

Common Variants of *cGKII/PRKG2* Are Not Associated with Gout Susceptibility

Masayuki Sakiyama, Hirotaka Matsuo, Toshinori Chiba, Akiyoshi Nakayama, Takahiro Nakamura, Seiko Shimizu, Emi Morita, Nana Fukuda, Hiroshi Nakashima, Yutaka Sakurai, Kimiyoshi Ichida, Toru Shimizu, and Nariyoshi Shinomiya

ABSTRACT. Objective. Recently, genetic analyses indicated the association between gout and cGMP-dependent protein kinase 2 (*cGKII/PRKG2*) gene in a Fukien-Taiwanese heritage population. However, no replication study has been reported in other ancestries. Therefore, we investigated this association in a Japanese population.

Methods. Genotyping of 4 variants (rs11736177, rs10033237, rs7688672, and rs6837293) of *cGKII* was performed in 741 male gout patients and 1302 male controls.

Results. *cGKII* variants have no association with gout.

Conclusion. Our replication study suggests that *cGKII* is not involved in gout susceptibility. (J Rheumatol First Release June 1 2014; doi:10.3899/jrheum.131548)

Key Indexing Terms:

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From the Department of Integrative Physiology and Bio-Nano Medicine, Department of Dermatology, Laboratory for Mathematics, and Department of Preventive Medicine and Public Health, National Defense Medical College, Tokorozawa; Department of Preventive Medicine, Nagoya University Graduate School of Medicine, Nagoya; Department of Pathophysiology, Tokyo University of Pharmacy and Life Sciences, Tokyo; Division of Kidney and Hypertension, Department of Internal Medicine, Jikei University School of Medicine, Tokyo; and Midorigaoka Hospital, Takatsuki, Japan.

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M. Sakiyama, MD, Department of Integrative Physiology and Bio-Nano Medicine, and Department of Dermatology, National Defense Medical College; H. Matsuo, MD, PhD; T. Chiba, MD; A. Nakayama, MD, Department of Integrative Physiology and Bio-Nano Medicine, National Defense Medical College; T. Nakamura, PhD, Laboratory for Mathematics, National Defense Medical College; S. Shimizu, PhD, Department of Integrative Physiology and Bio-Nano Medicine, National Defense Medical College; E. Morita, MD, PhD; N. Fukuda, BHE, Department of Preventive Medicine, Nagoya University Graduate School of Medicine; H. Nakashima, MD, PhD; Y. Sakurai, MD, PhD, Department of Preventive Medicine and Public Health, National Defense Medical College; K. Ichida, MD, PhD, Department of Pathophysiology, Tokyo University of Pharmacy and Life Sciences; Division of Kidney and Hypertension, Department of Internal Medicine, Jikei University School of Medicine; T. Shimizu, MD, PhD, Midorigaoka Hospital; N. Shinomiya, MD, PhD, Department of Integrative Physiology and Bio-Nano Medicine, National Defense Medical College.

Drs. Sakiyama and Matsuo contributed equally to this work.

Address correspondence to Dr. H. Matsuo, Department of Integrative Physiology and Bio-Nano Medicine, National Defense Medical College, 3-2 Namiki, Tokorozawa, Saitama 359-8513, Japan.

E-mail: hmatsuo@ndmc.ac.jp

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Gout, which is caused by hyperuricemia, is one of the most common types of inflammatory arthritis. Several genes associated with gout and serum uric acid (SUA) levels have been reported, including ATP-binding cassette transporter, subfamily G, member 2 (*ABCG2/BCRP*)^{1,2,3,4,5}, glucose transporter 9 (*GLUT9/SLC2A9*)^{6,7,8}, monocarboxylate transporter 9 (*MCT9/SLC16A9*)^{9,10}, and leucine-rich repeat-containing 16 A (*LRRC16A/CARMIL*)^{9,11}.

