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EXTENDED REPORT

Genome-wide association study of clinically defined gout identifies multiple risk loci and its association with clinical subtypes

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ABSTRACT

Objective Gout, caused by hyperuricaemia, is a multifactorial disease. Although genome-wide association studies (GWASs) of gout have been reported, they included self-reported gout cases in which clinical information was insufficient. Therefore, the relationship between genetic variation and clinical subtypes of gout remains unclear. Here, we first performed a GWAS of clinically defined gout cases only.

Methods A GWAS was conducted with 945 patients with clinically defined gout and 1213 controls in a Japanese male population, followed by replication study of 1048 clinically defined cases and 1334 controls.

Results Five gout susceptibility loci were identified at the genome-wide significance level ($p<5.0\times10^{-8}$), which contained well-known urate transporter genes (*ABCG2* and *SLC2A9*) and additional genes: rs1260326 ($p=1.9\times10^{-12}$; OR=1.36) of *GCKR* (a gene for glucose and lipid metabolism), rs2188380 ($p=1.6\times10^{-23}$; OR=1.75) of *MYL2-CUX2* (genes associated with cholesterol and diabetes mellitus) and rs4073582 ($p=6.4\times10^{-9}$; OR=1.66) of *CNIH-2* (a gene for regulation of glutamate signalling). The latter two are identified as novel gout loci. Furthermore, among the identified single-nucleotide polymorphisms (SNPs), we demonstrated that the SNPs of *ABCG2* and *SLC2A9* were differentially associated with types of gout and clinical parameters underlying specific subtypes (renal underexcretion type and renal overload type). The effect of the risk allele of each SNP on clinical parameters showed significant linear relationships with the ratio of the case–control ORs for two distinct types of gout (r=0.96 [$p=4.8\times10^{-4}$] for urate clearance and r=0.96 [$p=5.0\times10^{-4}$] for urinary urate excretion).

Conclusions Our findings provide clues to better understand the pathogenesis of gout and will be useful for development of companion diagnostics.

INTRODUCTION

Gout is a common disease caused by deposition of monosodium urate (MSU) crystal due to hyperuricaemia.[1] Humans have long suffered from gout as reported by Hippocrates 2500 years ago.[2] There have been many famous patients with gout such as Sir Isaac Newton[3] in the more recent past, and the numbers are still growing. From the pathophysiological point of view, gout can be classified into the renal underexcretion (RUE) type, the renal overload (ROL) type and the combined type based on clinical parameters[4] (see online supplementary figure S1).

So far the genome-wide association studies (GWASs) of serum uric acid (SUA) level[5–16] have identified a number of genetic loci including *SLC2A9* (also known as *GLUT9*) and *ABCG2* (also known as *BCRP*), and subsequent genetic and functional studies have revealed the biological and pathophysiological significance of *ABCG2*.[4 17 18] Previous GWASs of gout reported a significant association with single-nucleotide polymorphisms (SNPs) of *ABCG2*, *SLC2A9* with European ancestries,[14 15] and of *ALDH16A1* with Icelanders,[14] while another study with African-American and European ancestries reported no significantly associated SNPs of gout.[13] All of these studies were, however, performed with cases including self-reported patients with gout, in which clinical information was insufficient. Therefore, the relation to genetic heterogeneity underlying gout subtypes is also unclear. To better understand its genetic basis, we first performed a GWAS of clinically defined gout cases only. We then investigated the relationship between genetic variation and clinical types of gout.

METHODS

Subjects

In the present study, we avoided use of self-reported gout cases and collected only clinically defined gout

cases. All gout cases were clinically diagnosed as primary gout according to the criteria established by the American College of Rheumatology.[19] All patients were assigned from among the Japanese male outpatients at the gout clinics of Midorigaoka Hospital (Osaka, Japan), Kyoto Industrial Health Association (Kyoto, Japan) or Ryougoku East Gate Clinic (Tokyo, Japan). Patients with inherited metabolism disorders including Lesch–Nyhan syndrome were excluded. Finally, 1994 male gout cases were registered as valid case participants. As controls, 2547 individuals were assigned from among Japanese men with normal SUA level (≤7.0 mg/dL) and no gout history, who were obtained from BioBank Japan[11 20] and Japan Multi-Institutional Collaborative Cohort Study (J-MICC Study).[21]

Genotyping and quality control

Genome-wide genotyping was performed with Illumina HumanOmniExpress v1.0 (Illumina) in 946 cases and 1213 controls. Detailed methods of genotyping and quality control are shown in the online supplementary methods and figure S2. Finally, 570 442 SNPs passed filters for 945 cases and 1213 controls.

In total, 123 SNPs passing the significance threshold at $p<1.0\times10^{-5}$ in the GWAS stage were used for subsequent analyses. Among these SNPs, we examined their linkage disequilibrium (LD) and selected 16 SNPs for replication study (see online supplementary methods). These 16 SNPs were then genotyped by an allelic discrimination assay (Custom TaqMan Assay and By-Design, Applied Biosystems) with a LightCycler 480 (Roche Diagnostics).[18] After quality control, subsequent statistical analysis was performed with 1048 cases and 1334 controls.

Statistical analyses for GWAS

We conducted an association analysis using a 2×2 contingency table based on the allele frequency, and p value of association was assessed by χ^2 test. The quantile–quantile plot and the genomic inflation factor were used to assess the presence of systematic bias in the test statistics due to potential population stratification (see online supplementary methods and figure S3).

We then combined results from the GWAS and replication stages by meta-analysis.[22] The inverse-variance fixed-effects model meta-analysis was used for estimating summary OR. Cochran's Q test[23] and I^2 statistic[24 25] were examined to assess heterogeneity in ORs between GWAS and replication study. If heterogeneity was present by the statistical test ($p_{het}<0.05$) or measurement ($I^2>50\%$), we implemented DerSimonian and Laird random-effects model meta-analysis.[26] All the meta-analyses were performed using the STATA V.11.0. Genome-wide significance threshold was set to be $\alpha=5.0\times10^{-8}$ to claim evidence of a significant association. Detailed methods of imputation and per cent variance are shown in the online supplementary methods.

Subtype analyses

Gout contains two distinct types, 'ROL' type and 'RUE' type. The ROL type was defined when urinary urate excretion (UUE) was over 25.0 mg/h/1.73 m^2 (600 mg/day/1.73 m^2)[4 27–29] and their urate clearance (urate clearance/creatinine clearance ratio, FE_{UA}) was 5.5% or over. Also, the RUE type was determined when UUE was 25.0 mg/h/1.73 m^2 or under and FE_{UA} was under 5.5%.[4 30 31] Detailed methods of subtype analyses are described in the online supplementary methods.

RESULTS

Genome-wide association study

Clinical characteristics of participants in this study are shown in online supplementary tables S1–S3. GWAS with 945 clinically defined gout cases and 1213 controls identified SNPs in three loci showing evidence of associations at the genome-wide significance level ($p<5.0\times10^{-8}$): rs2728125 of *ABCG2* ($p=1.5\times10^{-27}$; OR=2.05), rs3775948 of *SLC2A9* ($p=6.7\times10^{-15}$; OR=1.64) and rs2188380 of *MYL2-CUX2* ($p=5.7\times10^{-13}$; OR=1.78, figures 1 and 2, table 1 and online supplementary figure S4).

Replication study was conducted with 1048 cases and 1334 controls. As a result, the three SNPs surpassing the genome-wide significance threshold in the GWAS stage were successfully replicated; rs2728125 ($p=8.3\times10^{-29}$; OR=2.03), rs3775948 ($p=7.6\times10^{-14}$; OR=1.57) and rs2188380 ($p=2.0\times10^{-12}$;

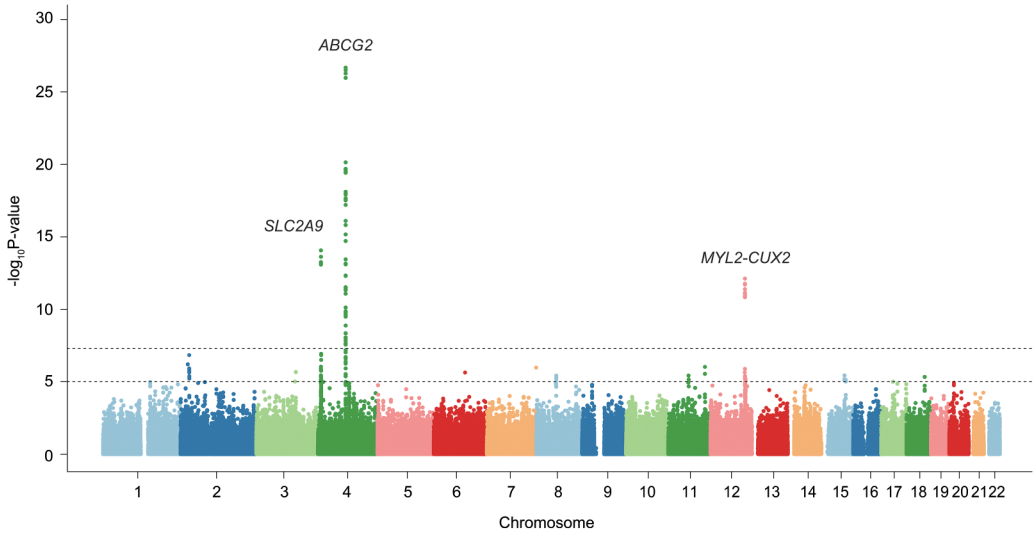

Figure 1 Manhattan plot of a genome-wide association analysis of gout. X-axis shows chromosomal positions. Y-axis shows -$\log_{10}$ p values. The upper and lower dotted lines indicate the genome-wide significance threshold ($p=5.0\times10^{-8}$) and the cut-off level for selecting single-nucleotide polymorphisms for replication study ($p=1.0\times10^{-5}$), respectively.

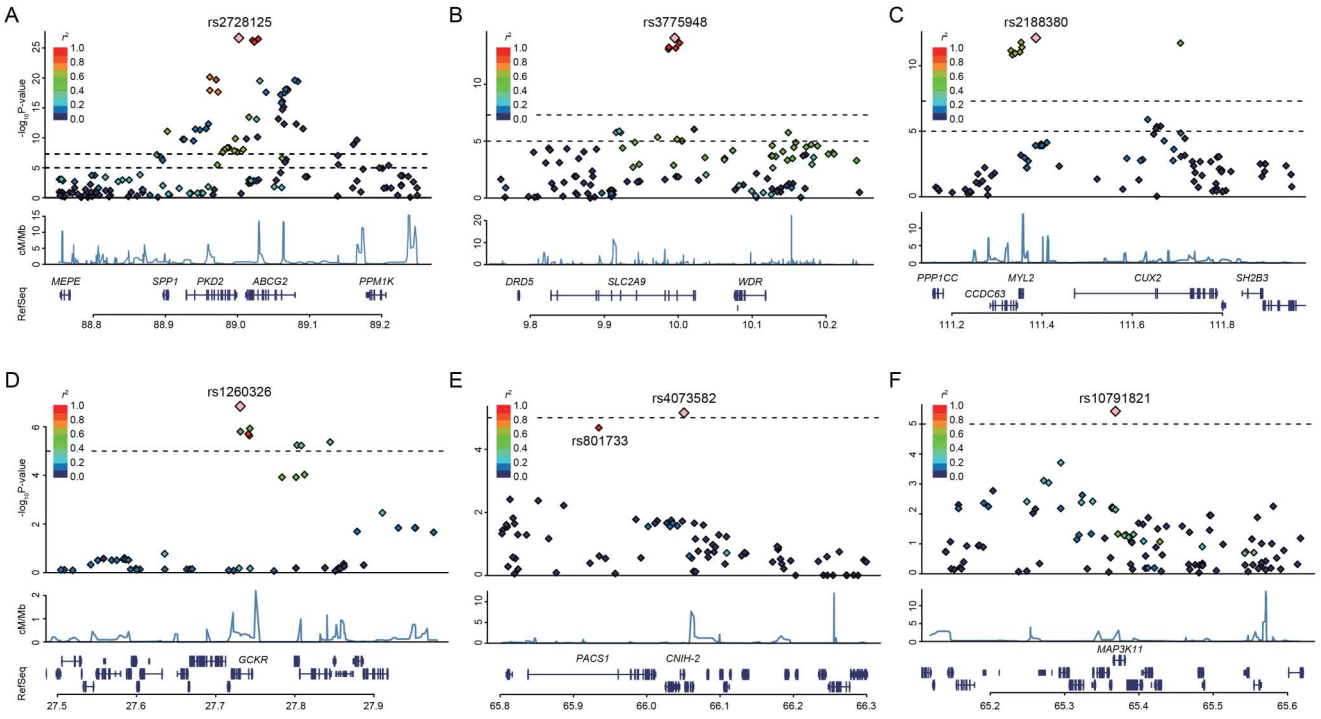

Figure 2 Regional association plots for six discovered loci of gout. Five regions exceeding the genome-wide significance level (A–E) and one region showing a suggestive association (F). The highest association signal in each panel is located on *ABCG2* (A), *SLC2A9* (B), *MYL2-CUX2* (C), *GCKR* (D), *CNIH-2* (E) and *MAP3K11* (F). Region within 250 kb from single-nucleotide polymorphism (SNP) showing lowest p value is displayed. (Top panel) Plots of -$\log_{10}$ p values for the test of SNP association with gout in the genome-wide association study stage. SNP showing the lowest p value is depicted as a pink diamond. Other SNPs are colour-coded according to the extent of linkage disequilibrium (measured in r^2) with SNP showing the lowest p value. (Middle panel) Recombination rates (centimorgans per Mb) estimated from HapMap Phase II data are plotted. (Bottom panel) RefSeq genes. Genomic coordinates are based on Genomic Reference Consortium GRCh37.

