

Association of DNA methyltransferase polymorphisms with susceptibility to primary gouty arthritis

XIAOWU ZHONG^{1-3*}, YUANHONG PENG^{4*}, CHENGJIAO YAO^{2*}, YUFENG QING⁴, QIBIN YANG⁴, XIAOLAN GUO^{1,3}, WENGUANG XIE^{1,2}, MINGCAI ZHAO¹⁻³, XIAOMING CAI⁵ and JING-GUO ZHOU^{3,4}

¹Department of Clinical Laboratory and ²Medicine Research Center, Affiliated Hospital of North Sichuan Medical College, Nanchong, Sichuan 637000; ³Department of Laboratory Medicine, North Sichuan Medical College, Nanchong, Sichuan 637007; ⁴Department of Rheumatology and Immunology of the Affiliated Hospital, North Sichuan Medical College, Nanchong, Sichuan 637000; ⁵Department of Biology, North Sichuan Medical College, Nanchong, Sichuan 637007, P.R. China

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Abstract. Gouty arthritis is the most common type of inflammatory and immune disease, and the prevalence and incidence of gout increases annually. Genetic variations in the DNA methyltransferases (DNMTs) gene have not, to the best of our knowledge, been reported to influence gene expression and to participate in the pathogenesis of gout. The aim of the present study was to investigate whether the *DNMT1*, *DNMT3A* and *DNMT3B* polymorphisms contribute to gout susceptibility. These polymorphisms were screened for in 336 gout patients and 306 healthy control subjects (from a South China population) for association with gout. The distribution frequencies of *DNMT1* rs2228611 AA genotype (P=0.007) and A allele (P=0.002; odds ratio=1.508, 95% confidence interval=1.158-1.964) were found to be significantly increased in the gout patients when compared with those in the healthy control subjects. The rs1550117 in *DNMT3A* and rs2424913 in *DNMT3B* exhibited no significant associations with gout susceptibility between the patients and control subjects. These results demonstrated that the *DNMT1* rs2228611 polymorphism may be involved in the pathogenesis of gout, while *DNMT3A* rs1550117 and *DNMT3B* rs2424913 did not show any obvious significance in the current study; thus, may not be used as risk factors to predict the susceptibility to gout. However, further studies are required to investigate the functions and regulatory mechanism of the polymorphisms of *DNMTs* in gout.

Introduction

Gout arthritis is the most common inflammatory and immune disease affecting 1-2% of adults worldwide, which is associated with elevated serum urate levels and the deposition of monosodium urate (MSU) in the joints (1). Previous studies show that gout is also associated with the genetic background, a purine rich diet and alcohol consumption (2-4), and epidemiological studies have suggested that the prevalence and incidence of gout are increasing globally (5,6). Over the past decade, significant scientific advances have been made in understanding the pathogenesis and treatment of gout. Being a complex disease, the identification of genetic and environmental risk factors for gout may facilitate with investigating the pathogenesis of gout. As with other diseases, epigenetic events or heritable changes in gene expression capacity without DNA sequence alterations may be evaluated to gain insight into the concrete pathogenesis of gout (7). DNA methylation is the most common epigenetic modification, and is important in transcription and the chromatin structure (8). The process of DNA methylation usually occurs at the CpG sites and the methyl group to the 5' position of a cytosine in a CpG dinucleotide conferred by DNA methyltransferases (DNMTs) (9). Three primary DNMTs, DNMT1, DNMT3A and DNMT3B, may influence global DNA methylation (10). In addition, DNMT1 is a primary enzyme for maintaining methylation patterns during DNA replication, whereas DNMT3A and DNMT3B act predominantly as the *de novo* methyltransferases, and create novel methylation patterns (11-13).