In addition, a recent genomewide analysis and a case-control study revealed a significant association between gout and the cGMP-dependent protein kinase 2 (*cGKII*, also known as *PRKG2*) gene¹². *cGKII* is expressed in several tissues, such as intestine and kidney, and is involved in the regulation of water and sodium secretion by epithelial tissues¹³. It is also known that a *cGKII* dysfunctional mutation causes dwarfism in cattle¹⁴.

However, no replication study has evaluated this relationship in other ancestries. We therefore investigated the association between gout and *cGKII* variants in Japanese gout cases and controls.

MATERIALS AND METHODS

Patients. Our study was approved by the institutional ethical committees, and all procedures involved in our study were performed in accordance with the Declaration of Helsinki. Informed consent in writing was obtained from each subject. A case-control study was conducted to examine the association between gout and *cGKII* gene. From the patients of Midorigaoka Hospital (Osaka, Japan) and Jikei University Hospital (Tokyo, Japan), 741 male Japanese patients with primary gout were collected. All gout cases were diagnosed according to the criteria established by the American College of Rheumatology¹⁵. For the control group, 1302 male Japanese individuals with normal SUA levels (≤ 7.0 mg/dl) and

no gout history were collected from the Japan Multi-Institutional Collaborative Cohort Study (J-MICC Study)¹⁶. The mean age (SD) of case and control groups was 55.0 years (\pm 13.2) and 52.7 years (\pm 8.4), respectively, and their mean body mass index was 24.6 kg/m² (\pm 3.5) and 23.2 kg/m² (\pm 2.8), respectively.

Genotyping. Genomic DNA was extracted from whole peripheral blood cells¹⁷. Our study focused on the following 4 single-nucleotide polymorphisms (SNP), which were previously reported to be associated with gout¹²: rs11736177, rs10033237, rs7688672, and rs6837293 of the *cGKII* gene. Genotyping of these 4 SNP was performed by TaqMan Assay-By-Design method (Life Technologies Corporation) with a LightCycler 480 (Roche Diagnostics)¹⁸. To confirm their genotypes, more than 30 samples were subjected to direct sequencing with the following primers: for 11736177, forward 5'-ACA TAA AAA TTT CCA ATG TCA ATG-3', and reverse, 5'-GCA TAT TCT CAC TCA TAG ATG GG-3'; for rs10033237, forward 5'-ATC ATC AGT CAT AAT GGC TCT TC-3', and reverse, 5'-AAG TGC TCA ATA GCC ATA TTT G-3'; for 7688672, forward 5'-GGG CCT TCT GAT CTG AAT C-3', and reverse, 5'-CTC TAA AGT TTT TTC CAG CTC TAT ATC-3'; for 6837293, forward 5'-CTG ATT TTA GTT GTG CCT TCC-3', and reverse, 5'-TCC TGA GTT ATA CTA GCC ACT TTT C-3'. DNA sequencing analysis was performed with a 3130 μ l Genetic Analyzer (Life Technologies Corporation)¹⁷.

Data analysis. Pairwise linkage disequilibrium (LD) among 4 SNP of *cGKII* were calculated using software R (version 3.0.2) (www.r-project.org/) with GenABEL software package. For other calculations in the statistical analysis, SPSS v.17.0J (IBM Japan Inc.) was used. The chi-square test was used for association analysis.

RESULTS

The genotyping results of *cGKII* 4 SNP for 741 patients with gout and 1302 controls are shown in Table 1. The call rates for rs11736177, rs10033237, rs7688672, and rs6837293 were 97.7%, 97.7%, 96.5%, and 96.6%, respectively. Their p values for Hardy-Weinberg equilibrium were 0.41, 0.46, 0.37, and 0.25, respectively. An extremely low p value that suggested mistyping was not obtained. The minor allele frequencies of the 4 SNP were more than 0.34 in both case and control groups, indicating that these SNP are very common in both groups. Because strong LD was observed among the 4 SNP ($D' = 0.851$ between rs11736177 and rs10033237, $D' = 0.990$ between rs11736177 and rs7688672, $D' = 0.988$ between rs11736177 and rs6837293, $D' = 0.850$ between rs10033237 and rs7688672, $D' = 0.842$ between rs10033237 and rs6837293, $D' = 0.995$ between rs7688672 and rs6837293), no correction for multiple testing was performed.