OR=1.73). Additionally, two SNPs showed significant associations at $p<3.1\times10^{-3}$ (=0.05/16) with Bonferroni correction; rs1260326 of *GCKR* ($p=2.8\times10^{-6}$; OR=1.32) and rs4073582 of *CNIH-2* ($p=1.6\times10^{-4}$; OR=1.55) as shown in table 1 and online supplementary table S4.

All five SNPs that showed significant associations in the replication study achieved genome-wide significance in the meta-analysis of GWAS and replication study (table 1): rs2728125 ($p_{meta}=7.2\times10^{-54}$; OR=2.04), rs3775948 ($p_{meta}=5.5\times10^{-27}$; OR=1.61), rs2188380 ($p_{meta}=1.6\times10^{-23}$; OR=1.75), rs1260326 ($p_{meta}=1.9\times10^{-12}$; OR=1.36) and rs4073582 ($p_{meta}=6.4\times10^{-9}$; OR=1.66). In addition, an intronic SNP of *MAP3K11* (rs10791821) showed a suggestive level of association ($p_{meta}=1.0\times10^{-7}$; OR=1.57). There was >80% power to detect a risk variant for OR=1.6 at the genome-wide significance level ($p<5.0\times10^{-8}$) for an SNP with a minor allele frequency of 0.35 (see online supplementary table S5). Imputation was also performed with the GWAS genotyping data for 1 Mb across the identified SNPs of novel loci (rs2188380 of *MYL2-CUX2*, rs1260326 of *GCKR*, rs4073582 of *CNIH-2* and rs10791821 of *MAP3K11*). SNPs that passed the significant threshold of GWAS stage ($p<1.0\times10^{-5}$) in this imputation are shown in online supplementary table S6A–D.

Two dysfunctional SNPs of *ABCG2*

We previously demonstrated that two dysfunctional SNPs of *ABCG2*, rs72552713 (Gln126Ter) and rs2231142 (Gln141Lys), were located on different haplotypes[4 18] and strongly associated with hyperuricaemia and gout.[4 18 32] Therefore, we additionally performed genotyping of these two SNPs by an allelic discrimination assay because SNPs are not on Illumina HumanOmniExpress V.1.0 (Illumina). SNP showing the highest significance in the present GWAS (rs2728125) was in strong LD with rs2231142 ($r^2=0.76$) but not in LD with rs72552713 ($r^2=0.03$). A multivariate logistic regression analysis including these three SNPs of *ABCG2* showed that rs2728125 no longer had a significant association (p=0.19), but rs72552713 and rs2231142, that is, two non-synonymous SNPs, remained highly significant (see online supplementary table S7A, B), indicating that rs2728125 was merely a surrogate marker for rs2231142. Therefore, we used these two non-synonymous variants for subsequent analyses.

Cumulative effect of risk alleles for gout

Accumulation of the number of risk alleles of the gout-associated SNPs (rs3775948, rs2188380, rs1260326, rs4073582, rs72552713 and rs2231142) increased the probability of gout logarithmically. When setting the reference category as having four or fewer risk alleles, ORs for having 5, 6, 7, 8 and 9 or more risk alleles were 1.79 ($p=3.5\times10^{-3}$), 3.16 ($p=2.3\times10^{-10}$), 5.10 ($p=9.7\times10^{-21}$), 10.1 ($p=5.3\times10^{-39}$) and 18.6 ($p=3.6\times10^{-45}$), respectively (see online supplementary figure S5 and table S8).

Subtype analysis of gout

We examined type-specific ORs and the case–subtype heterogeneity test.[33] The subgroup analysis (table 2) showed that the associations of two non-synonymous SNPs of *ABCG2* (rs72552713 and rs2231142) were stronger for the ROL type (ORs=4.35 and 3.37, respectively) than for the RUE type (ORs=1.28 and

Table 1 Five SNPs showing significant association at genome-wide significance level and one suggestive SNP

SNP¶	Chromosome	Position (bp)††	Gene	A1/A2‡‡	GWAS†				Replication study‡				Meta-analysis§	
					Freq.		OR (95% CI)	p Value	Freq.		OR (95% CI)	p Value	OR (95% CI)	p Value
					Cases	Controls			Cases	Controls				
rs2728125	4	89 001 893	*ABCG2*	C/T	0.40	0.25	2.05 (1.80 to 2.34)	1.5×10^{-27}	0.40	0.24	2.03 (1.79 to 2.30)	8.3×10^{-29}	2.04 (1.86 to 2.23)	7.2×10^{-54}
rs3775948	4	9 995 182	*SLC2A9*	G/C	0.68	0.56	1.64 (1.45 to 1.86)	6.7×10^{-15}	0.67	0.56	1.57 (1.40 to 1.77)	7.6×10^{-14}	1.61 (1.47 to 1.75)	5.5×10^{-27}
rs2188380	12	111 386 127	*MYL2-CUX2*	T/C	0.85	0.76	1.78 (1.52 to 2.08)	5.7×10^{-13}	0.86	0.78	1.73 (1.48 to 2.02)	2.0×10^{-12}	1.75 (1.57 to 1.96)	1.6×10^{-23}
rs1260326	2	27 730 940	*GCKR*	T/C	0.62	0.54	1.39 (1.23 to 1.57)	1.2×10^{-7}	0.61	0.55	1.32 (1.18 to 1.49)	2.8×10^{-6}	1.36 (1.25 to 1.48)	1.9×10^{-12}
rs4073582	11	66 050 712	*CNIH-2*	G/A	0.95	0.91	1.78 (1.39 to 2.29)	5.3×10^{-6}	0.94	0.91	1.55 (1.23 to 1.96)	1.6×10^{-4}	1.66 (1.40 to 1.96)	6.4×10^{-9}
rs10791821**	11	65 368 323	*MAP3K11*	G/A	0.94	0.90	1.75 (1.38 to 2.22)	2.8×10^{-6}	0.94	0.92	1.41 (1.12 to 1.77)	3.4×10^{-3}	1.57 (1.33 to 1.85)	1.0×10^{-7}

†945 gout cases and 1213 controls.
‡1048 gout cases and 1334 controls.
§Meta-analyses of the combined GWAS and replication samples (1993 gout cases and 2547 controls).
¶dbSNP rs number. A suggestive SNP is marked with '**'.
††SNP positions are based on the National Center for Biotechnology Information human genome reference sequence Build 37.4.
‡‡A1 is a risk-associated allele and A2 is a non-risk-associated allele.
Freq., frequency of A1; GWAS, genome-wide association study; SNP, single-nucleotide polymorphism.

1.88, respectively). The differences in ORs between the gout types were highly significant ($p=2.4\times10^{-5}$ and 1.0×10^{-7}, respectively). The association of rs3775948 of *SLC2A9* was stronger for the RUE type (OR=1.94) than for the ROL type (OR=1.38). The case–subtype heterogeneity test showed a significant difference in ORs ($p=2.7\times10^{-4}$). The other SNPs evidenced no significant differences. Then, associations between SNPs and clinical parameters (FE_{UA} and UUE) were assessed. Only SNPs that showed a significant difference in ORs between different gout types were significantly associated with FE_{UA} and UUE (table 2 and online supplementary figure S6, table S9); the gout risk alleles of *ABCG2* and *SLC2A9* were associated with increased and decreased levels of these parameters, respectively. The effect of the risk allele of each SNP on clinical parameters showed significant linear relationships with OR in the case–subtype heterogeneity test, which was an estimate of the ratio of the case–control ORs for the gout types (r=0.96 [$p=4.8\times10^{-4}$] for FE_{UA} and r=0.96 [$p=5.0\times10^{-4}$] for UUE) (figure 3).

DISCUSSION

Through the GWAS with clinically defined cases, we identified five gout-associated loci that showed different association patterns in subtype analysis. Previous GWASs of SUA[5–16] showed genome-wide significant associations with *ABCG2*, *SLC2A9* and *GCKR*. These genes were also reported to have significant associations with gout as a consequence of hyperuricaemia.[13–15] The present study revealed for the first time that three loci (*GCKR*, *MYL2-CUX2* and *CNIH-2*) were associated with gout at the genome-wide significance level. In particular, *MYL2-CUX2* and *CNIH-2* are novel loci for gout.

The total variance explained by the seven SNPs was estimated to be 9.0% (see online supplementary methods): three SNPs of well-known urate transporter genes (*SLC2A9* and *ABCG2*) with large effects accounted for 6.9%, and the four SNPs identified in this GWAS with modest effects explained 2.1%. Additional discoveries of unidentified genetic variants by performing a meta-analysis of GWAS data sets will improve the explained genetic variation of gout.

ABCG2 and *SLC2A9* are well-known urate transporter genes for urate excretion[17 18] and renal urate reabsorption,[34 35] respectively. *ABCG2* is identified to have an association with SUA levels by recent GWASs.[9–16] Subsequent genetic and functional analysis[17 18] revealed that ABCG2 is a high-capacity urate exporter and shows the reduced transport of urate by a common half-functional variant, rs2231142 (Gln141Lys). We also demonstrated that common dysfunctional genotype combinations of *ABCG2* gene (non-functional rs72552713 [Gln126Ter] and rs2231142) are a major cause of hyperuricaemia and gout,[18] especially for early-onset gout.[32] We earlier found that the risk alleles of these two SNPs reside on different haplotypes,[4 18] indicating independent risks of gout. Recently, these dysfunctional SNPs were revealed to decrease extrarenal (intestinal) urate excretion and to cause ROL hyperuricaemia,[4] through studies with hyperuricaemic patients[4] and *Abcg2*-knockout mice.[4 36] This is consistent with the fact that ABCG2 exporter is expressed on the apical membrane in several tissues, including intestine[37] and kidney,[38] which have urate-excreting functions in humans.

