Common variants of a urate-associated gene \textit{LRP2} are not associated with gout susceptibility

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Abstract A recent genome-wide association study revealed that there is an association between serum uric acid (SUA) levels and rs2544390, a common variant in low-density lipoprotein-related protein 2 (\textit{LRP2}/\textit{Megalin}) gene. Two other variants of \textit{LRP2}, rs2229268 and rs3755166, are also found to have associations with dyslipidemia and Alzheimer’s disease, respectively, which also could have a relationship with SUA in human. Although no studies report that \textit{LRP2} transports urate, \textit{LRP2} is a multi-ligand receptor and expresses in many tissues including kidney, suggesting a direct and/or indirect relationship with gout. In the present study, we investigated the association between gout and these variants of \textit{LRP2} with 741 clinically diagnosed male gout patients and 1,302 controls. As a result, the three common \textit{LRP2} variants, rs2544390, rs2229268 and rs3755166, showed no association with gout ($P =$ 0.76, 0.55, and 0.22, respectively). Our study is the first to reveal that an SUA-related gene \textit{LRP2} is not involved in gout susceptibility.

Keywords Gouty arthritis · Hyperuricemia · Hyperlipidemia · Urate exporter · Low-density lipoprotein receptor (LDLR) · LDLR gene family

Introduction

Gout is a common disease as a consequence of hyperuricemia. A recent genome-wide association study (GWAS) [1] with 8,868 Japanese revealed the association between serum uric acid (SUA) levels and rs2544390, which is a single nucleotide polymorphism (SNP) in low-density lipoprotein-related protein 2 (\textit{LRP2}, also known as \textit{Megalin}). \textit{LRP2} is a member of the low-density lipoprotein receptor [\textit{LDLR} (MIM606945)] gene family, and two SNPs of \textit{LRP2}, rs2229268 and rs3755166, are also found to have associations with dyslipidemia [2] and Alzheimer’s disease [3],...
respectively. In this study, we investigated the association between gout and these SNPs with clinically diagnosed gout patients and controls.

Subjects and methods

Subjects

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. As gout cases, 741 Japanese male individuals were collected from the outpatients of the gout clinics in either Jikei University Hospital (Tokyo, Japan) or Midorigaoka Hospital (Osaka, Japan). All of them were clinically diagnosed with primary gout according to the criteria established by the American College of Rheumatology [4]. As a control group, 1,302 Japanese male individuals with normal SUA (< 7.0 mg/dl) without gout history were collected from the Japan Multi-Institutional Collaborative Cohort Study (J-MICC Study) [5]. The mean age and body-mass index with standard deviation are 55.0 ± 13.2 years old and 24.6 ± 3.5 kg/m² for cases, respectively, and 52.7 ± 8.4 years old and 23.2 ± 2.8 kg/m² for controls, respectively.

Genetic analysis and statistical analysis

Genomic DNA was extracted from whole peripheral blood cells [6]. Genotyping of rs2544390, rs2229268, and rs3755166 in LRP2 gene was performed by an allelic discrimination assay (Custom Taqman MGB, Applied Biosystems) with a LightCycler 480 (Roche Diagnostics) [7]. To confirm their genotypes, more than 25 samples were subjected to direct sequencing with the following primers: for rs2544390, forward 5'-CGTCGTAGACCATGACACAG-3' and reverse, 5'-CTGCCAACCTGTGTTCGTGTGG-3'; for rs2229268, forward 5'-CTGCCAACCTGTGTTCGTGTGG-3' and reverse, 5'-TTCCCAACTTTTCAGGTAC-3'; for rs3755166, forward 5'-GTGTAAGGCCACTTGTGC-3' and reverse, 5'-GAAATGGACGAGGAAAG-3'. DNA sequencing analysis was performed with a 3130xl Genetic Analyzer (Applied Biosystems) [8]. The software R (version 3.0.2) (http://www.r-project.org/) with package GenABEL was used for the calculation of linkage disequilibrium (r²). For the calculations in the statistical analyses, SPSS v.17.0J (IBM Japan Inc., Tokyo, Japan) was used. The χ² test was used for association analysis.

