μmol/L	513.50±19.40		335.50±48.65		53.41	< 0.001

BMI: body mass index; DBP: Diastolic blood pressure; SBP: Systolic blood pressure; TC: Total cholesterol; TG: Triglyceride; Continuous variables analyzed using independent sample t-test; categorical variables analyzed using χ^2-test.

Table 2. Genotype characteristics of the eight nucleotide polymorphisms of *NALP3*

SNP	Alleles	MAF From dbSNP	MAF		P for HWE in controls
			Patients	Controls	
rs4612666	A/G	0.4109	0.3988	0.4271	0.07
rs3806268	A/G	0.3874	0.4919	0.4089	0.08
rs12239046	C/T	0.4067	0.4453	0.4494	0.42
rs10754558	C/G	0.3558	0.3704	0.3522	0.001
rs7512998	T/C	0.1359	0.1498	0.1336	0.16
rs12137901	T/C	0.2869	0.3117	0.2834	0.06
rs12565738	C/T	0.1661	0.1660	0.1457	< 0.001

MAF: Minor allele frequency.

patients and control subjects were 51.60±9.15 and 51.95±8.52 years, respectively. As expected, no significantly difference was found between patients with primary gout and control subjects in terms of age and gender ($P > 0.05$). When compared with control subjects, the patients with primary gout had a higher BMI, DBP, SBP, TG, urea nitrogen and uric acid by χ^2-test (All P value < 0.05) (**Table 1**). Additionally, no significant differences were found between the 2 groups in terms of tobacco smoking, alcohol drinking, TC and creatinine (All P value > 0.05).

By χ^2-test with one degree of freedom, the genotype distributions of rs4612666, rs3806268, rs12239046, rs7512998 and rs12137901 confirmed with Hardy-Weinberg equilibrium (P value < 0.05), while the rs10754558 and rs12565738 deviated from Hardy-Weinberg equilibrium (P value > 0.05) (**Table 2**). The minor allele frequencies of the seven SNPs in controls were similar with those in the general population, as found in the NCBI dbSNP databases.

The association between the seven SNPs of *NALP3* and risk of primary gout was shown in **Table 3**. By χ^2-test, there were no significant differences between patients with primary gout and control subjects in terms of genotype distributions of the seven SNPs (All P values < 0.05). According to logistic regression analysis, indi-

Table 3. Association between the seven SNPs of *NALP3* and risk of primary gout

SNP	Patients	%	Controls	%	x^2 value	*P* value	OR (95% CI)[1]	*P* value
rs4612666								
AA	82	33.20	74	29.96			Ref. (1.0)	-
AG	131	53.04	135	54.66			0.87 (0.58-1.22)	0.51
GG	33	13.36	38	15.38	0.82	0.66	0.78 (0.43-1.43)	0.40
rs3806268								
AA	56	22.67	77	31.17			Ref. (1.0)	-
AG	139	56.28	138	55.87			1.38 (0.89-2.15)	0.12
GG	52	21.05	39	12.96	4.79	0.09	1.83 (1.03-3.26)	< 0.03
rs12239046								
CC	72	29.15	78	31.58			Ref. (1.0)	-
CT	130	52.63	116	46.96			1.21 (0.79-1.86)	0.35
TT	45	18.22	53	21.46	1.69	0.43	0.92 (0.53-1.58)	0.75
rs10754558								
CC	110	44.53	116	46.96			Ref. (1.0)	-
CG	91	36.84	88	35.63			1.09 (0.72-1.65)	0.67
GG	46	18.62	43	17.41	0.31	0.86	1.13 (0.67-1.90)	0.63
rs7512998								
TT	182	73.68	188	76.11			Ref. (1.0)	-
TC	56	22.67	52	21.05			1.11 (0.71-1.75)	0.63
CC	9	3.64	7	2.83	0.50	0.78	1.33 (0.43-4.29)	0.58
rs12137901								
TT	128	51.82	136	55.06			Ref. (1.0)	-
TC	84	34.01	82	33.20			1.09 (0.72-1.64)	0.67
CC	35	14.17	29	11.74	5.87	0.05	1.28 (0.71-2.31)	0.37
rs12565738								
CC	185	74.90	191	77.33			Ref. (1.0)	-
CT	42	17.00	40	16.19			1.08 (0.65-1.80)	0.74
TT	20	8.10	16	6.48	0.59	0.75	1.29 (0.61-2.75)	0.47

[1]Adjusted for BMI, DBP, SBP, TG, urea nitrogen and uric acid.

viduals carrying with the *GG* genotype of rs3806268 were associated with increased risk of primary gout when compared with the *AA* genotype (OR=1.83, 95% CI=1.03-3.26). However, we did not find significant association of rs4612666, rs12239046, rs10754558, rs7512998, rs12137901 and rs12565738 with the development of primary gout (All *P* values > 0.05).

