Identification of rs671, a common variant of ALDH2, as a gout susceptibility locus

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Gout is a common disease resulting from hyperuricemia. Recently, a genome-wide association study identified an association between gout and a single nucleotide polymorphism (SNP) rs2188380, located on an intergenic region between MYL2 and CUX2 on chromosome 12. However, other genes around rs2188380 could possibly be gout susceptibility genes. Therefore, we performed a fine-mapping study of the MYL2-CUX2 region. From 8,595 SNPs in the MYL2-CUX2 region, 9 tag SNPs were selected, and genotyping of 1,048 male gout patients and 1,334 male controls was performed by TaqMan method. Eight SNPs showed significant associations with gout after Bonferroni correction. rs671 (Glu504Lys) of ALDH2 had the most significant association with gout ($P = 1.7 \times 10^{-18}$, odds ratio = 0.53). After adjustment for rs671, the other 8 SNPs no longer showed a significant association with gout, while the significant association of rs671 remained. rs671 has been reportedly associated with alcohol drinking behavior, and it is well-known that alcohol drinking elevates serum uric acid levels. These data suggest that rs671, a common functional SNP of ALDH2, is a genuine gout-associated SNP in the MYL2-CUX2 locus and that “A” allele (Lys) of rs671 plays a protective role in the development of gout.

Gout is a common disease resulting from hyperuricemia, and causes acute arthritis. Previous genetic and functional analyses revealed that ABCG2 dysfunctional variants caused gout1–3 due to decreased urate excretion in gut4 and kidney5. Genome-wide association studies (GWASs) of gout also showed genome-wide significant associations with ABCG2 and GLUT96–8. Recently, we revealed for the first time that the following 3 loci were associated with gout at the genome-wide significance level: rs1260326 of GCKR, rs4073582 of CNIH-2 and rs2188380 of MYL2-CUX28. Among them, 2 SNPs are located in gene regions: rs1260326 is a nonsynonymous single nucleotide polymorphism (SNP) (Leu446Pro) of GCKR, and rs4073582 is an intronic SNP of CNIH-2. On the other hand, rs2188380 is located on an intergenic region between MYL2 and CUX2. Additionally, we detected many SNPs showing significant associations with gout across the chromosome 12q24 region which were in strong linkage disequilibrium (LD) with rs2188380. MYL2 encodes a regulatory light chain associated with cardiac myosin β (or slow) heavy chain, and an association between MYL2 variant and high-density lipoprotein cholesterol metabolism was previously reported9. CUX2 regulates cell-cycle progression10 and plays important roles in neural progenitor development in the central nervous system10,11. An association between CUX2 and type 1 diabetes has also been reported12. However, there is a possibility that the other genes around rs2188380 of MYL2-CUX2 can be gout susceptibility genes. Therefore, we performed fine-mapping of the MYL2-CUX2 region and a further association analysis of gout.

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Results

From 8,595 SNPs in the MYL2-CUX2 region within 10 Mb across rs2188380, 45 SNPs in LD ($r^2 \geq 0.3$) with rs2188380 were selected (Supplementary Figure S1). Among these 45 SNPs and rs2188380, 9 tag SNPs were selected for association analysis (Fig. 1 and Supplementary Table S1). Genotyping results of the 9 tag SNPs for 1,048 gout patients and 1,334 controls were shown in Table 1. The call rates for the 9 SNPs were more than 95.0%. All the SNPs in the control group were in Hardy-Weinberg equilibrium ($P > 0.05$). Except for rs2555004, the other 8 SNPs showed significant associations at $P < 5.6 \times 10^{-3}$ ($= 0.05/9$) with the Bonferroni correction, and rs671 (Glu504Lys) of aldehyde dehydrogenase 2 ($ALDH2$) had the most significant association with gout ($P = 1.7 \times 10^{-18}$; odds ratio [OR] = 0.53; 95% confidence interval [CI]: 0.46–0.61, Table 1 and Supplementary Figure S2A).