SLC2A9 is a member of the glucose transporter (GLUT) family. SLC2A9 was found to transport urate,[7 34] and several GWAS have demonstrated an association of *SLC2A9* with SUA levels.[5–16] SLC2A9 has two isoforms, GLUT9L (long isoform) and GLUT9S (short isoform),[34] and is highly expressed in the kidney proximal tubules in humans.[39] Genetic and functional

Table 2 Associations of seven SNPs with gout types

SNP†	Gene	Freq.		ROL type vs controls*		RUE type vs controls*		Case–subtype heterogeneity test	
		ROL type	RUE type	OR (95% CI)	p Value	OR (95% CI)	p Value	OR (95% CI)	p Value‡
rs3775948	*SLC2A9*	0.62	0.70	1.38 (1.14 to 1.68)	1.0×10^{-3}	1.94 (1.63 to 2.31)	1.0×10^{-13}	0.66 (0.53 to 0.83)	**2.7×10^{-4}**
rs2188380	*MYL2-CUX2*	0.84	0.85	1.45 (1.11 to 1.89)	6.5×10^{-3}	1.47 (1.16 to 1.86)	1.2×10^{-3}	0.92 (0.68 to 1.25)	0.60
rs1260326§	*GCKR*	0.60	0.62	1.25 (1.04 to 1.50)	0.016	1.35 (1.15 to 1.58)	3.0×10^{-4}	0.94 (0.77 to 1.14)	0.51
rs4073582	*CNIH-2*	0.95	0.94	1.96 (1.30 to 2.95)	1.2×10^{-3}	1.51 (1.09 to 2.08)	0.013	1.26 (0.80 to 1.99)	0.32
rs10791821	*MAP3K11*	0.93	0.95	1.37 (0.96 to 1.96)	0.084	1.79 (1.26 to 2.54)	1.2×10^{-3}	0.79 (0.51 to 1.23)	0.30
rs72552713§	*ABCG2*	0.067	0.029	4.35 (2.82 to 6.72)	3.0×10^{-11}	1.28 (0.78 to 2.12)	0.32	2.90 (1.77 to 4.75)	**2.4×10^{-5}**
rs2231142§	*ABCG2*	0.50	0.38	3.37 (2.76 to 4.12)	2.8×10^{-32}	1.88 (1.58 to 2.24)	2.5×10^{-12}	1.76 (1.43 to 2.17)	**1.0×10^{-7}**

*We performed multivariate logistic regression analyses, in which all seven SNPs, alcohol drinking and body mass index were included in the model. In total, 1613 patients with gout and 1334 controls with genotypes for rs72552713 and rs2231142 of *ABCG2*, which were not on the Illumina OmniExpress platform, were used. Also, 375 and 509 patients with gout were grouped into ROL type and RUE type, respectively.
†dbSNP rs number.
‡p Values <0.05 are shown in bold.
§Non-synonymous SNPs (rs1260326, Leu446Pro; rs72552713, Gln126Ter; and rs2231142, Gln141Lys).
Freq., frequency of risk-associated allele; ROL, renal overload; RUE, renal underexcretion; SNP, single-nucleotide polymorphism.

analysis[34 35] with patients with renal hypouricaemia (RHUC) revealed that RHUC is caused by dysfunctional mutations in *SLC2A9*, which decrease urate reabsorption in the renal proximal tubules. For example, non-functional mutations of either GLUT9L (Arg198Cys and Arg380Trp) or GLUT9S (Arg169Cys and Arg351Trp, corresponding to Arg198Cys and Arg380Trp in GLUT9L), which were found from patients with RHUC, dramatically reduced the urate transport activity.[34] Therefore, SLC2A9 plays an important role in renal urate reabsorption.[34] Thus, *SLC2A9* is a causative gene for RHUC type 2,[34 40] which was confirmed by the report of homozygous mutations in patients with RHUC type 2.[35] In our subtype analysis, OR of RUE type was higher than that of ROL type (OR=1.94 and 1.38, respectively, table 2), which is compatible with the fact that SLC2A9 is a transporter for urate reabsorption in human kidney.

Glucokinase regulatory protein (GCKR) controls the activity of glucokinase, which is a major glucose sensor for insulin secretion. GCKR regulates the first step of glycolysis, the phosphorylation of glucose to glucose-6-phosphate.[41 42] Glucokinase activity is controlled by GCKR, which binds to glucokinase and suppresses its function in the postabsorptive phase. On the other hand, this binding is loosened in the postprandial phase, so that glucokinase could adopt the glycolysis.[43] So far, the gout risk allele of rs1260326 (Leu446Pro) has been reported to be associated with lower fasting glucose levels, and inversely, higher levels of triglyceride[43–45] and SUA.[10 12 15] An association of *GCKR* with dyslipidaemia has also been reported.[46]

MYL2 encodes a regulatory light chain associated with cardiac myosin β (or slow) heavy chain. *MYL2* mutations are associated with mid-left ventricular-type hypertrophic cardiomyopathy. In addition, its association with high-density lipoprotein

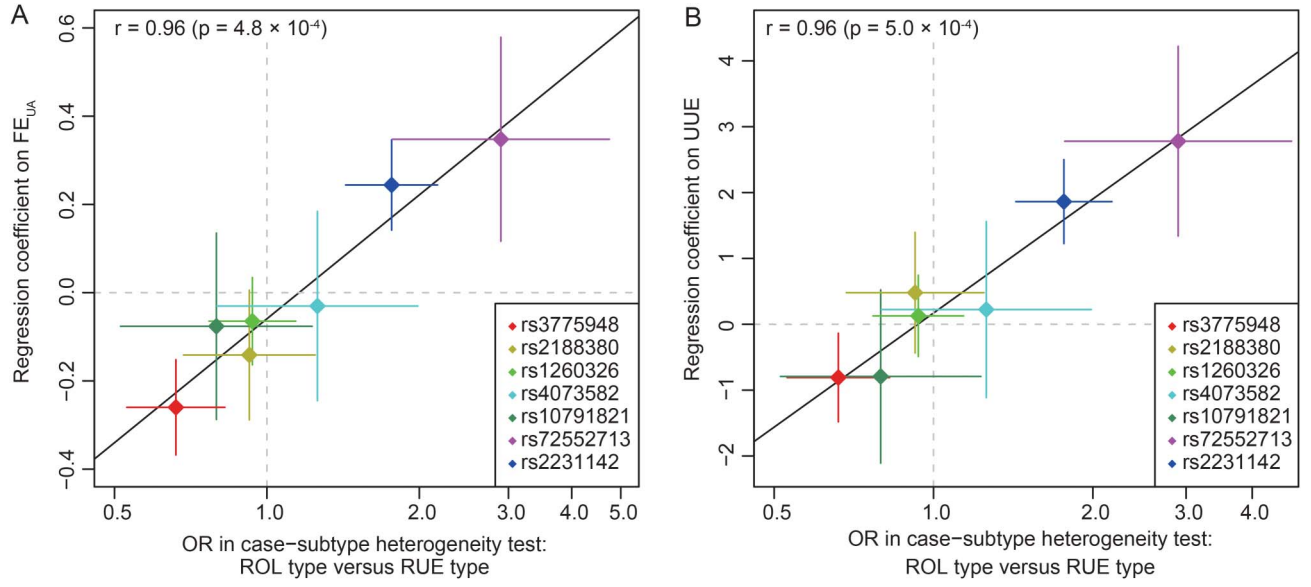

Figure 3 Relationships between effects of risk alleles on clinical parameters and ORs in case–subtype heterogeneity test. (A) FE_{UA} and (B) urinary urate excretion (UUE). The seven single-nucleotide polymorphisms (SNPs) listed in table 2 were examined. OR in case–subtype heterogeneity test is an estimate of the ratio of the case–control OR for the renal overload (ROL) type to that for the renal underexcretion (RUE) type. If an SNP has a stronger effect for the ROL type than for the RUE type, it takes a value >1. Diamonds and lines represent point estimates and their 95% CIs. Pearson's correlation coefficient (r) between the effect on clinical parameters and natural logarithm of OR in case–subtype heterogeneity test and its significance were examined. FE_{UA}, fractional excretion of urate clearance.

cholesterol metabolism was previously reported.[47] CUX2 regulates cell-cycle progression[48] and plays important roles in neural progenitor development in the central nervous system.[48 49] Its association with type 1 diabetes has also been reported.[50] Thus, rs2188380 of *MYL2-CUX2* showed an association with gout because *MYL2* and *CUX2* might influence such metabolic pathways. Rs2188380 locates near rs653178 of *ATXN2* (see online supplementary figure S7), which was reported by Köttgen *et al*[15] to have an association with SUA. Rs653178 is, however, monomorphic in the Japanese population of the HapMap project,[51] and we also confirmed it in our samples by genotyping >250 replication cases. Conversely, rs2188380 of *MYL2-CUX2* is monomorphic in European and African populations,[51] while rs2188380 is a common variant in the Japanese population (table 1). Therefore, this SNP was identified as a novel locus of gout in the present study. The differences in study populations could be one of the reasons why rs2188380 was not found in a large European-driven GWAS on urate and gout.[15] Further analyses including fine mapping and functional analysis are required in this region.

CNIH-2 regulates the function of glutamate receptors of the AMPA-subtype assembly at the cell surface of various neurons and glial cells.[52 53] CNIH-2 modulates AMPA receptor gating by increasing its cell surface expression. The newly identified rs4073582 of *CNIH-2* was in strong LD with rs801733 in *PACS1* (r^2=0.97, figure 2E and see online supplementary figure S4E), which is reported to be associated with severe obesity.[54] Accordingly, *PACS1* can also be a good candidate for a gout susceptibility gene. Additional genetic dissection and functional analysis will be needed to determine whether these genes or others could play roles with true causality at this locus. Since Okada *et al*[16] previously reported the association between SUA and rs504915 of *NRXN2*, which is near *CNIH-2* and *MAP3K11*, we examined their relationships. They are not in strong LD (see online supplementary table S10), and the association of rs4073582 and rs10791821 remained significant after adjustment with rs504915 (see online supplementary table S11). Therefore, rs4073582 of *CNIH-2*, rs10791821 of *MAP3K11* and rs504915 of *NRXN2* are revealed to be independent of each other.

MAP3K11, also known as mixed lineage kinase 3 (MLK3), is a MAP kinase member and plays a significant role in the activation of c-Jun N-terminal kinase (JNK), a stress-activated protein kinase.[55] Signalling from the small GTP-binding proteins Rac1 and Cdc42 induces MLK3 to activate the MEKK-SEK-JNK kinase cascade. Interestingly, the JNK pathway is activated when monocytes/macrophages phagocytose MSU crystals,[56] which cause gouty arthritis. The SNP rs10791821 of *MAP3K11* has been associated with the expression level of *MAP3K11* in monocytes,[57] and therefore, is likely to be a regulatory SNP. However, further study is required to confirm precise involvement of MAP3K11 in the development of gout.

Other genes (*CCDC63*, *C2orf16*, *ZNF512*, *RAB1B*, *EHBP1L1* and *KCNK7*) near each of the novel loci, which are found by imputation analysis (see online supplementary table S6A–D), could also be candidate genes of gout, and further studies including functional analyses are warranted.

Most of the gout-related genes are also associated with SUA.[15] In the present study design, to identify novel gout risk loci, clinically defined gout and normouricaemic controls were recruited. Therefore, further investigations with different study designs will be needed to identify gout loci associated with crystal deposition and inflammation.

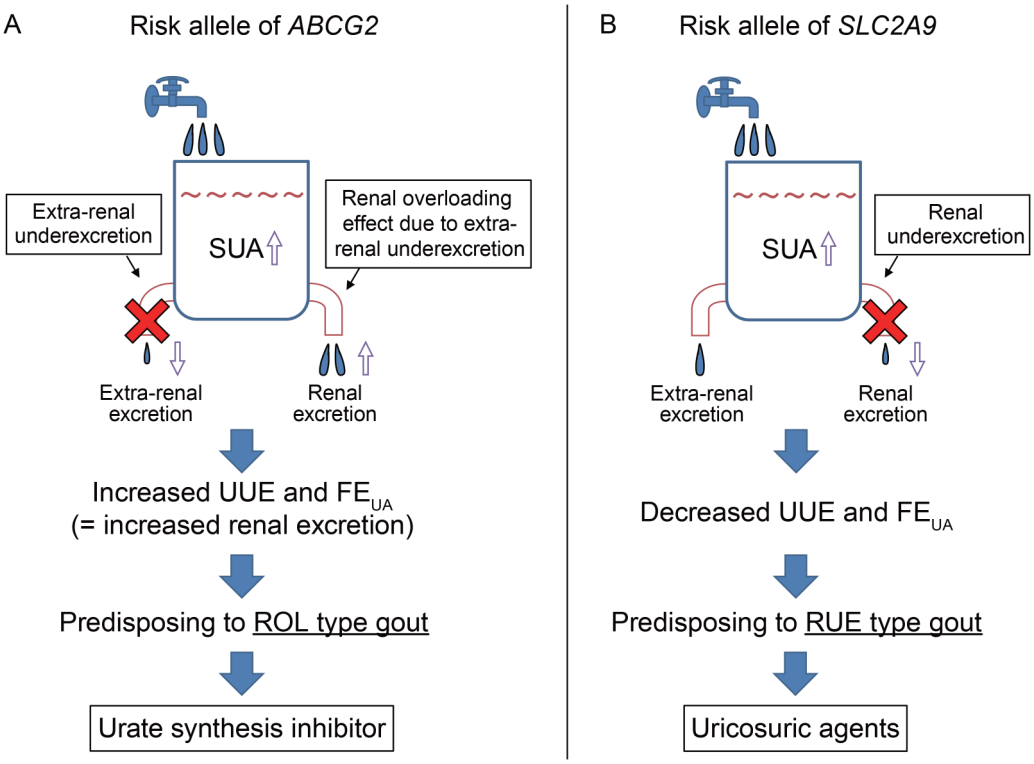

Figure 4 Differential effects by risk allele on clinical parameters and gout. (A) The risk alleles of *ABCG2* increase UUE and FE_{UA}, which leads to the overloading effect on renal urate excretion and increases the risk of the ROL-type gout. Therefore, patients with risk alleles for the ROL-type gout would be given urate synthesis inhibitors. (B) The risk allele of *SLC2A9* reduces UUE and FE_{UA}, which reflects a decreased renal urate excretion, thereby increasing the risk of the RUE-type gout. Patients with risk alleles for the RUE-type gout would be administered uricosuric agents. FE_{UA}, fractional excretion of urate clearance; ROL, renal overload; RUE, renal underexcretion; SUA, serum uric acid; UUE, urinary urate excretion.