In addition, DNA methylation and its regulatory enzymes have been implicated in a diverse set of biological processes, including X chromosome inactivation, genomic imprinting, as well as autoimmunity (14-17). Mutation of the human *DNMTs* alters gene expression and may provide insight into the mechanism of various diseases, such as centromere instability, acute myeloid leukemia and immunodeficiency (18-20). Therefore, it DNMTs may be important in the pathogenesis of autoimmune diseases. The single nucleotide polymorphism (SNP) 14463G>C of the *DNMT1* gene was demonstrated to be associated with higher lupus disease activity (21). And Nam *et al* (22)

Correspondence to: Professor Jing-Guo Zhou, Department of Rheumatology and Immunology of the Affiliated Hospital, North Sichuan Medical College, 63 Wenhua Road, Nanchong, Sichuan 637000, P.R. China
E-mail: jgzhou@nsmc.edu.cn

*Contributed equally

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identified that the -283C/T polymorphism at the promoter of the *DNMT3B* gene was associated with progression of joint destruction in rheumatoid arthritis (22). However, thus far, to the best of our knowledge, no studies have investigated the *DNMT* polymorphism association with gouty arthritis. In the present study, 336 primary gout patients and 306 healthy control subjects were employed to investigate the three SNPs of the *DNMT* gene and the susceptibility to primary gouty arthritis in a Chinese Han population, and aimed to gain a comprehensive understanding of their clinical relevance with regard to the development of gout.

Materials and methods

Subjects and ethical approval. All participants were recruited from the Department of Rheumatology, the Affiliated Hospital of North Sichuan Medical College (Nanchong, China) between February 2013 and April 2015. The study group consisted of 336 primary gouty arthritis patients and 306 healthy control subjects from a Chinese Han population. The gout patients were confirmed by a clinical endocrinology physician, according to the American College of Rheumatology classification criteria (23). The patients were not receiving any systemic anti-inflammatory treatment, or medication to control the production and elimination of uric acid prior to obtaining blood samples. The age-matched healthy control subjects, who had no history of gout or any systemic inflammatory disease, were enrolled in the present study. All the participants were of Chinese Han descent. Venous blood samples (2 ml) from all participants were obtained in the morning, following an overnight fasting of at least 12 h, and collected into sterile, single-use, anticoagulant-coated tubes and immediately sent to the laboratory for genetic testing. This study was approved by the Ethics Committee of the Affiliated Hospital of the North Sichuan Medical College. All participants provided written informed consent.

Clinical and laboratory examination. All of the clinical data and measurements were assessed and carefully recorded at the Department of Clinical Laboratory, the Affiliated Hospital of North Sichuan Medical College. The body mass index (BMI) was defined as weight in kilograms divided by the square of height in meters. The plasma biochemical values of all the individuals were measured using 7170S Automatic Chemistry Analyzer (Hitachi Co., Ltd., Tokyo, Japan). In addition, the plasma hematological values were obtained according to routine laboratory testing methods. All measurements were performed by an expert who was blinded to the study.

Genomic DNA preparation. Genomic DNA was obtained using the TIANamp Blood DNA kit (Tiangen Biotech, Co., Ltd., Beijing, China) according to the manufacturer's instructions. DNA was isolated from the whole blood samples (2 ml) of the 336 primary gout patients and 306 healthy control subjects in each collection tube, and eluted to a final elution volume of 50 μ l with Tris-HCl buffer (10 mmol/l; pH 8.0). The optical densities of the DNA samples were quantified using a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, Inc., Wilmington, DE, USA) to determine the

DNA concentration. The DNA samples were stored at -80°C until use.

Analysis of the *DNMT* polymorphisms. The rs2228611 in *DNMT1*, rs1550117 in *DNMT3A* and rs2424913 in *DNMT3B* were selected from the NCBI SNP Database (<http://www.ncbi.nlm.nih.gov/snp/>). The detailed information regarding these SNPs in *DNMTs* gene is presented in Table I. SNP genotyping was performed using a TaqMan real-time polymerase chain reaction (PCR) assay (Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the protocol described by Qing *et al* (24,25). The TaqMan assays for the *DNMT* gene variants were designed using Primer Express software version 3.0 (Thermo Fisher Scientific, Inc.). The two probes labeled with a fluorescent reporter dye (VIC or FAM) and a non-fluorescing quencher/minor groove binder were used. During the primer extension phase of PCR, the 5' nuclease activity of the Taq polymerase cleaves and releases the fluorescence of the reporter dye. Upon termination of PCR, the samples were genotyped by analyzing the fluorescence of the two dyes on the ABI PRISM® 7900HT Sequence Detection System (Thermo Fisher Scientific, Inc.). TaqMan real-time PCR was performed according to the manufacturer's standard PCR protocol. The 5.0- μ l PCR reaction system was composed as follows: 1 ng Total genomic DNA was mixed with the supplied 2X TaqMan Universal PCR Master Mix No AmpErase UNG and TaqMan Assay Mix to a final volume of 5.0 μ l. The PCR reactions were performed in a 96-well plate on the ABI PRISM® 7900HT Sequence Detection system (Thermo Fisher Scientific, Inc.). The PCR procedure was performed as follows: Initial denaturation at 95°C for 10 min, amplification was conducted for 40 cycles at 92°C for 15 sec and 60°C for 60 sec. The investigator who performed the genotyping was blinded to the patients' clinical status. Additionally, the DNA of the gout patients and healthy control subjects were genotyped together on the same plates with duplicates samples (15%) to assess intraplate variation and interpolate genotype quality. No discrepancies in the genotypes were detected.