The association analyses of the 4 *cGKII* variants (rs11736177, rs10033237, rs7688672, and rs6837293) showed no significant association with gout in the allele frequency model ($p = 0.52, 0.73, 0.50,$ and $0.96,$ respectively; Table 1).

In the dominant and the recessive models, all 4 SNP of the *cGKII* gene also had no association with gout (Table 1).

DISCUSSION

We performed a replication study about the relation of *cGKII* gene to gout, and first demonstrated that the 4 *cGKII* variants, rs11736177, rs10033237, rs7688672, and rs6837293, had no significant association with gout susceptibility.

The most established function of *cGKII* is the regulation of renin and aldosterone secretion^{13,19}. Thus, dysfunction of the *cGKII* gene could cause hypertension through the renin-angiotensin-aldosterone system. As a result, hypertension might lead to hyperuricemia through muscle glycolysis²⁰. However, in this pathway, the relationship between *cGKII* and gout/hyperuricemia is not direct. Therefore, even if there is an association between *cGKII* and gout/hyperuricemia, it could be an indirect and weak consequence.

The *cGKII* gene was located on 4q13.1-q21.1 and first identified by Chang, *et al* to have an association with gout in a Fukien-Taiwanese heritage population¹². They found that chromosome 4q21 contains a locus significantly linked with gout (D4S3243 at 81 289 553 bp; $p = 0.004$; logarithm of odds score = 5.13) in a Taiwanese family through genomewide scan methods. In a subsequent case-control study, they analyzed 29 SNP around this marker to confirm their relationships with gout. Among them, 4 SNP of *cGKII* gene showed a significant association with gout¹². However, there are no replication studies indicating an association between *cGKII* gene and gout in other ancestries. Our present study revealed that the *cGKII* gene does not contribute to the gout susceptibility in a Japanese population. This opposite result would be because of the difference in sample size and population group between each study. In addition, the true functional and pathogenic gene could not be *cGKII*, but other genes located in the candidate region on

Table 1. Association analysis of 4 common variants of *cGKII/PRKG2* gene in gout patients and controls.

			Genotypes						Allele Frequency Model				Dominant Model**		Recessive Model***	
			Case			Control			MAF				p	OR (95% CI)	p	OR (95% CI)
	A1* A2*	A1/A1	A1/A2	A2/A2	A1/A1	A1/A2	A2/A2	Case	Control	p	OR (95% CI)					
rs11736177	A	C	209	369	150	369	606	293	0.46	0.47	0.52	0.96 (0.84–1.09)	0.85	1.02 (0.83–1.25)	0.20	0.86 (0.69–1.08)
rs10033237	A	G	303	346	85	535	553	174	0.35	0.36	0.73	0.98 (0.85–1.12)	0.63	1.05 (0.87–1.26)	0.16	0.82 (0.62–1.08)
rs7688672	G	A	208	369	153	357	593	291	0.46	0.47	0.50	0.96 (0.84–1.09)	0.90	1.01 (0.83–1.24)	0.20	0.87 (0.69–1.08)
rs6837293	C	T	205	366	154	372	591	286	0.46	0.47	0.96	1.00 (0.88–1.14)	0.48	1.08 (0.88–1.32)	0.39	0.91 (0.73–1.13)

*The major allele is referred to as A1 and the minor allele as A2. **Dominant model indicates genotype A1/A1 versus A1/A2 or A2/A2. ***Recessive model indicates genotype A1/A1 or A1/A2 versus genotype A2/A2. MAF: minor allele frequency.

chromosome 4q21 reported in a Fukien-Taiwanese heritage population¹².

Although further studies of *cGKII* are necessary to reveal the relationship between *cGKII* variants and gout, our finding suggests that *cGKII* variants are not strong genetic risks for gout.

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