We further investigated the cumulative effect of risk alleles of the five significant loci (*ABCG2*, *SLC2A9*, *MYL2-CUX2*, *GCKR* and *CNIH-2*) on gout risk. The result showed that individuals with five or more risk alleles had a higher risk for gout compared with those having four or fewer risk alleles. The more risk alleles in an individual, the higher became the risk of gout.

Furthermore, the relationship between genetic variation and clinical types of gout was investigated. The results of subtype analyses (table 2, figure 3 and online supplementary figure S6, table S9) indicate that the alleles closely associated with the risk of specific gout type represented differential effects on clinical parameters (FE_{UA} and UUE). This allows the estimation of disturbed urate excretion pathways. An increase of FE_{UA} and UUE by the risk alleles of *ABCG2* leads to the overloading effect on renal urate excretion and causes the ROL-type gout (figure 4A). These estimations are consistent with our previous finding obtained from *Abcg2*-knockout mouse models and hyperuricaemic patients.[4] In contrast, the reduction of FE_{UA} and UUE by the risk allele of *SLC2A9* reflects a decreased renal urate excretion, thereby increasing the risk of the RUE-type gout (figure 4B). The present study demonstrated that the combination of GWAS of patients with clinically defined gout with actual clinical data is an effective method to analyse genetic heterogeneity among different types of gout.

In summary, we conducted the first GWAS using patients with clinically defined gout only and identified five loci containing two novel loci. Moreover, identified SNPs showed differential effects on different gout types and affected clinical parameters underlying specific types. Thus, genetic testing for gout may well be introduced into future companion diagnostics. For example, patients with risk alleles for ROL-type gout would be given urate synthesis inhibitors[31 58] such as allopurinol and febuxostat, while patients with risk alleles for RUE-type gout would be administered uricosuric agents[31 58] including benzbromarone and lesinurad, a selective uric acid reabsorption inhibitor that has just finished its phase III study.[59 60] Exploring genetic heterogeneity among different gout types will deepen understanding of the aetiology of gout and serve to categorise patients for future personalised treatment.

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Contributors HM, KY, HNakaoka, AN, MS, TC, II and NS conceived and designed the experiments. ATakahashi, TN, HNakashima, YT, TT, YS, HS, YKanai, TH and MK assisted with research design. HM, AN, MS, TC, ATokumasu, KIchida, HOoyama and TS collected and analysed clinical data of cases. ATakahashi, GY, RO, EM, MN, NH and MK collected and analysed clinical data of controls. HM, KY, AN, MS, TC, YT, ID, SS, JA, YKawamura, STerashige, HOgata, STatsukawa, YN and NS performed genetic analysis. HNakaoka, ATakahashi, TN, HNakashima and YS performed statistical analysis. HM, KY, HNakaoka, AN, MS, TC, ATakahashi, TN, HNakashima, MK, and NS analysed the data. TN, HNakashima, HOnoue, KIwaya, TI, TT, KInoue, YKato and II provided intellectual input and assisted with the preparation of the manuscript. HM, KY, HNakaoka, AN, MS, TC, ATakahashi and NS wrote the paper.

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Competing interests HM, TT and NS have a patent pending based on the work reported in this paper. Other authors have declared that no competing interests exist.

Patient consent Obtained.

Ethics approval All procedures involved in this study were approved by the institutional ethical committees of National Defense Medical College, Nagoya University and RIKEN, and all procedures involved were performed in accordance with the Declaration of Helsinki.

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Supplementary Materials:

Supplementary Figure S1 Classification of gout.

Supplementary Figure S2 Result of principal component analysis including case and control samples used in this GWAS and four HapMap populations.

Supplementary Figure S3 Quantile-Quantile plot of p value distribution for association.

Supplementary Figure S4 Regional association plots for six discovered loci (Larger version of Figure 2).

Supplementary Figure S5 Cumulative effect of six gout-associated SNPs for developing gout.

Supplementary Figure S6 Relationship between clinical parameters (FE_{UA} and UUE) and the SNPs of *SLC2A9* and *ABCG2*.

Supplementary Figure S7 Extended regional association plot of *MYL2-CUX2* locus.

Supplementary Table S1 Clinical characteristics of case and control.

Supplementary Table S2 Clinical parameters for each specific type of gout patient.

Supplementary Table S3 Concomitant diseases of gout patients.

Supplementary Table S4 Summary of GWAS and replication study of 16 SNPs.

Supplementary Table S5 Power of the present study design.

Supplementary Table S6A Imputation analysis near *MYL2-CUX2* locus.

Supplementary Table S6B Imputation analysis near *GCKR* locus.

Supplementary Table S6C Imputation analysis near *CNIH-2* locus.

Supplementary Table S6D Imputation analysis near *MAP3K11* locus.

Supplementary Table S7A Univariate and multivariate logistic regression including three SNPs of *ABCG2*.

Supplementary Table S7B Association analysis of gout with *ABCG2* dysfunctional missense variants.

Supplementary Table S8 Magnitude of gout risk associated with the number of risk alleles.

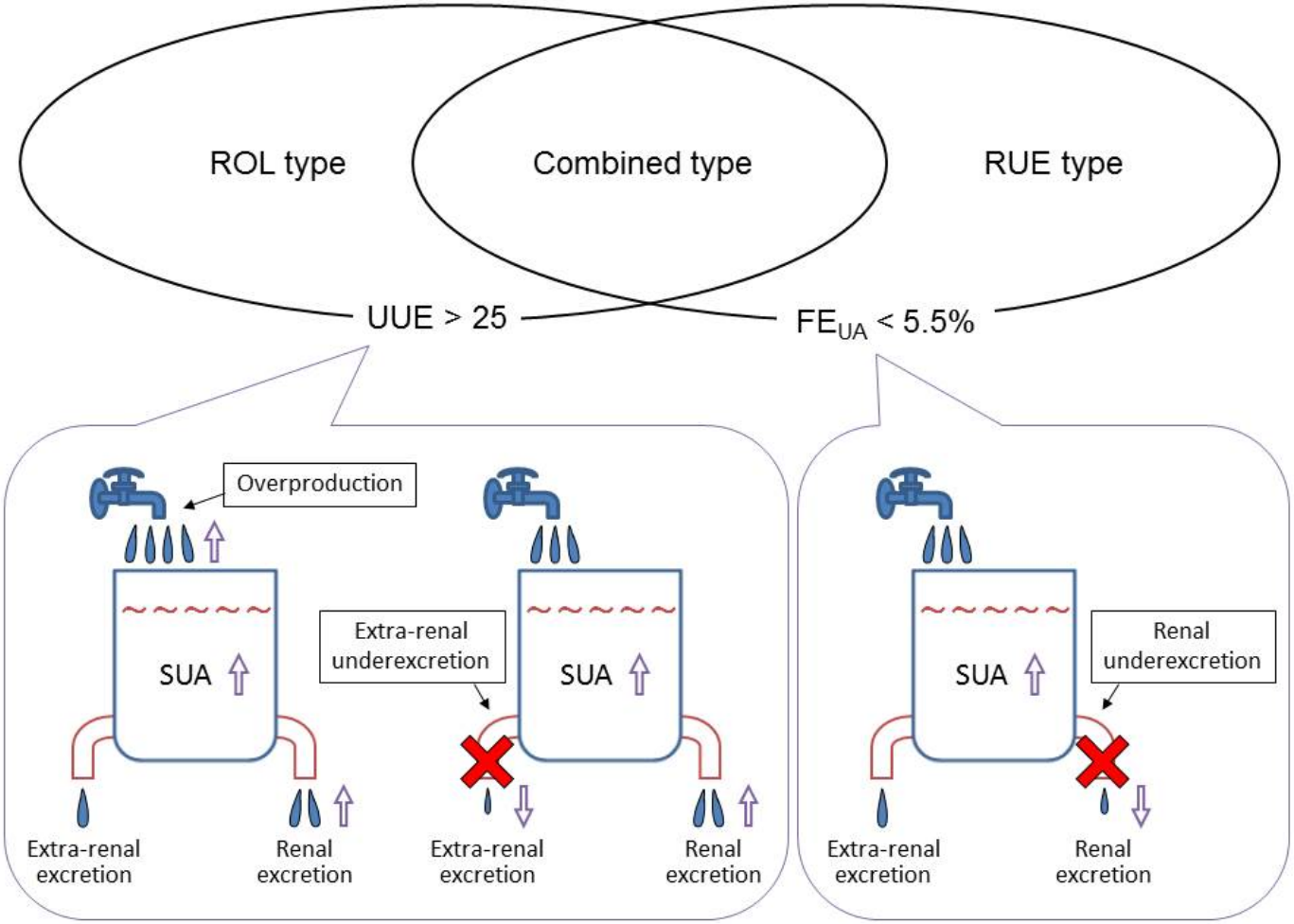
Supplementary Table S9 Association analysis between seven SNPs and three uric acid-related parameters.

Supplementary Table S10 Linkage disequilibrium among rs4073582 of *CNIH-2*, rs10791821 of *MAP3K11*, and rs504915 of *NRXN2.*

Supplementary Table S11 Multivariate logistic regression analysis adjusted with rs504915 of *NRXN2.*