Statistical analysis. All statistical analysis was performed using SPSS statistical software, version 22.0 (IBM SPSS, Armonk, NY, USA). Student's t-test was performed to compare the clinical parameters between patients with gout and the healthy control subjects. Each *DNMTs* polymorphism was analyzed for deviations from the Hardy-Weinberg equilibrium (HWE) using χ^2 test. And the χ^2 analysis was used to compare the allele and genotype frequencies between the cases and control subjects. The odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to assess the associations between genotypes and gout patients. Genotype and allele frequencies were performed using the SHEsis software (<http://analysis.bio-x.cn/myAnalysis.php>) (26). P<0.05 was considered to indicate a statistically significant difference.

Results

Subject characteristics. A total of 642 subjects were enrolled in the present study, including 306 healthy individuals and 336 patients with primary gout. The clinical characteristics

Table I. SNPs from DNA methyltransferases.

Gene	SNP	Location	Chromosome	Chromosome position (bp)	From	To	Change	Codon
<i>DNMT1</i>	rs2228611	Coding region	19	10156401	G	A	Pro463Pro	463
<i>DNMT3A</i>	rs1550117	5' near gene	2	25343038	G	A	25565907A>G	-
<i>DNMT3B</i>	rs2424913	Intron	20	32786453	C	T	31374259C>T	-

SNP, single nucleotide polymorphisms.

Table II. Characteristics of the study samples (mean \pm standard deviation).

Characteristic	Gout (n=336)	Control (n=306)	P-value
Age (years)	47.64 \pm 9.63	46.02 \pm 7.99	>0.05
Body mass index (kg/m ²)	25.22 \pm 2.39	23.02 \pm 2.14	<0.01
Serum uric acid (μ mol/l)	515.91 \pm 80.51	324.60 \pm 34.59	<0.01
Monocyte count ($\times 10^9/l$)	0.66 \pm 0.20	0.45 \pm 0.13	<0.01
Neutrophile granulocyte counts ($\times 10^9/l$)	4.97 \pm 1.42	3.26 \pm 0.56	<0.01
White blood cell count ($\times 10^9/l$)	8.30 \pm 1.44	6.16 \pm 0.99	<0.01
Lymphocyte count ($\times 10^9/l$)	1.86 \pm 0.73	1.82 \pm 0.56	>0.05
Serum glucose (mmol/l)	6.07 \pm 0.90	5.10 \pm 0.44	<0.01
High-density lipoprotein (mmol/l)	1.09 \pm 0.34	1.27 \pm 0.17	<0.01
Low-density lipoprotein (mmol/l)	2.53 \pm 0.78	2.80 \pm 0.39	<0.01
Very low-density lipoprotein (mmol/l)	1.13 \pm 0.57	0.55 \pm 0.23	<0.01
Triglycerides (mmol/l)	2.12 \pm 1.15	1.11 \pm 0.32	<0.01
Total cholesterol (mmol/l)	4.67 \pm 0.92	4.48 \pm 0.49	>0.05

Data were compared using Student's t-test and P<0.05 was considered to indicate a statistically significant difference.

and laboratory data of participants are presented in Table II. No significant differences were observed in age, lymphocyte count (LY) and total cholesterol (TC) between the patients and controls (P>0.05). The gout patients had significantly higher BMIs, serum uric acid (sUA), monocyte counts (MO), neutrophile granulocyte counts (GR), white blood cell counts (WBC), serum glucose (GLU), low-density lipoprotein (LDL-C), very low-density lipoprotein (VLDL) and triglycerides (TG) compared with the healthy control subjects (P<0.01). However, the levels of high-density lipoprotein (HDL-C) were markedly higher in healthy control subjects compared with the gout patients (P<0.01).

