Investigation on the association between *NLRP3* gene polymorphisms and susceptibility to primary gout

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Genet. Mol. Res. 14 (4): 16410-16414 (2015)
Received August 10, 2015
Accepted October 25, 2015
Published December 9, 2015
DOI http://dx.doi.org/10.4238/2015.December.9.10

ABSTRACT. We conducted a case-control study to investigate the association between 3 common *NALP3* polymorphisms (rs10754558, rs7512998, and rs12137901) and the susceptibility to primary gout. A total of 320 patients with primary gout and 320 controls were included in this study. The genotyping of *NALP3* rs10754558, rs7512998, and rs12137901 were conducted by polymerase chain reaction-restriction fragment length polymorphism. Comparison analysis showed that primary gout patients were more likely to have higher body mass index, prevalence of hypertension, blood glucose, triglycerides, urea nitrogen, and uric acid ($P < 0.05$). Logistic regression analysis revealed no significant association between the *NALP3* rs10754558, rs7512998, and rs12137901 polymorphisms and the risk of gouty arthritis. In conclusion, we found no significant association between *NALP3* gene polymorphisms and the risk of primary gout.

Key words: *NLRP3*; Polymorphism; Primary gout

INTRODUCTION

Primary gout has become a common metabolic disease, which is manifested by higher urate concentration in the serum and acute arthritis in and around the joints (Terkeltaub, 1993; Bieber and Terkeltaub, 2004). In China, the incidence of primary gout has rapidly increased in recent years because of dietary and lifestyle changes. Previous studies reported that the prevalence of gout in eastern China is approximately 2% in men and 0.5% in women, while it was less than 0.1% overall 10 years ago (Miao et al., 2008). The development of gout is caused by multiple factors, including environmental and genetic factors (Qing et al., 2013; Cai et al., 2014; Li et al., 2014; Roddy and Choi, 2014).

Interleukin-1β (IL-1β) is released by blood monocytes stimulated by monosodium urate monohydrate to mediate the inflammatory response. Moreover, caspase-1 catalyzes inactive proIL-1β and influences the production of active IL-1β. The expression of LRR and PYD domains-containing protein 3 (NALP3) is involved in the activation of caspase-1 and the production of IL-1β. Previous studies reported an association between NALP3 gene polymorphisms and the susceptibility to gout, but the results have been inconsistent (Miao et al., 2009; Meng et al., 2013). Therefore, we conducted a case-control study to investigate the association between 3 common *NALP3* polymorphisms (rs10754558, rs7512998, and rs12137901) and the susceptibility to primary gout.

MATERIAL AND METHODS

Patients

A total of 320 patients with primary gout were recruited for this study from the Huaihe Hospital of Henan University. Primary gout was diagnosed according to preliminary criteria for the classification of gout of the American Rheumatism Association (Wallace et al., 1977). Gout was defined as uric acid levels >420 mM in males and post-menopausal women and as >350 mM in pre-menopausal women.

Additionally, 320 controls were collected from individuals who received health check-up examinations. All patients were confirmed to have no history of primary gout, hyperuricemia, or other inflammation-related diseases.

The demographic and clinical characteristics were collected from medical records. Informed consent was obtained from all participants they were enrolled in the study. The Ethical Committee of Huaihe Hospital of Henan University approved the study protocols, and all participants provided written informed consent according to the Declaration of Helsinki.

DNA extraction and genotyping

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). The genotyping of *NALP3* rs10754558, rs7512998, and rs12137901 was conducted by polymerase chain reaction-restriction fragment length polymorphism. The primers for *NALP3* rs10754558, rs358294199, and rs35829419 were designed using Sequenom Assay Design 3.1 software (San Diego, CA, USA). Amplification 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.

Statistical analysis

The Statistical Analyses System package (version 8.01; SAS Institute, Cary, NC, USA) was used for statistical analysis. Differences between continuous variables were expressed by the mean ± SD, while those between categorical variables were evaluated using frequencies and percentages. Deviations from Hardy-Weinberg equilibrium of *NALP3* rs10754558, rs7512998, and rs12137901 were evaluated by χ^2-test. Logistic regression was used to assess the influence of *NALP3* rs10754558, rs7512998, and rs12137901 genetic polymorphisms on the susceptibility to primary gout. Associations were determined based on odds ratios and their 95% confidence intervals. 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

Patient demographic and clinical characteristics

The mean ages of the 320 primary gout patients and 320 controls were 52.6 ± 10.5 and 50.5 ± 9.2 years, respectively. Comparative analysis revealed that primary gout patients were more likely to have a higher body mass index, prevalence of hypertension, blood glucose, triglycerides, urea nitrogen, and uric acid (P < 0.05) (Table 1). However, there were no significant differences between the 2 groups in terms of sex, age, total cholesterol, and creatinine.

Table 1. Demographic and clinical characteristics of the primary gout patients and controls.

