Note

A Common Variant of Organic Anion Transporter 4 (OAT4/SLC22A11) Gene Is Associated with Renal Underexcretion Type Gout

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Summary: Gout, a common disease, is a consequence of hyperuricemia, and increases the risks of hypertension, cardiovascular diseases, cerebrovascular diseases, and renal failure. Gout can be classified into 3 types: the renal underexcretion (RUE) type, renal overload type, and combined type. RUE type is a major type of gout; however, its genetic causes are still unclear. Since human organic anion transporter 4 (OAT4/SLC22A11) is expressed in the kidney and mediates urate transport, we investigated the effects of a common variant of OAT4/SLC22A11 on the susceptibility to gout. Five hundred and forty-five Japanese male gout cases and 1,115 male individuals as a control group were genotyped with rs17300741, a single nucleotide polymorphism in the OAT4/SLC22A11 gene. The association analysis of rs17300741 showed no significant association for all gout cases; however, there was a slight but significant association for RUE type gout cases (p = 0.049). These results also suggest that OAT4 contributes to urate transport at the apical membrane of renal proximal tubule cells in humans. Our findings make it clear for the first time that a common variant of OAT4/SLC22A11 is associated with RUE type gout, a major gout subtype.

Keywords: SLC transporter; urate transporter; gouty arthritis; subtype analysis; clinical classification

Introduction

Gout is a common disease resulting from hyperuricemia which causes acute arthritis,1) Gout and hyperuricemia increase the risk for hypertension,2,3) cardiovascular diseases,4) cerebrovascular diseases,5) and renal failure.6) Gout is classified into the renal underexcretion (RUE) type, renal overload (ROL) type, and combined type gout.7) RUE type is a major group of gout; however, the genetic causes of this type are still unclear. Human organic anion transporter 4 (OAT4/SLC22A11) is expressed in the kidney and

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mediates the transport of xenobiotics, endogenous organic anions, and urate. While genome-wide association studies (GWAS) have revealed an association between SUA levels and a single nucleotide polymorphism (SNP) in OAT4/SLC22A1 (rs17300741), it remains to be clarified whether OAT4/SLC22A1 contributes to the susceptibility to gout. In this study, therefore, we investigated the effects of a common variant of OAT4/SLC22A1I on the susceptibility to gout in patients and healthy volunteers.

Materials and Methods

Patients: All procedures were carried out in accordance with the standards of the institutional ethical committees involved in this project and the Declaration of Helsinki. Written informed consent was obtained from each subject participating in this study. A case-control study was conducted to examine the association between gout and the OAT4/SLC22A1I gene. For cases, 545 male gout patients were assigned from the outpatients of gout clinics in Midorigaoka Hospital (Osaka, Japan) between July 2009 and June 2010. All of them were clinically diagnosed with primary gout according to the criteria established by the American College of Rheumatology.10) For the control group, 1,115 males with normal serum uric acid (SUA) (≤7.0 mg/dl) and without a history of gout were registered from the Japan Multi-Institutional Collaborative Study (J-MICC Study).11) The details and participants in this study are shown in Supplemental Table 1.

Clinical parameters for urate handling: Patients classified as having RUE gout were characterized by a low (<5.5%) fractional excretion of urate clearance (urate clearance/creatinine clearance ratio, FEUA)12) on the basis of the normal FEUA range (5.5–11.1%)13) and ROL gout was defined by high (>25 mg/h/1.73 m²) urinary urate excretion (UUE) as previously described.7,14-16) Combined type gout was classified when a patient’s UUE and FEUA met the criteria of both RUE and ROL gout. Patients who met the single criterion of either RUE or ROL gout, excluding combined type gout, were defined as RUE type or renal ROL type gout, respectively.