Results

Table 1 shows the alleles of LRP2 variants, rs2544390, rs2229268, and rs3755166. The call rates for rs2544390, rs3755166, and rs2229268 were 98.2%, 98.1%, and 99.5%, respectively. P values for Hardy–Weinberg equilibrium of these SNPs were 0.039, 0.095, and 0.134. P values that suggested mistyping were not obtained. The minor allele frequencies (MAFs) for the three LRP2 variants were more than 0.27 in both case and control groups, indicating these SNPs are very common in both these groups. No strong linkage disequilibrium was observed between these three SNPs (r² = 0.0014 between rs2544390 and rs2229268, r² = 0.0013 between rs2229268 and rs3755166, r² = 0.15 between rs3755166 and rs2544390, respectively), showing that these SNPs are independent of each other.

Discussion

Our study demonstrated that the three LRP2 variants, rs2544390, rs2229268, and rs3755166, had no association with gout.

Table 1 Association analysis of LRP2 variants, rs2544390, rs2229268, and rs3755166 in gout patients

| Chromosomal positionsa (bp) | Allelesb | P value | OR | 95 % CI |
|----------------------------|----------|---------|----|--------|
|                           | Case     |         |    |        |
|                           | 1        | 2       | MAF |         |         |
|                           | 1        | 2       | MAF |         |         |
| rs2544390 170204846        | 747      | 717     | 0.490 | 1.314 | 1.236 | 0.485 | 0.758 | 1.020 | 0.897–1.160 |
| rs2229268 170025083        | 1,051    | 423     | 0.287 | 1.829 | 705   | 0.278 | 0.552 | 1.044 | 0.906–1.204 |
| rs3755166 170219881        | 702      | 778     | 0.474 | 1.277 | 1,307 | 0.494 | 0.223 | 1.083 | 0.953–1.231 |

MAF minor allele frequency, OR odds ratio, CI confidence interval

a SNPs positions are based on NCBI human genome reference sequence build 37.5. LRP2 is located on chromosome 2q31.1
b The major allele was referred to as allele 1 and the minor allele as 2. Allele 1 is C and allele 2 is T in rs2544390. Allele 1 is A and allele 2 is G in rs2229268. Allele 1 is T and allele 2 is G in rs3755166
Recent GWAS of SUA [9, 10] identified several genes including GLUT9/SLC2A9 and ABCG2/BCRP, which have been revealed to have associations with urate disorders such as renal hypouricemia [11, 12] and gout [13, 14]. Recent reports also show the significance of transporter genes such as ABCG2 [15, 16], NPT1/SLC17A1 [17], MCT9/SLC16A9 [18], and OAT4/SLC22A11 [19], for the pathogenesis of gout. LRP2 was first reported to have the association with SUA in the GWAS by Kamatani et al. [1]. Although we found no studies reporting that LRP2 transports urate, LRP2 variants could have an association with gout risks because gout is a consequence of hyperuricemia. Moreover, it is also demonstrated that LRP2 is a multi-ligand receptor and is expressed in various tissues, mainly in the kidney, especially in glomeruli and proximal tubular cells. As LRP2 has a role of renal reabsorption for its ligands such as insulin [20], LRP2 variants could have an association with SUA variation with increasing insulin resistance.

LRP2 is originally found as a member of the low-density lipoprotein (LDL) receptor family and has been suggested to mediate endocytosis of LDL. Indeed, Mii et al. [2] reported that one variant of LRP2, rs2229268, has an association with serum LDL levels in humans, indicating the direct association between LRP2 and LDL. Since dyslipidemia is known as a risk to increase the insulin resistance, rs2229268 seems to have an association with SUA variation. Otherwise, LRP2 could be associated with SUA variation through the endocytosis of urate-binding proteins.

Interestingly, Wang et al. [3] previously reported the association between rs3755166 in LRP2 and Alzheimer’s disease in a Chinese population. LRP2, whose ligand ApoE [21] is known for the risk of Alzheimer’s disease [22, 23],is expressed in brain and facilitates the clearance of the Aβ peptide, that is, the cause of Alzheimer’s disease [24]. Together with the fact that urate has anti-oxidant effects, LRP2 variants carrying the risk of Alzheimer’s disease might have an association with SUA variation.

However, the present study first revealed that the common variants of LRP2 have no association with gout susceptibility. Although LRP2 was first reported to have an association with SUA in Japanese population [1], there are no replication studies indicating an association between LRP2 and SUA in other ancestry such as a European population. It is possible that the present study failed to show these associations due to the limited sample (2,043 individuals). Although further studies of LRP2 are necessary to reveal the relationship between LRP2 variants and gout, our study at least revealed that LRP2 is not a strong genetic risk for gout.

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Conflict of interest The authors declare that they have no conflict of interest.

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