Next, the multivariate logistic regression analyses were performed to evaluate whether there was an additional association signal after the adjustment for the most significantly associated SNP rs671. We set the significance threshold as $\alpha = 6.3 \times 10^{-3}$ ($= 0.05/8$) with the Bonferroni correction. While the significant association of rs671 remained, the other 8 SNPs no longer showed a significant association with gout after the adjustment for rs671 (Table 2 and Supplementary Figure S2B). Among rs671 and 6 tagged SNPs (rs3782886, rs11066015, rs4646776, rs11066132, rs2074356 and rs11066280) shown in Supplementary Table S2, rs671 is the most promising functional SNP because only rs671 is a nonsynonymous variant (Glu504Lys). rs4766566 had a nominally significant association ($P = 0.032$), but did not pass the significant threshold for multiple hypothesis testing (Table 2). Additionally, the OR for rs4766566 became closer to 1.0 after the adjustment for rs671 (from 0.59 to 0.82; Tables 1 and 2). These data suggest that rs671 (Glu504Lys) of $ALDH2$ is a genuine gout-associated SNP in the MYL2-CUX2 locus.

Figure 1. Linkage disequilibrium heat map of 46 SNPs. We examined the linkage disequilibrium (LD) between each pair of 46 SNPs and searched the SNPs that were tagging other SNPs with strong LD ($r^2 \geq 0.8$). The 9 tag SNPs (rs2978484, rs16940688, rs2071629, rs2188380, rs11065783, rs3809297, rs4766566, rs671 and rs2555004), which are shown in bold and boxes, were selected for association analysis.
was improved by applying the dominant model (P = 7.1 × 10⁻¹²). Actually, the statistical significance of the association between rs671 and gout is partly due to alcohol drinking. This alcohol metabolism may be of great importance in the development of gout. Therefore, it is reasonable that this SNP has not been detected in the previous GWASs of gout in Europeans and African Americans due to its low frequency. We showed that rs671 of *ALDH2* enzyme is a crucial enzyme in the alcohol metabolism. Alcohol is oxidized to acetaldehyde by alcohol dehydrogenase, and acetaldehyde is further metabolized to acetate by aldehyde dehydrogenase, which largely depends on *ALDH2*. rs671 of *ALDH2* enzyme (Glu504Lys) is one of the reasons why alcohol drinking elevates serum uric acid (SUA) levels. Thus, the association between rs671 and gout is partly due to alcohol drinking.

The allelic frequencies of rs671 of *ALDH2* differ among populations: the 504Lys allele ("A" allele) is common in East Asians including Japanese, but quite rare in other populations such as European and African descendants.

### Table 1. Association analysis of gout

| SNP | Position | Gene       | rs2288380 | rs4766566 | rs11065783 | rs11940688 | rs2071629 | rs11106783 | rs2389297 | rs7978484 |
|-----|----------|------------|-----------|-----------|------------|------------|-----------|------------|-----------|-----------|
| rs2188380 | 111386127 | MYL2-CUX2 | G/A       | G/A       | G/A        | G/A        | G/A       | G/A        | G/A       | G/A       |
| rs7978484 | 109738076 | FOXN4     | 0.35      | 0.65      | 0.14       | 0.17       | 0.14      | 0.17       | 0.14      | 0.17      |
| rs16940688 | 11360321 | TCHP-GIT2 | 0.36      | 0.85      | 0.07       | 0.13       | 0.07      | 0.13       | 0.07      | 0.13      |
| rs2071629 | 11351186 | MYL2      | 0.73      | 0.90      | 0.12       | 0.23       | 0.12      | 0.23       | 0.12      | 0.23      |
| rs11106783 | 111396249 | MYL2-CUX2 | 0.47      | 1.00      | 0.25       | 0.31       | 0.25      | 0.31       | 0.25      | 0.31      |
| rs3890297 | 111609727 | CUX2      | 0.52      | 0.79      | 0.17       | 0.28       | 0.17      | 0.28       | 0.17      | 0.28      |
| rs7978484 | 111706877 | CUX2      | 0.43      | 1.00      | 0.25       | 0.36       | 0.25      | 0.36       | 0.25      | 0.36      |
| rs671    | 112247766 | ALDH2     | 0.30      | 0.91      | 0.18       | 0.29       | 0.18      | 0.29       | 0.18      | 0.29      |
| rs2555004 | 114686845 | RBM19-TRX5| 0.31      | 0.82      | 0.21       | 0.20       | 0.21      | 0.20       | 0.21      | 0.20      |