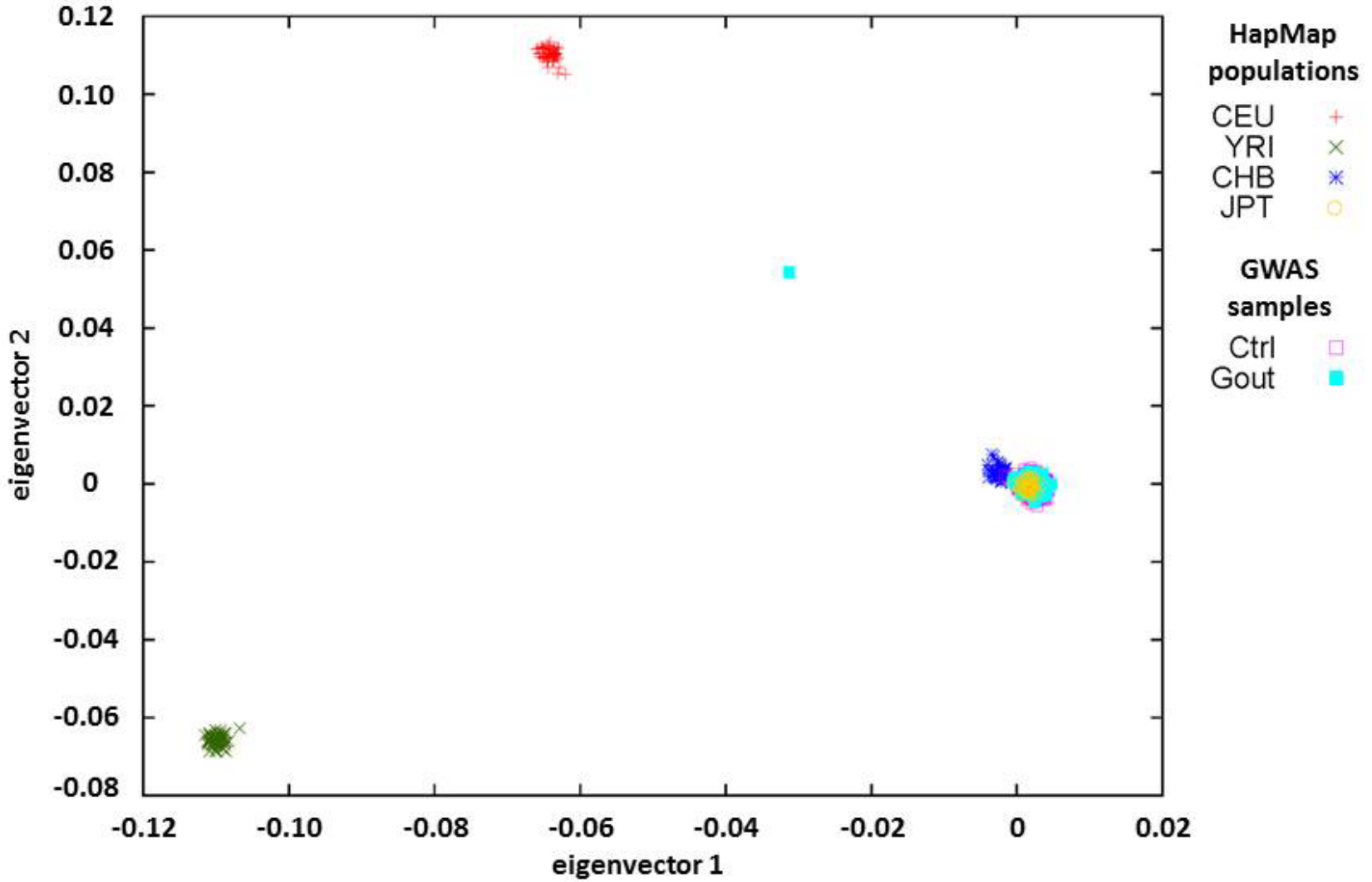
Supplementary Methods

References for Supplementary Methods

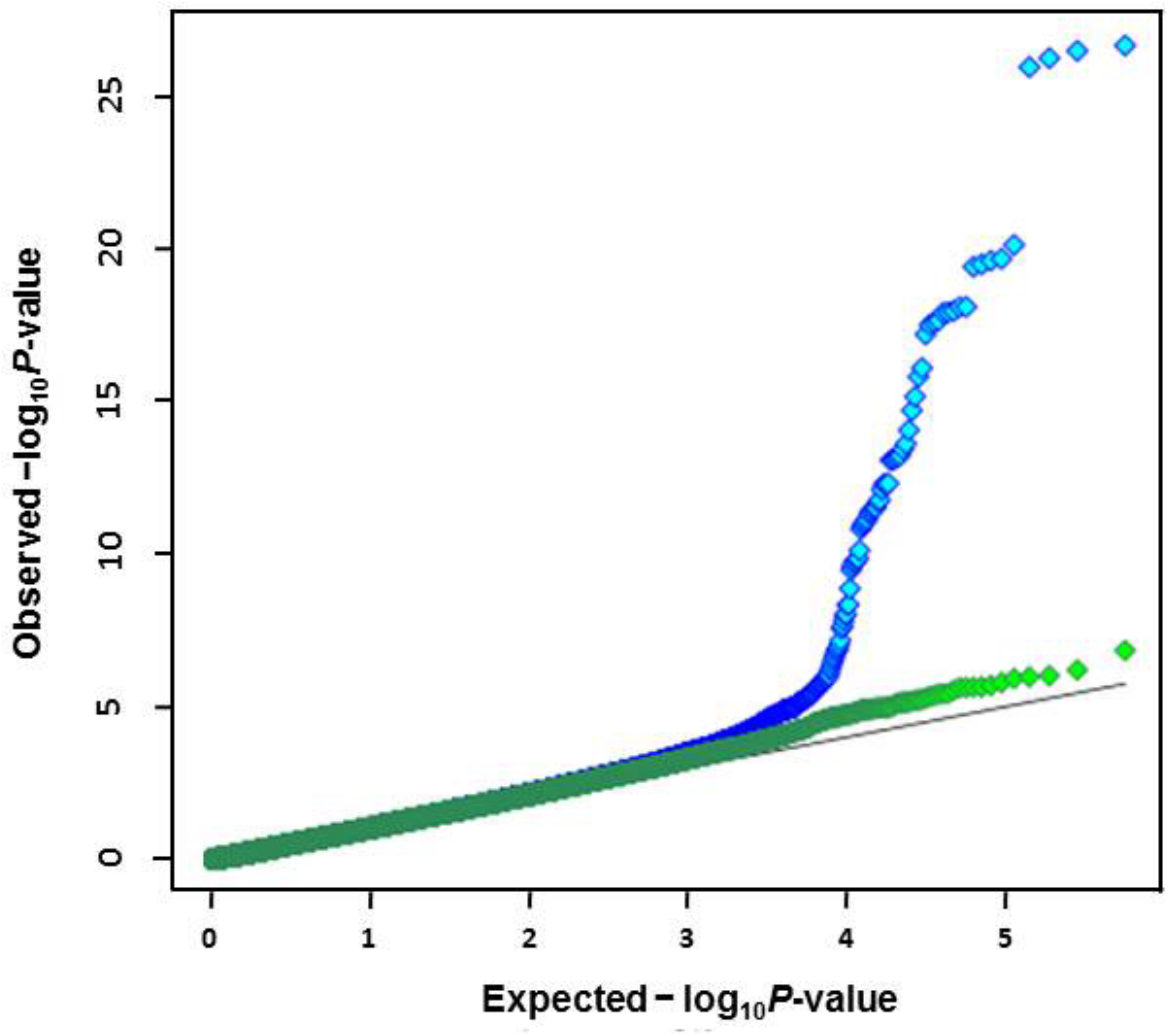


Supplementary Figure S1 Classification of gout.

Gout can be pathophysiologically classified into the 'renal overload' (ROL) type, the 'renal underexcretion' (RUE) type, or the combined type, based on the clinical parameters, UUE and FE_{UA}. The unit of UUE is 'mg/hr/1.73m^2.' Urate is an end-product of purine metabolism, and excreted from kidney (renal excretion pathway) or from extra-renal tissues (extra-renal excretion pathway). Therefore, elevated SUA with high UUE (>25 mg/hr/1.73m^2) is caused by renal urate overload (overproduction and/or extra-renal underexcretion of urate), while increased SUA with low FE_{UA} (<5.5%) results from renal urate underexcretion. SUA, serum uric acid. UUE, urinary urate excretion. FE_{UA}, fractional excretion of urate clearance.

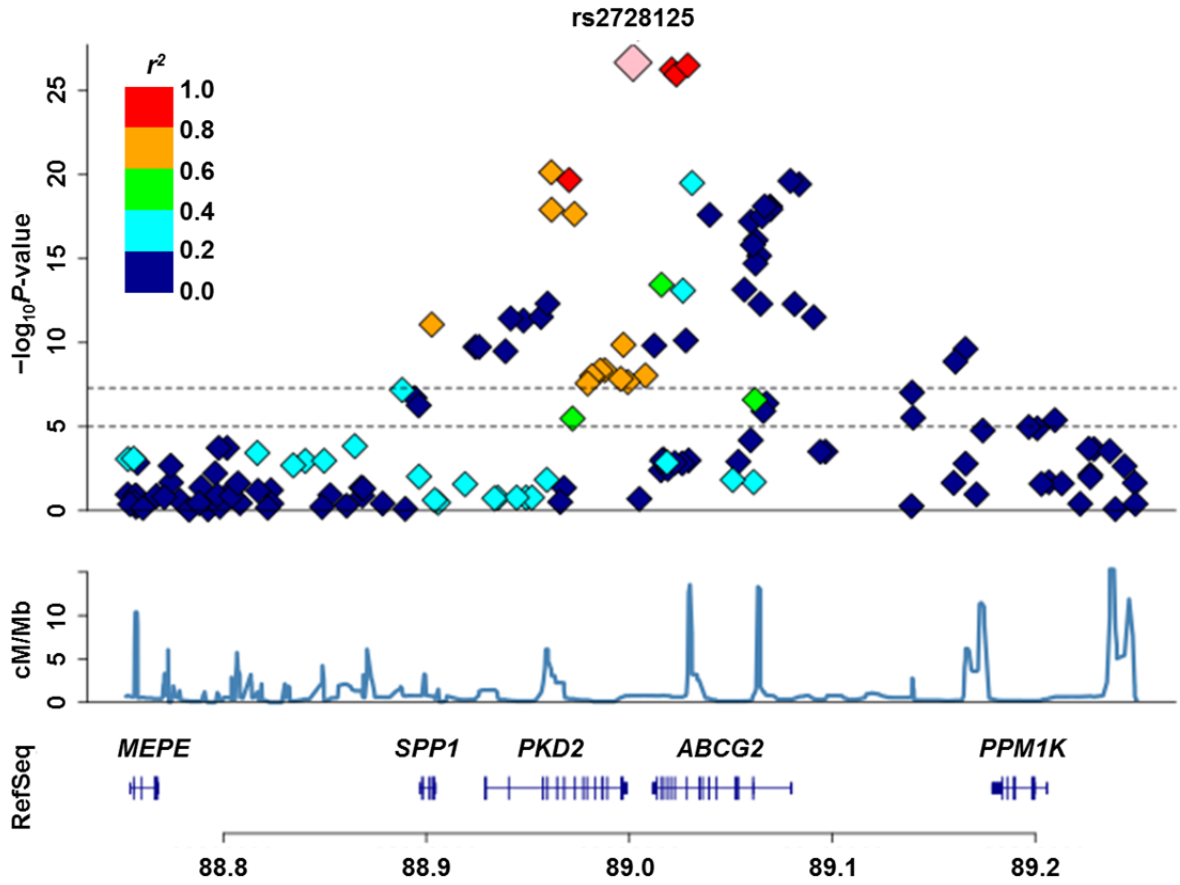


Supplementary Figure S2 Result of principal component analysis including case and control samples used in this GWAS and four HapMap populations. JPT, Japanese in Tokyo, Japan. CEU, CEPH (Utah residents with ancestry from northern and western Europe). YRI, Yoruba in Ibadan, Nigeria. CHB, Han Chinese in Beijing, China.

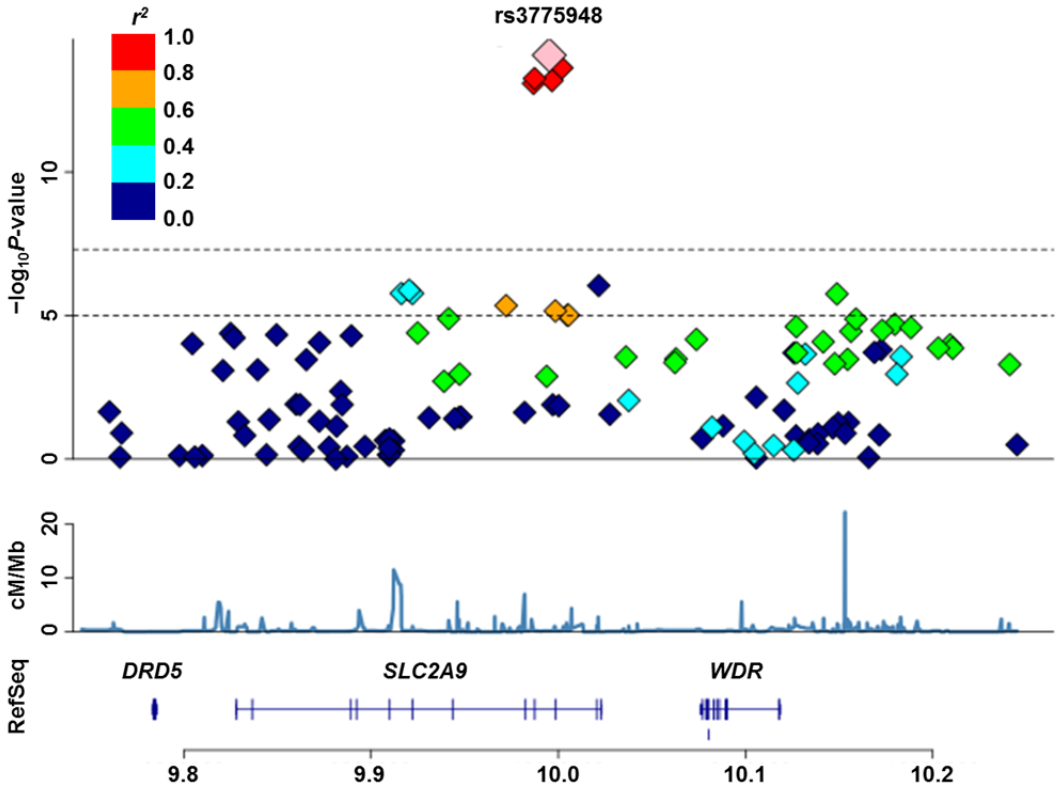


Supplementary Figure S3　Quantile-Quantile plot of p value distribution for association.