Associations between DNMT gene SNPs and gout risk. In the present study, three *DNMT* gene SNPs were obtained from the NCBI SNP database: The rs2228611 SNP, in the coding region of *DNMT1*, is located on chromosome 19, while the rs1550117 is located near the 5' region of *DNMT3A* on chromosome 2. The third SNP, rs2424913 was within the intron of *DNMT3B* on chromosome 20 (Table I). All the genotyped distributions were consistent with those expected from the HWE analysis between all investigated groups (P>0.05). As shown in Table III, the distribution frequencies of the rs2228611 AA genotype ($\chi^2=9.378$; P=0.007) and A allele ($\chi^2=9.772$; P=0.002 and OR=1.508, 95% CI=1.158-1.964) in the gout patients were

significantly increased compared with the healthy control subjects, which indicated significant differences of the genotype and allele frequencies of rs2228611 in *DNMT1* in patients as compared with the healthy control subjects.

In the control subjects, the frequencies of GG, AG and AA genotypes, and G and A alleles of the *DNMT3A* gene rs1550117 were 0.680, 0.307, 0.013, 0.833 and 0.167, respectively, while the frequencies of GG, AG and AA genotypes, and G and A alleles in the patients with gout were 0.652, 0.330, 0.018, 0.817 and 0.183, respectively. In addition, the *DNMT3B* gene rs2424913 distributions of the genotype and allele did not show any associations with gout susceptibility between patients and control subjects ($\chi^2=1.312$ and 1.266; P=0.531 and 0.252). Of the gout patients, 64 (0.190) were heterozygous for CT, 8 (0.024) were homozygous for C and 264 (0.786) for T. In addition, the numbers and frequencies of CC, CT and TT genotypes in the healthy control subjects were 9 (0.029), 68 (0.222) and 229 (0.748), respectively.

Discussion

Gout is a type of inflammatory arthritis caused by deposition of MSU crystals in the tissues and organs, and its prevalence and incidence has increased across various regions of the world in recent years (6). However, the precise pathogenesis of gout has

Table III. Association analysis of *DNMT1*, *DNMT3A* and *DNMT3B* SNPs in gout.

Gene	Cohort		χ^2	P-value	OR (95% CI)
	Patient	Control			
<i>DNMT1</i> rs2228611					
G/G	179 (0.533)	197 (0.644)			
A/G	136 (0.405)	100 (0.327)			
A/A	21 (0.062)	9 (0.029)	9.378	0.007	
G	494 (0.735)	484 (0.807)			
A	178 (0.265)	118 (0.193)	9.772	0.002	1.508 (1.158-1.964)
<i>DNMT3A</i> rs1550117					
G/G	219 (0.652)	208 (0.680)			
A/G	111 (0.330)	94 (0.307)			
A/A	6 (0.018)	4 (0.013)	0.594	0.707	
G	549 (0.817)	510 (0.833)			
A	123 (0.183)	102 (0.167)	0.692	0.441	0.893 (0.669-1.192)
<i>DNMT3B</i> rs2424913					
C/C	8 (0.024)	9 (0.029)			
C/T	64 (0.190)	68 (0.222)			
T/T	264 (0.786)	229 (0.748)	1.312	0.531	
C	80 (0.119)	86 (0.141)			
T	592 (0.881)	526 (0.859)	1.266	0.252	1.210 (0.873-1.677)

SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

not been clarified. Therefore, further studies investigating gout will facilitate a better understanding of its pathogenesis and treatment. In the present study, the concentration of sUA, MO, GR, WBC, GLU, LDL-C, VLDL and TG were identified to be significantly higher in the gout patients compared with those in the healthy control subjects, which indicates that a close association between sUA levels and gout exists. Certain studies have demonstrated that genetic variation of ATP binding cassette subfamily G member 2 (*ABCG2*), solute carrier family 2 member 9 (*SLC2A9*), and solute carrier family 22 member 12 (*SLC22A12*) genes are involved in control of sUA levels, and the SNPs of *ABCG2*, *SLC2A9* and *SLC22A12* genes may increase the risk for an individual to develop gout (27-29). However, only ~10% of patients with hyperuricaemia would eventually develop gout (30) while the remaining 90% patients would not, which prompts investigation of the molecular basis involved in the metabolism and regulation of urate to further reveal the pathogenesis of gout.