### Table 2. Multivariate logistic regression analysis of gout including rs671 and each of the 8 SNPs

| SNP A | rs671 | P value* | OR (95% CI) | P value* | OR (95% CI) |
|-------|-------|----------|-------------|----------|-------------|
| rs7978484 | 2.6 × 10⁻¹⁰ | 0.57 (0.46–0.70) | rs2071629 | 1.6 × 10⁻¹¹ | 0.54 (0.47–0.63) |
| rs16940688 | 2.1 × 10⁻⁷ | 0.56 (0.47–0.67) | rs2188308 | 6.8 × 10⁻¹⁰ | 0.48 (0.41–0.58) |
| rs1106783 | 3.1 × 10⁻³ | 0.59 (0.46–0.76) | rs3890297 | 3.2 × 10⁻⁶ | 0.61 (0.50–0.75) |
| rs7978484 | 9.4 × 10⁻⁶ | 0.52 (0.45–0.60) |
Japanese as the other 4 previously reported loci (ABCG2, SLC2A9, GCKR and CNHI-2) of gout, and further investigations in East Asian populations will be able to warrant these findings.

In summary, Glu504Lys polymorphism (rs6761), a common dysfunctional SNP of ALDH2, is identified as a genuine gout-associated polymorphism in the MYL2-CUX2 locus, and “A” allele (Lys) of rs6761 plays a protective role in the development of gout.

Methods

Study participants. This study was approved by the institutional ethical committees (National Defense Medical College and Nagoya University), and all procedures involved in this study were performed in accordance with the Declaration of Helsinki with written informed consent from each subject.

1,048 gout cases were assigned from the Japanese male outpatients at the gout clinics of Kyoto Industrial Health Association (Kyoto, Japan), or Ryugokou East Gate Clinic (Tokyo, Japan). All patients were clinically diagnosed as primary gout according to the criteria established by the American College of Rheumatology. Patients with inherited metabolism disorders including Lesch–Nyhan syndrome were excluded. For the control group, 1,334 Japanese males with normal SUA (< 7.0 mg/dl) and without a history of gout were collected from the participants in the Shizuoka area in the Japan Multi-Institutional Collaborative Cohort Study (J-MICC Study) (version 3.1.1) (http://www.r-project.org/). The details of participants in this study are shown in Supplementary Table S3.

Selection of SNPs. First, 8,595 SNPs within 10 Mb across rs2188380 were selected using HapMap phase III JPT samples (http://hapmap.ncbi.nlm.nih.gov/); then, the pairwise LD was calculated between rs2188380 and the 8,595 SNPs (Supplementary Figure S1). After 8,550 SNPs in weak LD were excluded, the other 45 SNPs showing moderate to strong LD ($r^2 > 0.3$) with rs2188380 remained. Next, we examined the LD between each pair of these 46 SNPs (Fig. 1), and searched for the SNPs that were tagging other SNPs with strong LD ($r^2 > 0.8$). Finally, in addition to rs2188380, we selected 8 SNPs (rs7978484, rs16940688, rs2071629, rs1065783, rs3809297, rs4766566, rs671 and rs2555004) for association analysis (Supplementary Table S1).

Genetic analysis. Genomic DNA was extracted from whole peripheral blood cells. Genotyping of the 8 SNPs was performed by the TaqMan method (Life Technologies Corporation, Carlsbad, CA USA) with a LightCycler 480 (Roche Diagnostics, Mannheim, Germany). To confirm their genotypes, DNA sequencing analyses were performed with the primers shown in Supplementary Table S4. Direct sequencing was performed with a 3130xl Genetic Analyzer (Life Technologies Corporation) (version 3.1.1) (http://www.r-project.org/).

Statistical analyses. The associations between SNPs and gout were examined with logistic regression analyses. For the robustness of the statistical test, random re-sampling methods with computer simulation are often applied. In this study, the permutation test was used for random re-sampling in a case-control study with replacement for 1,000,000 times, and the robustness of statistics was confirmed. All the logistic regression analyses and chi-square tests were performed with SPSS v.22.0J (IBM Japan Inc., Tokyo, Japan) and the software R (version 3.1.1) (http://www.r-project.org/). We examined the pairwise LD using PLINK v1.07.

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Author Contributions

M.S., H.M., H.N. and A.N. conceived and designed this study. S.K., R.O., H.O. and T.S. collected samples and analyzed clinical data. M.S., H.M., K.Y. and A.N. performed genetic analysis. M.S., H.N. and T.N. performed statistical analyses. K.Y. and N.S. provided intellectual input and assisted with the preparation of the manuscript. M.S., H.M. and H.N. wrote the manuscript. M.S. and H.M. contributed equally to this work.

Additional Information

Supplementary information accompanies this paper at http://www.nature.com/srep

Competing financial interests: Yes, there is potential competing interest: H.M. and N.S. have a patent pending based on the work reported in this paper.

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