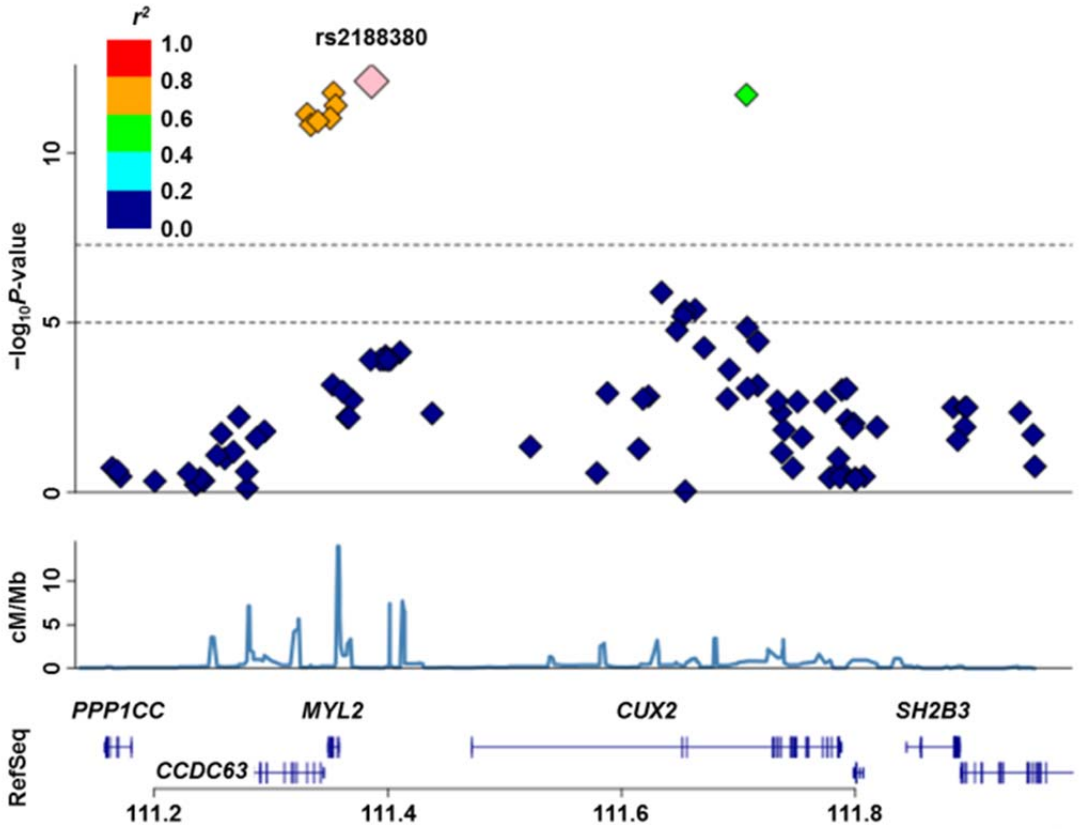
Results for all SNPs tested are plotted in blue. Results excluding SNPs within 500 kb from the SNPs strongly associated at $p < 5.0 \times 10^{-8}$ (rs2728125 of *ABCG2*, rs3775948 of *SLC2A9*, and rs2188380 of *MYL2-CUX2*) are plotted in green.



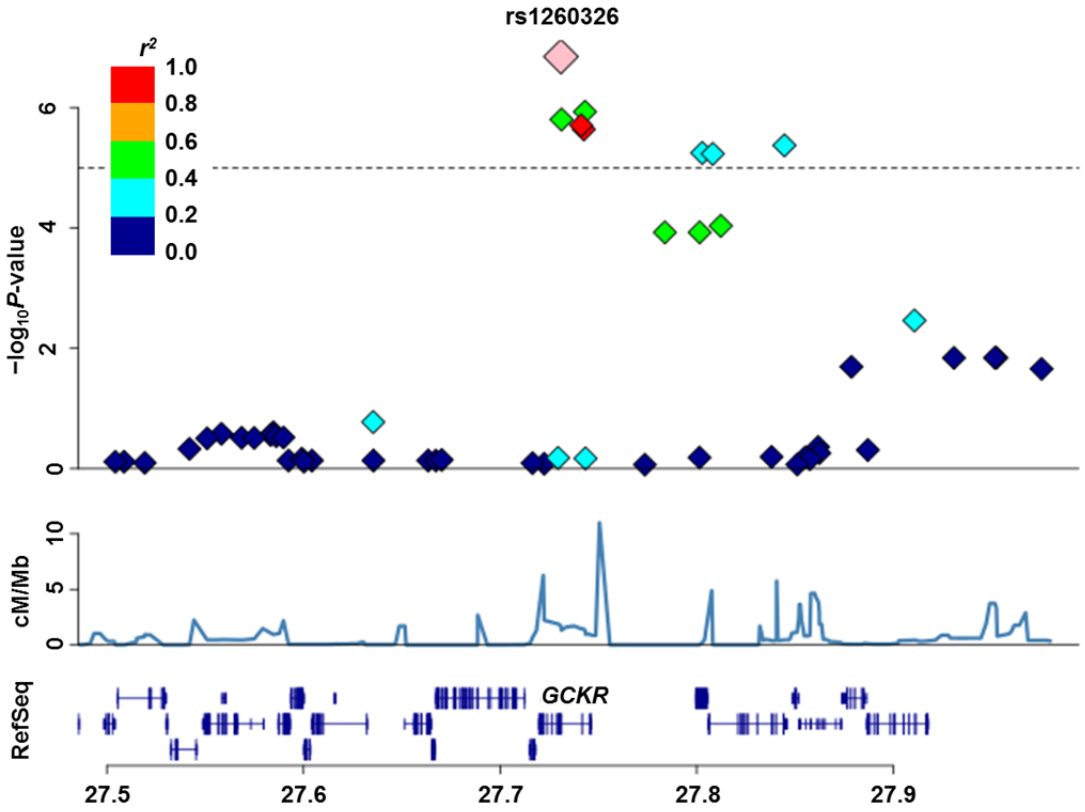
Supplementary Figure S4A Regional association plots for six discovered loci (Larger version of Figure 2A; *ABCG2* locus).



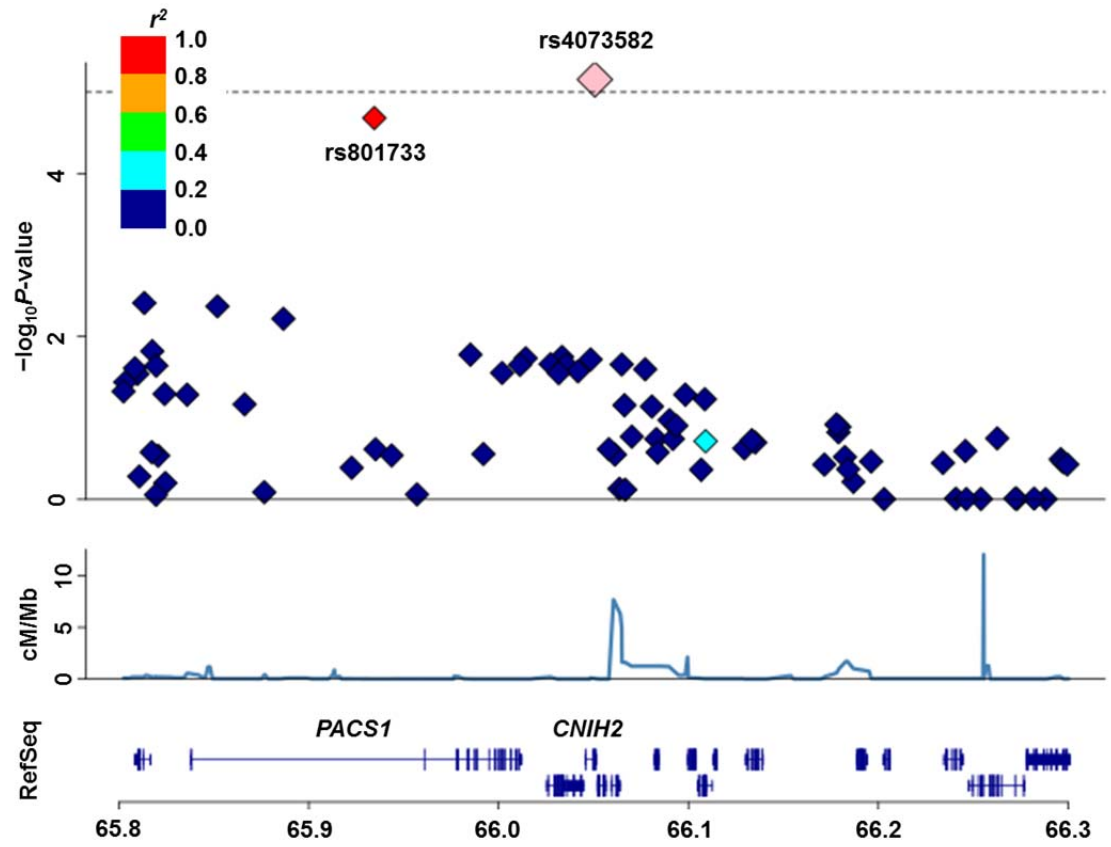
Supplementary Figure S4B Regional association plots for six discovered loci (Larger version of Figure 2B; *SCL2A9* locus).



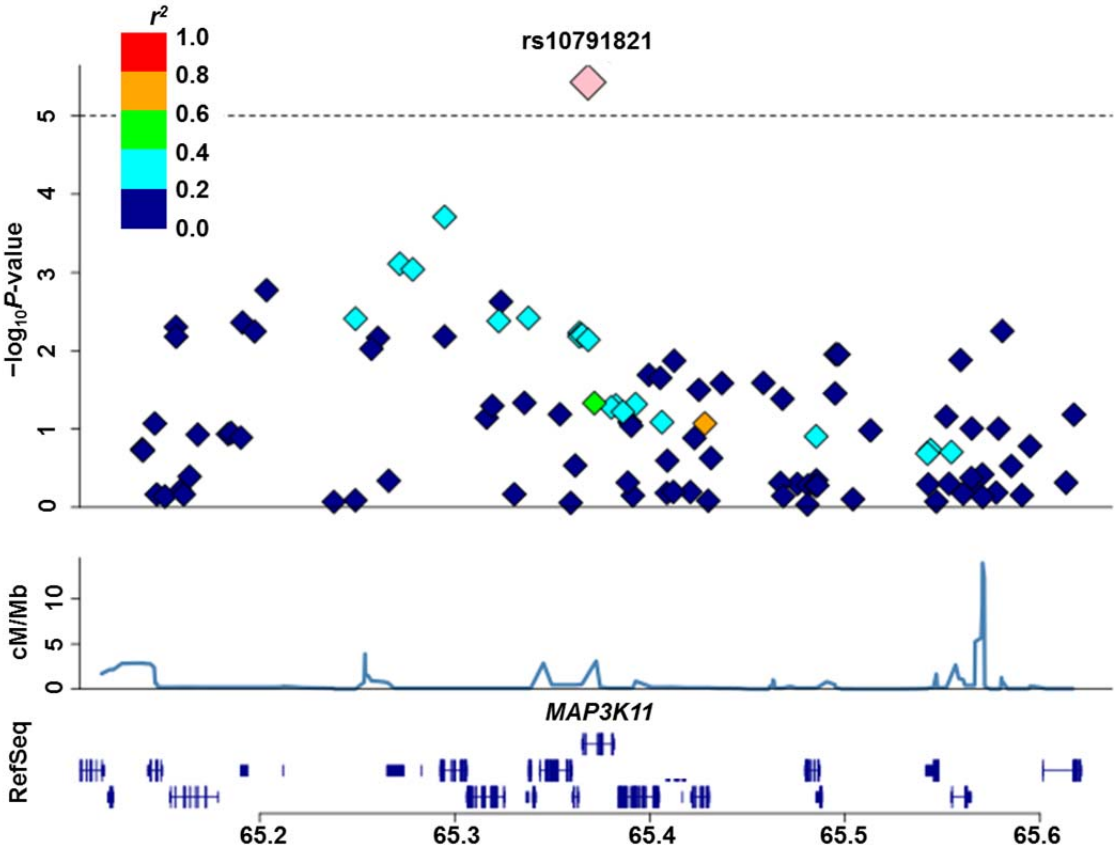
Supplementary Figure S4C Regional association plots for six discovered loci (Larger version of Figure 2C; *MYL2-CUX2* locus).