We further analyzed the association between rs3806268 and risk of primary gout stratified by BMI, DBP, SBP, TG, TC, urea nitrogen, creatinine and uric acid, and no significant interaction was found between rs3806268 and demographic and clinical characteristics (*P* values > 0.05).

Discussion

It is well known that polymorphisms have an effect on the regulation of gene expression, which could contribute to the differences between individuals in the susceptibility to a disease and its severity. Gout is a polygenic heterogeneity disease, and the pathogenesis of gout showed individualization in human. The distributions of SNP loci in different races showed genetic heterogeneity. More than 30 different single nucleotide polymorphisms (SNPs) located on the exon3 of *NLRP3* gene have been reported, which encodes the nucleotide binding site domain and boundary regions [13]. In our study, we found that the *GG* genotype of rs3806268 in *NLRP3* gene was associ-

ated with an increased risk of primary gout in a Chinese population.

NLRP3 are known to be responsible for the recognition of several molecular patterns inducing the assembling of inflammasome, the activation of caspase-1 and finally the secretion of the pro-inflammatory cytokine *IL-1β*, and consequently innate immune response [14]. Polymorphisms in *NLRP3* were previously associated with inflammation-related diseases, such as ulcerative colitis, inflammatory bowel disease, juvenile idiopathic arthritis, neonatal-onset multisystem inflammatory disease and type 2 diabetes [15-20]. Zhang et al. conducted a study in a Chinese population, and have reported that rs10754558 and rs10925019 of *NLRP*3 contribute to the development of ulcerative colitis [15]. Varghese et al. conducted a study in Caucasian, and they have reported that no association was found between *NLRP3* polymorphisms and development of inflammatory bowel disease [16]. Yang et al. have reported that rs4353135 polymorphism may contribute to the pathophysiology of juvenile idiopathic arthritis in a Taiwanese population [17]. Dehghan et al. reported that SNPs in *NLRP3* may play a critical role in regulation of inflammation underlying cardiovascular disease [19]. These above mentioned studies have suggested that *NLRP3* polymorphisms were associated to susceptibility to inflammation-related diseases. Therefore, we hypothesized that functional SNPs in *NALP3* inflammasome may involve in the development of primary gout.

Only one previous study reported the association of *NALP3* polymorphisms with the susceptibility to primary gout in human [12]. Meng et al. have conducted a study including 480 cases with primary gout and 480 control subjects to investigate the association between genetic variants in 17 SNPs of *NALP3* and the susceptibility to the development of primary gout in a Chinese population. However, they did not find any association between them [12]. In our study, we also conducted a study in a Chinese population, and we found that the *GG* genotype of rs3806268 in *NLRP3* was associated with the development of primary gout, whereas no significant association was identified for the remaining six SNPs. The discrepancies of our results may be related to variation in origin of population, sample size, genotyping method and also by chance. Further clinical genetic studies are greatly needed to be performed to confirm our results.

Several limitations should be considered in our study. First, subject selection bias may overestimate the true size of effect or lead to spurious findings. Although there may exist selection bias for hospital-based study design, both cases and controls were matched on age and sex, which may have minimized the bias. Second, we investigated the association between selected seven SNPs in *NLRP3* and development of primary gout, but the associations of the functional SNPs in *NLRP3* with risk factors of gouty arthritis were not assessed. Therefore, a more detailed data are needed to analyze their association. Third, the rs10754558 and rs12565738 deviated from Hardy-Weinberg equilibrium which suggests that the sample size may not better represent the general population. Four, the sample size of the present study was small and all were selected from Chinese population, and thus the results could not representative of other ethnicities. Therefore, the results of our study should be repeated in other populations with more sample sizes.

In conclusion, we found a significant association between rs3806268 in *NLRP3* gene and the risk of primary gout in a Chinese population. Further clinical and genetic studies are required to investigate the mechanisms underlying the association between *NALP3* polymorphisms and the development of primary gout.

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Disclosure of conflict of interest

None.

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