Variables	Patients (N = 320)	%	Controls (N = 320)	%	*t* or χ^2 value	P value
Age, years						
<55	173	54.1	187	58.4		
≥55	147	45.9	133	41.6	1.24	0.26
Gender						
Male	219	68.4	210	65.6		
Female	101	31.6	110	34.4	0.57	0.44
BMI, kg/m^2		24.5 ± 2.6		22.7 ± 3.5	7.38	<0.05
Hypertension						
No	170	53.1	232	72.5		
Yes	150	46.9	88	27.5	25.71	<0.05
Blood glucose, mM		6.1 ± 1.9		5.3 ± 1.3	6.22	<0.05
TG, mM		2.5 ± 2.1		1.4 ± 0.9	8.61	<0.05
TC, mM		5.1 ± 1.5		5.2 ± 1.6	0.82	0.21
Urea nitrogen, mM		6.05 ± 2.3		5.7 ± 1.5	2.28	<0.05
Creatinine, mM		90.6 ± 31.5		93.2 ± 20.6	1.24	0.11
Uric acid, mM		484.2 ± 126.6		317.4 ± 62.4	21.14	<0.05

The genotype frequencies of *NALP3* rs10754558, rs7512998, and rs12137901 are shown in Table 2. Genotype distributions were found to be in line with Hardy-Weinberg equilibrium (P > 0.05). We found no significant difference in the genotype distributions of *NALP3* rs10754558, rs7512998, and rs12137901 between cases and controls (all P values > 0.05, Table 2). According to logistic regression analysis, no significant association was found between the *NALP3* rs10754558, rs7512998, and rs12137901 polymorphisms and the risk of gouty arthritis.

Table 2. Association between the *NALP3* gene polymorphisms and susceptibility to primary gout.

Variables	Patients	%	Controls	%	HWE (P value)[2] in controls	OR (95%CI)[1]	P value
rs10754558							
CC	136	42.5	146	45.6		-	-
CG	138	43.1	134	41.9		1.11 (0.78-1.56)	0.56
GG	46	14.4	40	12.5	0.29	1.23 (0.74-2.06)	0.39
rs7512998							
TT	164	51.3	175	54.7		-	-
TC	129	40.3	124	38.8		1.11 (0.79-1.56)	0.53
CC	27	8.4	21	6.6	0.87	1.37 (0.72-2.66)	0.31
rs12137901							
CC	146	45.6	159	49.7		-	-
CT	128	40.0	123	38.4		1.13 (0.80-1.61)	0.46
TT	46	14.4	38	11.9	0.07	1.32 (0.79-2.21)	0.26

[1]Adjusted for age, gender, body mass index, hypertension, blood glucose, triglycerides, urea nitrogen, and uric acid.

DISCUSSION

It is well-known that multiple mutations in the NALP3 gene are associated with hereditary inflammatory periodic fever syndromes in humans, such as Muckle-Wells syndrome, cold induced autoinflammatory syndrome 1, and neonatal-onset multisystem inflammatory disease (Stojanov and Kastner, 2005; Masters et al., 2006; Pétrilli and Martinon, 2007; Church et al., 2008). There are more than 30 single-nucleotide polymorphisms (SNPs) in exon 3 of the NLRP3 gene, and these SNPs encode the nucleotide binding site domain and boundary regions (Aróstegui et al., 2004). Previous studies have reported an association between NALP3 gene polymorphisms and the susceptibility to gout (Miao et al., 2009; Meng et al., 2013), but the results of these studies are inconsistent. In our study, we found no significant difference between *NALP3* polymorphisms and primary gout, suggesting that mutations in the NALP3 inflammasome may not be involved in the susceptibility to gouty development.

Several previous studies have reported the role of NALP3 gene polymorphisms in the development of gout in different ethnicities (Miao et al., 2009; Meng et al., 2013). Meng et al. (2013) conducted a study in a Chinese population including 480 cases with primary gouty and 480 control subjects to investigate the association between 17 SNPs in *NALP3* and the susceptibility of gouty development. However, they found no significant association between NLRP3 gene polymorphisms and the risk of primary gouty arthritis. Miao et al. (2009) reported that functional mutations in NALP3 inflammasome may be genetic markers for gout. In our study, we investigated the role of 3 SNPs of *NLRP3* in the etiology of gout, but found no significant association between NALP3 gene polymorphisms and the susceptibility to primary gouty. The discrepancies in these results may have been caused by differences in case and control selection, study design, and sample size.

Several limitations should be considered in our study. First, all patients and controls were selected from a single hospital, and thus selection bias may have influenced the results of our study. Second, additional SNPs may be involved in the susceptibility to primary gout and interact with the NALP3 gene polymorphisms. However, we only investigated the association between NALP3 gene polymorphisms and primary gout risk. Third, the sample size was relatively small in our study, which may have limited the statistical power in finding differences between groups. Therefore, further multicenter studies including larger sample sizes are greatly needed to confirm our findings.

In conclusion, we found no significant association between NALP3 gene polymorphisms and the risk of primary gout. Further genetic studies including larger sample sizes are greatly needed to investigate the association between NALP3 gene polymorphisms and the development of primary gout.

Conflicts of interest

The authors declare no conflict of interest.

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