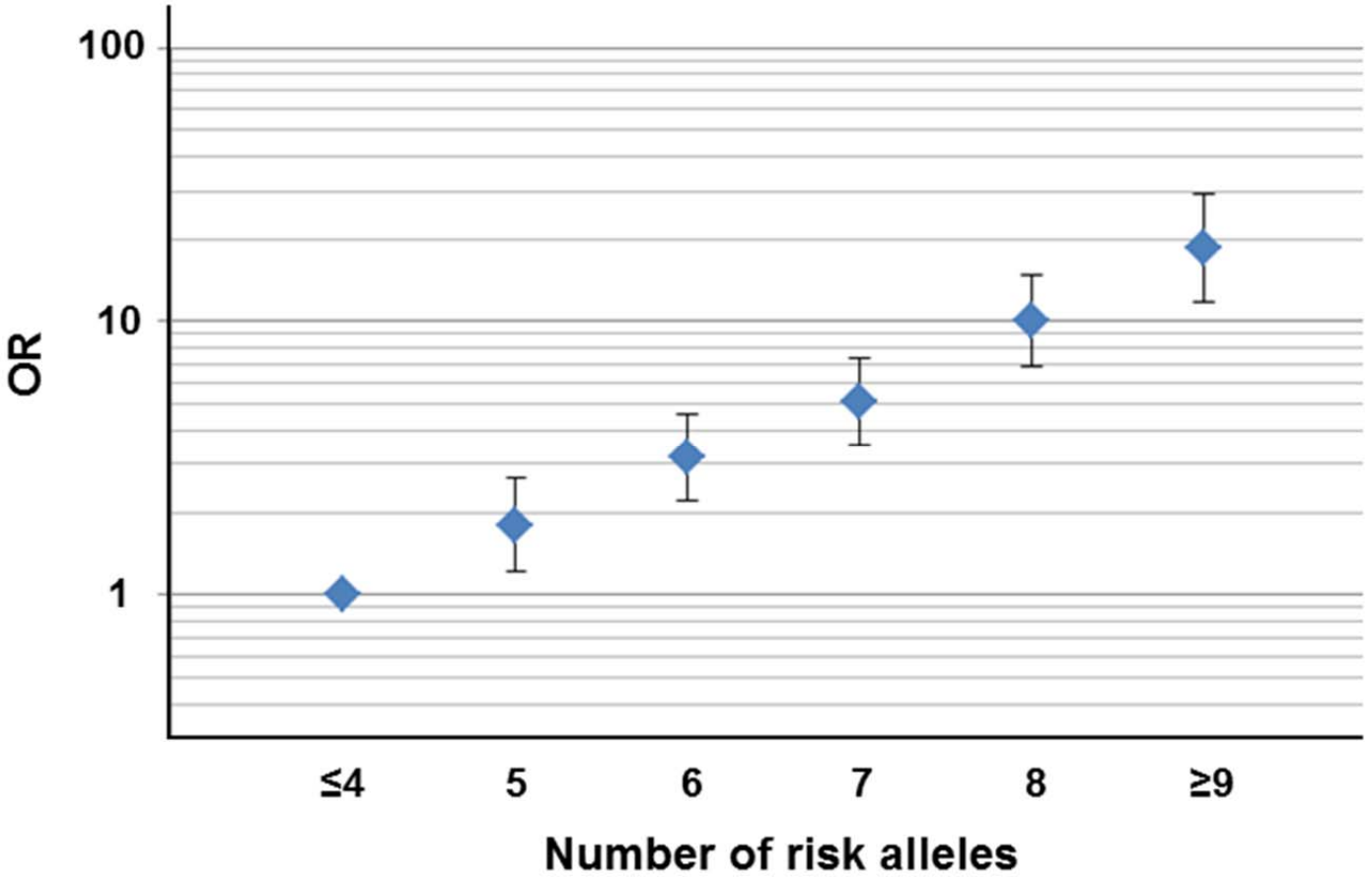
Supplementary Figure S4D Regional association plots for six discovered loci (Larger version of Figure 2D; *GCKR* locus).



Supplementary Figure S4E Regional association plots for six discovered loci (Larger version of Figure 2E; *CNIH-2* locus).

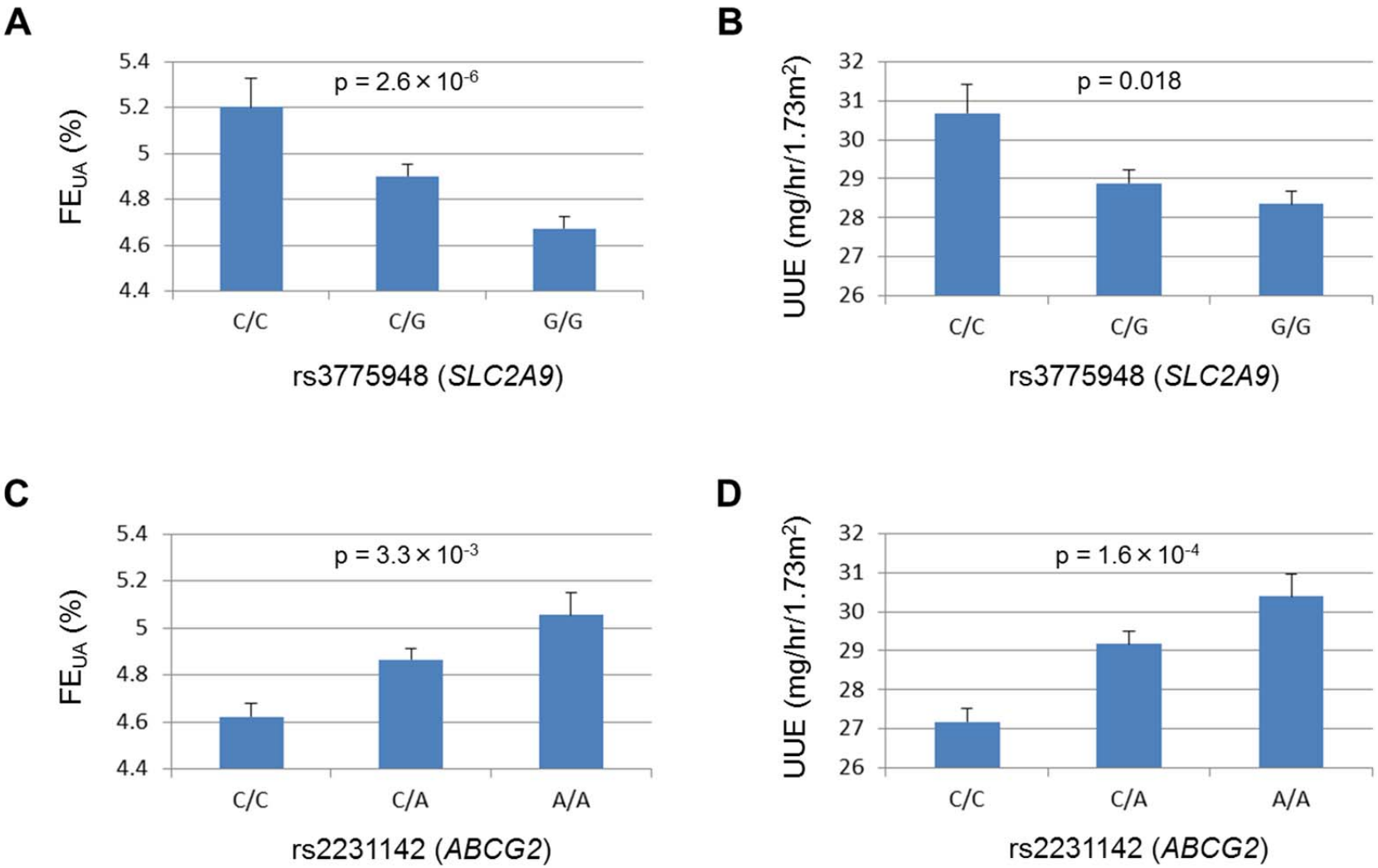


Supplementary Figure S4F Regional association plots for six discovered loci (Larger version of Figure 2F; *MAP3K11* locus).



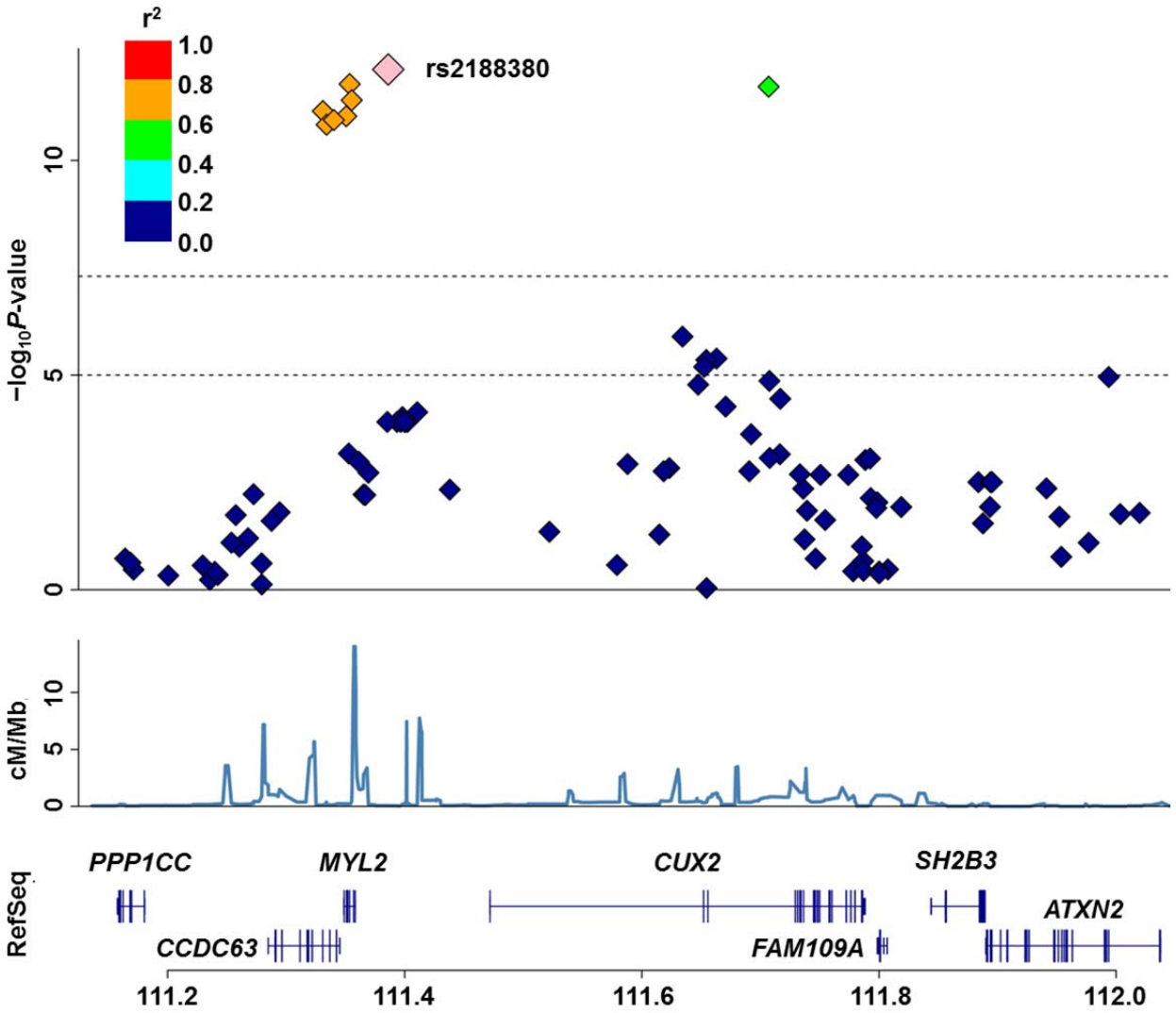
Supplementary Figure S5　Cumulative effect of six gout-associated SNPs for developing gout.

A genetic risk score was defined as the number of risk alleles over the following six SNPs: four SNPs identified by the present study (rs3775948 of *SLC2A9*, rs2188380 of *MYL2-CUX2*, rs1260326 [Leu446Pro] of *GCKR*, and rs4073582 of *CNIH-2*) and two nonsynonymous SNPs (rs72552713 [Gln126Ter] and rs2231142 [Gln141Lys] of *ABCG2*), which have been established as strong gout risk SNPs.



Supplementary Figure S6　Relationship between clinical parameters (FE_{UA} and UUE) and the SNPs of *SLC2A9* and *ABCG2*.

FE_{UA} and UUE significantly decreased as the risk allele of *SLC2A9* (rs3775948) increased (A, B), whereas they increased as the risk allele of *ABCG2* (rs2231142) increased (C, D). Because RUE type and ROL type are defined by FE_{UA} and UUE (Supplementary Figure S1), these are consistent with the results shown in Supplementary Table S9. All bars show mean ± SEM.



Supplementary Figure S7　Extended regional association plot of *MYL2-CUX2* locus.

Near rs2188380 of *MYL2-CUX2*, there is rs653178 of *ATXN2*, which was previously reported in European ancestry to have an association with SUA, although rs653178 is monomorphic in a Japanese population. Conversely, rs2188380 identified in the present study with Japanese is monomorphic in European and African populations.

