Common Variants of cGKI//PRKG2 Are Not Associated with Gout Susceptibility

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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. (First Release June 1 2014; J Rheumatol 2014;41:1395–7; doi:10.3899/jrheum.131548)

Key Indexing Terms:
- GOUTY ARTHRITIS
- HYPERURICEMIA
- URIC ACID
- SINGLE NUCLEOTIDE POLYMORPHISMS

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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)
no gout history were collected from the Japan Multi-Institutional Collaborative Cohort Study (J-MICC Study)\textsuperscript{16}. The mean age (SD) of case and control groups was 55.0 years (± 13.2) and 52.7 years (± 8.4), respectively, and their mean body mass index was 24.6 kg/m\textsuperscript{2} (± 3.5) and 23.2 kg/m\textsuperscript{2} (± 2.8), respectively.

**Genotyping.** Genomic DNA was extracted from whole peripheral blood cells\textsuperscript{17}. Our study focused on the following 4 single-nucleotide polymorphisms (SNP), which were previously reported to be associated with gout\textsuperscript{12}: 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)\textsuperscript{18}. To confirm their genotypes, more than 30 samples were subjected to direct sequencing with the following primers: for 11736177, forward 5’-CTG ATT TTA GTT GTG CCT TCC-3’, and reverse, 5’-GCA TAT TCT CAC TCA TAG ATG GG-3’; for 10033237, forward 5’-ATC ATC AGT CAT AAT GGC TCT TC-3’, and reverse, 5’-GGG CCT TCT GAT CTG AAT C-3’, and for 6837293, forward 5’-GGG CCT TCT GAT CTG AAT C-3’, and reverse, 5’-CTG ATT TTA GTT GTG CCT TCC-3’. DNA sequencing analysis was performed with a 3130xl Genetic Analyzer (Life Technologies Corporation)\textsuperscript{19}. To confirm their genotypes, more than 30 samples were subjected to direct sequencing with the following primers: for 11736177, forward 5’-CTG ATT TTA GTT GTG CCT TCC-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’-CTG ATT TTA GTT GTG CCT TCC-3’, and for 6837293, forward 5’-CTG ATT TTA GTT GTG CCT TCC-3’, and reverse, 5’-TCC TGA GTT ATA TCA GCC ACT TTT C-3’. DNA sequencing analysis was performed with a 3130xl Genetic Analyzer (Life Technologies Corporation)\textsuperscript{19}.

**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’ = 0.850 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.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Allele Frequency Model</th>
<th>Dominant Model**</th>
<th>Recessive Model***</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case</td>
<td>Control</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>rs11736177</td>
<td>A</td>
<td>C</td>
<td>209 369 150 369 606 293</td>
</tr>
<tr>
<td>rs10033237</td>
<td>A</td>
<td>G</td>
<td>303 346 85 535 553 174</td>
</tr>
<tr>
<td>rs7688672</td>
<td>G</td>
<td>A</td>
<td>208 369 153 357 593 291</td>
</tr>
<tr>
<td>rs6837293</td>
<td>C</td>
<td>T</td>
<td>205 366 154 372 591 286</td>
</tr>
</tbody>
</table>


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\textsuperscript{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 glycosynthesis\textsuperscript{20}. 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 population\textsuperscript{12}. 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 gout\textsuperscript{12}. 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
chromosome 4q21 reported in a Fukien-Taiwanese heritage population.

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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