Genetic and statistical analyses: Genomic DNA was extracted from whole peripheral blood cells.17) Genotyping of rs17300741, an SNP in OAT4/SLC22A1I gene, was performed by TaqMan Assay-By-Design method (Life Technologies Corporation, Carlsbad, CA) with a LightCycler 480 (Roche Diagnostics, Mannheim, Germany).18) To confirm their genotypes, direct sequencing was performed for more than 200 samples with the following primers: forward, 5'-TGTTAACAACGCGCCAGTGG-AATCACCCTGAAACTGCGGAG-3', and reverse 5'-CAGGAAAC-AGCTATGACCGACCTCCGAGTGG-3'. DNA sequencing analysis was performed with a 3130 × 1 Genetic Analyzer (Life Technologies Corporation).19)

For all calculations in the statistical analysis, SPSS v. 17.0J (IBM Japan Inc., Tokyo, Japan) were used. The χ² test was used for association analysis.

Results

Results of genotyping with rs17300741 are shown in Table 1 (545 gout patients and 1,115 healthy controls) and Supplemental Figure 1. The call rate for rs17300741 was 98.7%. Its p value for Hardy-Weinberg equilibrium was 0.035. A p value that suggested mistyping was not obtained. Among the 545 gout patients, urate handling data such as UUE and FEUA were available in 461 cases. These cases could be classified into subtype groups as RUE type gout, ROL type gout or combined type gout (Supplemental Table 2).

Table 1. Association analysis of OAT4 variant, rs17300741, in gout patients

<table>
<thead>
<tr>
<th>Genotype*</th>
<th>Gout cases</th>
<th>Controls</th>
<th>Allele frequency mode</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1/1 1/2 2/2 MAF</td>
<td>1/1 1/2 2/2 MAF</td>
<td>p-value OR 95% CI</td>
</tr>
<tr>
<td>All gout</td>
<td>493 47 2 0.0470</td>
<td>1013 79 5 0.0406</td>
<td>0.388 1.17 0.82 1.66</td>
</tr>
<tr>
<td>RUE type gout</td>
<td>143 19 1 0.0644</td>
<td>1013 79 5 0.0406</td>
<td>0.049 1.63 1.00 2.66</td>
</tr>
<tr>
<td>ROL type gout</td>
<td>135 11 0 0.0377</td>
<td>1013 79 5 0.0406</td>
<td>0.813 0.93 0.49 1.75</td>
</tr>
<tr>
<td>Comb. type gout</td>
<td>102 8 0 0.0364</td>
<td>1013 79 5 0.0406</td>
<td>0.762 0.89 0.43 1.87</td>
</tr>
</tbody>
</table>

CI, confidence interval; Comb, combined; MAF, minor allele frequency; OR, odds ratio; ROL, renal overload; RUE, renal underexcretion.

The minor allele was referred to as allele 2 and the major allele as 1. Allele 1 is A and allele 2 is G in rs17300741.

Discussion

In our study, an association between gout and rs17300741 was not found in all gout cases. This finding is consistent with a report by Stark et al.20) that rs17300741 does not contribute to the susceptibility to gout. However, an association was observed in RUE type gout cases in this study. Our findings make it clear for the first time that a common variant of OAT4/SLC22A1I, which encodes a solute carrier family transporter, has a weak but significant association with RUE type gout susceptibility.

OAT4 is expressed on the apical membrane of renal proximal tubule cells and placenta.21,22) This transporter functions as an organic anion/dicarboxylate exchanger and is responsible for the reabsorption of organic anions driven by an outwardly directed dicarboxylate gradient. OAT4 may act as an entry route for urate into the renal proximal tubule cells because it is considered to be an asymmetric carrier.23) Hagos et al.24) reported OAT4 to be a low-affinity urate transporter, using cells stably expressing OAT4 and OAT4-expressing oocytes in plasma-equivalent concentrations. Furthermore, recent GWAS have revealed an association between SUA levels and rs17300741 in OAT4/SLC22A1I,8,9) as was confirmed by a replication study.25) While the functional role of rs17300741 is still unknown and further studies are necessary, it may well be possible that this intronic SNP would regulate OAT4/SLC22A1I gene expression or be a surrogate marker for other functional SNPs.
Taken together with our findings, a common variant of OAT4/SLC22A11 is associated with RUE type gout, and OAT4 contributes to urate transport at the apical membrane of renal proximal tubule cells in humans.

We revealed the association between disease and gene by the subtype analysis of gout. Thus, our data also showed the great importance of subtype analysis, especially in common diseases such as gout.

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References

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