With increasing evidence suggesting that the epigenetic processes may contribute to promoting cancer and autoimmunity, *DNMT* gene polymorphisms were associated with various types of cancer and autoimmune diseases in different populations worldwide (31,32). *DNMT1* rs2228611 SNPs were associated with increased susceptibility to developing ovarian cancer, while there were no significant associations identified between rs2228611 and gastric cancer risk in the cases and controls (33,34). Previous studies of *DNMT3A* rs1550117 demonstrated that the SNPs may modulate the *DNMT3A* gene expression and contribute to genetic susceptibility to gastric and colorectal cancer

development (35,36). The rs2424913 of the *DNMT3B* gene are significantly associated with a negative risk of colorectal cancer development in the African population (37). Although the genotype distribution of *DNMT3B* rs2424913 demonstrated no significant differences between the children with immune thrombocytopenia (ITP) and the healthy individuals, the frequencies of the T allele were significantly increased in children with ITP (38). Conversely, Piotrowski *et al* (39) identified that the rs1550117 of *DNMT3A* exhibited a significant association with SLE in a Polish population when compared with healthy individuals, which indicated that this SNP may protect against SLE development. Recently, using Gene Expression Microarrays, *DNMT* gene (*DNMT1*, *DNMT3A* and *DNMT3B*) expression was demonstrated to be markedly different in patients with gout and in healthy control subjects (data not shown), which indicated that *DNMT1*, *DNMT3A* and *DNMT3B* may be associated with the risk of gout. To determine the association between *DNMT* genes and gout, the distribution of the polymorphisms between gout patients and healthy control subjects from a Chinese Han population were detected. The distribution frequencies of rs2228611 AA genotype and A allele were identified to be significantly increased in gout patients compared with healthy control subjects, which suggest that the AA genotype and A allele of rs2228611 were associated with the risk of gout in the Han Chinese population. To the best of our knowledge, this study is the first to investigate on the association of common SNPs in the *DNMT1* gene and risk of gout in a Chinese Han population. Therefore, it is hypothesized that the *DNMT1* rs2228611 polymorphism may be important in modulating *DNMT1* gene expression,

causing a genome-wide alteration of DNA methylation status, and influencing the transcription of other genes. Furthermore, the *DNMT1* rs2228611 polymorphism may contribute to susceptibility to gout development. However, the functional effects of the *DNMT1* rs2228611 polymorphism require further investigation.

In the present study, whether rs1550117 in the *DNMT3A* gene and rs2424913 in the *DNMT3B* gene are considered to be genetic susceptibility factors of gout was evaluated. However, no evidence for the involvement of *DNMT3A* rs1550117 and *DNMT3B* rs2424913 polymorphisms in susceptibility to gout was identified. Furthermore, the genotypes of these two polymorphisms demonstrated a similar distribution trend between the gout patients and the healthy control subjects in the Chinese Han population, which indicates that *DNMT3A* rs1550117 and *DNMT3B* rs2424913 may not be used to evaluate susceptibility to gout.

In conclusion, the association between *DNMT* gene polymorphisms and gout susceptibility remains unknown. In the present study, *DNMT1* rs2228611, *DNMT3A* rs1550117 and *DNMT3B* rs2424913 were investigated in Chinese Han patients with gout using TaqMan technology. The results demonstrated that the *DNMT1* rs2228611 polymorphism may be an important genetic risk factor for the development of gout, while *DNMT3A* rs1550117 and *DNMT3B* rs2424913 did not appear to predict a susceptibility to gout. Multicenter studies are required to investigate the present findings further and the underlying biological function of these genes requires further investigation.

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