

Concise report

Genetic association of polymorphism rs1333049 with gout

Binbin Wang^{1,2,3,*}, Dongmei Meng^{1,*}, Jing Wang^{2,3}, Shiguo Liu¹, Sirui Zhou^{2,3}, Zhimin Miao¹, Lin Han¹, Nan Chu¹, Kun Zhang³, Xu Ma^{2,3,4,#} and Changgui Li^{1,#}

Abstract

Objective. We suspect that genes or loci that contribute to coronary artery disease (CAD) may also play a role in the pathogenesis of gout, since hyperuricaemia leads to gout, and serum uric acid (SUA) levels are potential risk factors for CAD. The single nucleotide polymorphism (SNP) rs1333049 (C/G) on chromosome 9p21 has been implicated in previous studies to be associated with CAD. The aim of this study was to evaluate the relationship between this SNP and gout pathogenesis.

Methods. Nine hundred Chinese Han were recruited for this study (461 gout patients and 439 gout-free individuals). The rs1333049 SNP and surrounding sequences were PCR sequenced.

Results. There was a clear link between the rs1333049 genotypic and allelic frequencies between gout cases and controls ($\chi^2 = 6.81$, $df = 2$, $P = 0.033$ by genotype; $\chi^2 = 6.63$, $df = 1$, $P = 0.01$ by allele). There was a significantly increased risk of gout in carriers of the CC genotype (odds ratio = 1.43, 95% CI 1.07, 1.91).

Conclusion. To the best of our knowledge, our findings are the first to establish an association of rs1333049 with gout in a Chinese Han population. Meanwhile, this SNP is homologous to miR-519 and miR-520.

Key words: rs1333049, Gout, Association analysis.

Introduction

Gout is characterized by joint pain, inflammation and painful tophi, and can result in joint destruction and disability if untreated [1]. Uric acid is the end product of purine metabolism in humans, and serum levels are determined primarily by endogenous metabolism (synthesis and cell turnover), and the rate of excretion and re-absorption in

the kidney [2]. Exogenous purine ingestion is also important in determining uric acid levels.

Niskanen *et al.* [3] suggested that elevated serum uric acid (SUA) levels potentially increase the risk for coronary artery disease (CAD). Similarly, Baker *et al.* [4] suggested that increased SUA is likely an independent risk factor for CVD, and Krishnan *et al.* [5] evaluated the role of hyperuricaemia in a gout–CVD link. We suspected that genes contributing to CAD might play a role in gout pathogenesis. The single nucleotide polymorphism (SNP) rs1333049 on chromosome 9p21 has been shown to have a strong association with CAD in a variety of populations based on various genome-wide studies [6].

SNPs residing within genes encoding miRNAs and miRNA-target sites in mRNAs have recently been discovered. Glinsky *et al.* [7] identified a unique set of 10 miRNAs that have at least three homologous sequence counterparts among 29 top-scoring disease-associated SNPs. Among those identified are rs1333049, miR-519 and miR-520, which have been linked to CAD [7]. Given that rs1333049 has been implicated in CAD, we sought to identify the relationship between this SNP and gout pathogenesis.

¹Shandong Provincial Key Laboratory of Metabolic Diseases, Qingdao Key Laboratory of Common Diseases Gout Laboratory, Affiliated Hospital of Medical College, Qingdao University, Qingdao, Shandong, ²Graduate School, Peking Union Medical College, ³Center of Genetics, National Research Institute for Family Planning, Beijing and ⁴World Health Organization Collaborating Centre for Research in Human Reproduction, Beijing, China.

Submitted 20 September 2010; revised version accepted 4 March 2011.

Correspondence to: Xu Ma, Center for Genetics, National Research Institute for Family Planning, 12, Dahuisi Road, Haidian, Beijing, 100081 China. E-mail: nicgr@263.net or Changgui Li, Gout laboratory, Medical School Hospital of Qingdao University, 16 Jiangsu Road, Qingdao 266003, China. E-mail: lichanggui@medmail.com.cn

*Binbin Wang and Dongmei Meng contributed equally to this work.

#Xu Ma and Changgui Li contributed equally to this work.

Methods

A total of 900 age-matched subjects (461 gout patients and 439 gout-free individuals) were recruited for this study from Qingdao University (Qingdao, China). The study protocol conformed to the ethics guidelines of the 1975 Declaration of Helsinki and was approved by the Ethics Committee of the National Research Institute for Family Planning. Informed consent was obtained from all participants. Clinical features of patients were recorded, and all participants were measured for blood glucose, creatinine, uric acid, total cholesterol (TC) and triglycerides (TG) in the plasma using an automated multichannel chemistry analyser (Toshiba 200, Tokyo, Japan). Urea nitrogen was also measured in the urine. We calculated the BMI to assess obesity. Hyperuricaemia was defined as uric acid levels >420 µmol/l in males and post-menopausal women, and as >350 µmol/l in pre-menopausal women.

The rs1333049 SNP and its surrounding sequences of all subjects were amplified by PCR and sequenced using an ABI 3730XL (Applied Biosystems, Foster City, CA, USA) to perform genetic analysis. A subset of 96 samples were genotyped by Taqman (Assay ID: C__1754666_10; Applied Biosystems, Foster City, CA, USA) to confirm the genotyping data (shown in supplementary data, available at *Rheumatology* Online) [8].

Statistical analyses were carried out using the Statistical Package for Social Sciences (SPSS 10.0).

Differences between non-contiguous variables, genotype distribution and allele frequency were tested by chi-square analysis. Student's *t*-test was used to assess clinical data parameters between different genotypes. Significant differences are indicated by $P < 0.05$.

Results

The allele frequency distribution was in Hardy-Weinberg equilibrium in both gout patients and controls. The results showed that gout patients had significantly higher proportions of males, abnormal levels of TGs, TC, blood glucose, obesity, hypertension and hyperuricaemia than controls (Table 1, $P < 0.05$).

There were significant differences in rs1333049 genotypic and allelic frequencies between gout cases and controls ($\chi^2 = 6.81$, $df = 2$, $P = 0.033$ by genotype; $\chi^2 = 6.63$, $df = 1$, $P = 0.01$ by allele) (Table 2). There was a significantly increased risk of gout in carriers of the CC genotype [odds ratio (OR) = 1.43, 95% CI 1.07, 1.91]. Furthermore, the present study tests the association dependent on known CAD risk factors, such as BMI and dyslipidaemia, but the result did not reach significance.

Discussion

As previously described, rs1333049 at 9p21 is associated with CAD [6], Alzheimer's disease [9] and ischaemic

TABLE 1 Demographic and clinical characteristics [mean (s.d.)] of the study population

Parameter	Gout ($n = 461$)	Control ($n = 439$)	<i>P</i> -value
Gender: male/female, n (%)	421 (91.3%)/40 (8.7%)	297 (67.7%)/142 (32.3%)	<0.001
Age, years	52.9 (13.3)	51.4 (11.5)	0.078
BMI, kg/m ²	27.1 (3.61)	23.3 (3.26)	<0.001
Systolic blood pressure, mmHg	138 (20.9)	119 (12.2)	<0.001
Diastolic blood pressure, mmHg	88.3 (12.4)	78.5 (10.9)	<0.001
Blood glucose, mmol/l	6.09 (1.62)	5.05 (0.81)	<0.001
Uric acid, µmol/l	504.9 (139.2)	298.8 (69.5)	<0.001
TC, mmol/l	5.41 (1.3)	4.04 (5.1)	<0.001
TG, mmol/l	2.36 (1.8)	1.74 (1.6)	<0.001
Creatinine, mmol/l	90.9 (30.1)	92.5 (10.5)	0.312
Urea nitrogen, mmol/l	16.5 (10.8)	13.9 (2.94)	0.001

TABLE 2 Genotype distribution and relative allele frequencies of the rs1333049 polymorphism in Chinese with gout ($n = 461$) and controls ($n = 439$)

Group	n	Genotype frequency, n (%)			Allele frequency, n (%)	
		G/G	C/G	C/C	G	C
Gout	461	90 (19.5)	222 (48.2)	149 (32.3)	402 (43.6)	520 (56.4)
Controls	439	107 (24.4)	222 (50.6)	110 (25)	436 (49.7)	442 (50.3)
		$\chi^2 = 6.81$, $df = 2$, $P = 0.033$ ($P = 0.091$ adjusted by BMI value, TC level and TG level)			$\chi^2 = 6.63$, $df = 1$, $P = 0.01$ OR = 1.26, 95% CI 1.06, 1.54	

stroke [10]. The current study focused on the association of rs1333049 with gout, potentially broadening the scope of diseases to which locus 9p21 contributes. The present study establishes the association between SNP rs1333049 and the development of gout. According to the results of the present study, the association with gout did not reach significance when dependent on known CAD risk factors; this result demonstrated that the rs1333049 variant is not associated directly with gout development *per se*, but influences dyslipidaemia and/or BMI, which themselves are either risk factors or a consequence of gout.

MiR-519 is known to target HuR mRNA, which can stabilize and modulate its translation [11]. The over-expression of miR-519 specifically lowered HuR nascent translation, illustrating how regulation of post-transcriptional regulatory factors (RBPs) are regulated by another type of post-transcriptional regulator (miRNAs), as another mechanism to control cell division.

MiR-520 clusters among a large target group of genes with similar function based on Gene Ontology (GO) analysis. The functions of genes in this cluster include cell growth arrest, down-regulation of cellular metabolic processes, down-regulation of transcription and small GTPase-mediated signal transduction [12]. The miRNA miR-520h is highly expressed in haematopoietic stem cells (HSCs) from human umbilical cord blood, and promotes differentiation of HSCs into progenitor cells through inhibition of *ABCG2* expression [13], but the function of the broader miR-520 cluster is less well known. Interestingly, the *ABCG2* gene is linked to gout development according to Dehghan's results [14] and has been replicated in a Chinese Han male population [14].

In conclusion, this study is the first to implicate an SNP associated with CAD in the pathogenesis of gout. Meanwhile, the rs1333049 SNP is homologous to miR-519 and miR-520, which are known to exert effects by regulating mRNA expression. However, its precise role in gout pathogenesis will need further study.

Rheumatology key messages

- Rs1333049 has sequence homology to miR-519 and miR-520.
- Rs1333049 was related to gout in a Chinese Han population.

Acknowledgements

Funding: This work was supported by the National Basic Research Program of China (2010CB534902), the National Infrastructure Program of Chinese Genetic Resources (2006DKA21300), the National Natural Science Foundation of China (30570890) and the National Natural Science Foundation of China (30871192).

Disclosure statement: The authors have declared no conflicts of interest.

Supplementary data

Supplementary data are available at *Rheumatology* Online.

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