Supplementary Table S1 Clinical characteristics of case and control

	GWAS stage		Replication stage	
	Case	Control	Case	Control
Number	945	1213	1048	1334
Age (year)	46.5 ± 11.2	65.8 ± 8.4	44.9 ± 11.4	52.4 ± 8.6
Body-mass index (kg/m^2)	25.1 ± 3.6	21.8 ± 3.3	25.0 ± 3.5	23.2 ± 2.7
Serum uric acid (mg/dl)	8.5 ± 1.2	5.5 ± 1.1	8.6 ± 1.3	5.6 ± 0.9

Plus-minus values are means ± SD

Supplementary Table S2 Clinical parameters for each specific type of gout patient

	Number (%)	SUA	FE_{UA}	UUE
ROL type	375 (23.2%)	8.18 ± 1.10	6.73 ± 1.25	35.8 ± 8.3
RUE type	509 (31.6%)	8.38 ± 1.16	3.84 ± 0.79	20.5 ± 3.5
Combined type	664 (41.2%)	8.83 ± 1.25	4.40 ± 0.70	31.8 ± 6.9
Normal type	65 (4.0%)	7.72 ± 1.12	6.46 ± 0.90	21.2 ± 3.5
All	1613 (100%)	8.49 ± 1.22	4.85 ± 1.46	28.7 ± 8.9

Plus-minus values are means ± SD

SUA, serum uric acid; FE_{UA}, fractional excretion of urate clearance; UUE, urinary urate excretion; ROL, renal overload; RUE, renal underexcretion.

Among 1993 clinically-defined cases, 1613 patients had information such as clinical parameters (UUE and FE_{UA}) to classify their clinical subtypes.

Supplementary Table S3 Concomitant diseases of gout patients

Number (%)	Hypertension	Dyslipidemia	Diabetes mellitus	Ischemic heart disease	Stroke	Renal impairment*
GWAS stage	249 (26.3%)	42 (4.4%)	37 (3.9%)	19 (2.0%)	9 (0.95%)	11 (1.2%)
Replication stage	317 (30.2%)	58 (5.5%)	42 (4.0%)	26 (2.5%)	9 (0.86%)	4 (0.38%)
All	566 (28.4%)	100 (5.0%)	79 (4.0%)	45 (2.3%)	18 (0.90%)	15 (0.75%)

*Renal impairment is defined with high serum creatinine (≥1.5 mg/dl).

Supplementary Table S4 Summary of GWAS and replication study of 16 SNPs

					GWAS[§]				Replication study[‖]				Meta-analysis[††]		Heterogeneity	
					Freq.				Freq.							
SNP*	Chr.	Position (bp)[†]	Gene	A1/A2[‡]	Cases	Controls	OR (95% CI)	p Value	Cases	Controls	OR (95% CI)	p Value[‡‡]	OR (95% CI)	p Value[g]	Cochran's Q	I^2 (%)
rs1260326**	2	27730940	*GCKR*	T/C	0.62	0.54	1.39 (1.23-1.57)	1.2×10^{-7}	0.61	0.55	1.32 (1.18-1.49)	**2.8×10^{-6}**	1.36 (1.25-1.48)	**1.9×10^{-12}**	0.57	0
rs16835687	3	124327247	*KALRN*	G/A	0.17	0.12	1.48 (1.24-1.75)	7.8×10^{-6}	0.15	0.12	1.22 (1.03-1.45)	0.019	1.34 (1.12-1.62)	1.9×10^{-3}	0.12	58.2
rs4637240	3	127150025	*PLXNA1-TPRA1*	C/T	0.87	0.81	1.5 (1.27-1.77)	1.8×10^{-6}	0.84	0.83	1.08 (0.92-1.26)	0.35	1.27 (0.92-1.76)	0.15	4.3×10^{-3}	87.7
rs3775948**	4	9995182	*SLC2A9*	G/C	0.68	0.56	1.64 (1.45-1.86)	6.7×10^{-15}	0.67	0.56	1.57 (1.40-1.77)	**7.6×10^{-14}**	1.61 (1.47-1.75)	**5.5×10^{-27}**	0.63	0
rs2728125**	4	89001893	*ABCG2*	C/T	0.40	0.25	2.05 (1.80-2.34)	1.5×10^{-27}	0.40	0.24	2.03 (1.79-2.30)	**8.3×10^{-29}**	2.04 (1.86-2.23)	**7.2×10^{-54}**	0.90	0
rs6916538	6	100618009	*MCHR2-SIM1*	A/G	0.51	0.44	1.34 (1.19-1.51)	2.0×10^{-6}	0.48	0.46	1.09 (0.97-1.23)	0.13	1.21 (0.99-1.48)	0.062	1.7×10^{-2}	82.4
rs731305	7	157682840	*PTPRN2*	C/T	0.63	0.56	1.36 (1.20-1.54)	8.9×10^{-7}	0.61	0.61	1.02 (0.91-1.15)	0.70	1.18 (0.89-1.56)	0.25	1.1×10^{-3}	90.7
rs7007064	8	63099432	*ASPH-NKAIN3*	A/C	0.76	0.70	1.38 (1.21-1.59)	3.2×10^{-6}	0.73	0.75	0.90 (0.79-1.03)	0.12	1.12 (0.73-1.70)	0.61	8.8×10^{-6}	94.9
rs10791821***	11	65368323	*MAP3K11*	G/A	0.94	0.90	1.75 (1.38-2.22)	2.8×10^{-6}	0.94	0.92	1.41 (1.12-1.77)	3.4×10^{-3}	1.57 (1.33-1.85)	1.0×10^{-7}	0.19	41.2
rs4073582**	11	66050712	*CNIH-2*	G/A	0.95	0.91	1.78 (1.39-2.29)	5.3×10^{-6}	0.94	0.91	1.55 (1.23-1.96)	**1.6×10^{-4}**	1.66 (1.40-1.96)	**6.4×10^{-9}**	0.43	0
rs11216561	11	117685575	*FXYD2-DSCAML1*	C/T	0.11	0.07	1.70 (1.38-2.10)	7.2×10^{-7}	0.10	0.08	1.33 (1.09-1.63)	5.7×10^{-3}	1.50 (1.18-1.91)	1.0×10^{-3}	0.098	63.4
rs2188380**	12	111386127	*MYL2-CUX2*	T/C	0.85	0.76	1.78 (1.52-2.08)	5.7×10^{-13}	0.86	0.78	1.73 (1.48-2.02)	**2.0×10^{-12}**	1.75 (1.57-1.96)	**1.6×10^{-23}**	0.81	0
rs9806234	15	74663033	*CYP11A1-SEMA7A*	G/A	0.62	0.55	1.34 (1.18-1.51)	3.1×10^{-6}	0.58	0.58	1.02 (0.91-1.15)	0.70	1.17 (0.90-1.52)	0.24	1.9×10^{-3}	89.7
rs931794	15	78826180	*AGPHD1*	A/G	0.67	0.60	1.33 (1.18-1.51)	6.5×10^{-6}	0.64	0.64	1.00 (0.89-1.13)	0.97	1.16 (0.87-1.53)	0.31	1.3×10^{-3}	90.4
rs13755	17	37223458	*PLXDC1*	A/G	0.17	0.13	1.47 (1.24-1.73)	8.3×10^{-6}	0.14	0.16	0.87 (0.74-1.02)	0.088	1.13 (0.67-1.88)	0.65	1.2×10^{-5}	94.8
rs242676	18	58754937	*MC4R-CDH20*	A/G	0.72	0.65	1.36 (1.19-1.55)	3.9×10^{-6}	0.70	0.69	1.01 (0.89-1.14)	0.88	1.17 (0.88-1.57)	0.29	1.3×10^{-3}	90.4

Chr., chromosome; Freq., frequency of A1; OR, odds ratio; CI, confidence interval.

*dbSNP rs number. SNPs at genome-wide significance levels are marked with "**" and a suggestive SNP is marked with "***"

[†]SNP positions are based on NCBI human genome reference sequence Build 37.4.

[‡]A1 is a risk-associated allele and A2 is a non-risk-associated allele.

[§]945 gout cases and 1213 controls.

[‖]1048 gout cases and 1334 controls.

[††]The inverse-variance fixed-effects model meta-analysis was used for estimating summary OR. If heterogeneity was present by the statistical test ($p_{het} < 0.05$) or measure ($I^2 > 50\%$), we performed DerSimonian and Laird random-effects model meta-analysis.

[‡‡]p values smaller than 3.1×10^{-3} (adjusting for 16 tests with Bonferroni correction) in replication study are shown in bold letters. p values smaller than 5.0×10^{-8} in meta-analyses are shown in bold letters.

Supplementary Table S5 Power of the present study design

	Minor allele frequency of control		
Odds ratio	0.2	0.3	0.4
2.0	99.5%	100.0%	100.0%
1.8	92.7%	98.2%	99.0%
1.6	57.1%	76.9%	83.0%
1.4	8.7%	18.6%	24.5%

The significance thresholds are calculated to be $\alpha = 1.0 \times 10^{-5}$ and 3.1×10^{-3} (= 0.05/16) for GWAS and replication stages, respectively.

Supplementary Table S6A Imputation analysis near *MYL2-CUX2* locus

SNP*	Chr.	Position (bp)†	Gene	p Value	r²‡
rs12227162	12	111367244	*MYL2-CUX2*	4.4×10^{-14}	0.92
rs149607519	12	111389437	*MYL2-CUX2*	6.3×10^{-14}	0.99
rs17550549	12	111357471	*MYL2*	6.4×10^{-14}	0.61
rs10849917	12	111357074	*MYL2*	6.8×10^{-14}	0.60
rs2188380	12	111386127	*MYL2-CUX2*	7.2×10^{-14}	-
rs7488411	12	111356598	*MYL2*	7.2×10^{-14}	0.60
rs12311834	12	111354700	*MYL2*	7.7×10^{-14}	0.56
rs10849916	12	111355206	*MYL2*	7.7×10^{-14}	0.58
rs3782888	12	111355606	*MYL2*	8.3×10^{-14}	0.60
rs117607209	12	111354703	*MYL2*	8.9×10^{-14}	0.59
rs61943010	12	111354623	*MYL2*	1.1×10^{-13}	0.60
rs11065773	12	111355100	*MYL2*	1.1×10^{-13}	0.60
rs61943000	12	111354106	*MYL2*	1.3×10^{-13}	0.60
rs61942999	12	111353762	*MYL2*	1.3×10^{-13}	0.60
rs2301610	12	111353556	*MYL2*	1.3×10^{-13}	0.60
rs11065747	12	111326666	*CCDC63*	1.3×10^{-13}	0.56
rs2078847	12	111352478	*MYL2*	1.3×10^{-13}	0.60
rs11065770	12	111351937	*MYL2*	1.3×10^{-13}	0.60
rs916164	12	111351599	*MYL2*	1.4×10^{-13}	0.60
rs11065769	12	111351439	*MYL2*	1.4×10^{-13}	0.60
rs2071629	12	111351186	*MYL2*	1.4×10^{-13}	0.60
rs3858704	12	111705893	*CUX2*	3.9×10^{-13}	0.50
rs4766566	12	111706877	*CUX2*	4.0×10^{-13}	0.50
rs11065746	12	111326655	*CCDC63*	5.1×10^{-13}	0.60
rs3825389	12	111350771	*MYL2*	5.4×10^{-13}	0.60
rs3782889	12	111350655	*MYL2*	5.5×10^{-13}	0.60
rs3782890	12	111350531	*MYL2*	5.5×10^{-13}	0.60
rs3782891	12	111350457	*MYL2*	5.6×10^{-13}	0.60
rs11065766	12	111349849	*MYL2*	5.6×10^{-13}	0.60
rs12231049	12	111349223	*MYL2*	5.8×10^{-13}	0.60
rs117139109	12	111346382	*CCDC63*	5.9×10^{-13}	0.59
rs11065750	12	111331016	*CCDC63*	7.2×10^{-13}	0.60
rs11065749	12	111329227	*CCDC63*	7.2×10^{-13}	0.60
rs10774609	12	111325265	*CCDC63*	9.3×10^{-13}	0.59
rs11065751	12	111331156	*CCDC63*	1.0×10^{-12}	0.60
rs10849914	12	111325437	*CCDC63*	1.4×10^{-12}	0.59
rs78900292	12	111341921	*CCDC63*	1.5×10^{-12}	0.59

rs117528052	12	111341920	*CCDC63*	1.5×10^{-12}	0.59
rs11065752	12	111331165	*CCDC63*	1.6×10^{-12}	0.60
rs11065753	12	111331685	*CCDC63*	1.6×10^{-12}	0.60
rs7312231	12	111333224	*CCDC63*	1.6×10^{-12}	0.60
rs10849915	12	111333622	*CCDC63*	1.6×10^{-12}	0.60
rs1858881	12	111334117	*CCDC63*	1.7×10^{-12}	0.60
rs6489821	12	111334318	*CCDC63*	1.7×10^{-12}	0.60
rs2339598	12	111341925	*CCDC63*	1.8×10^{-12}	0.58
rs11065758	12	111341026	*CCDC63*	1.8×10^{-12}	0.58
rs6489822	12	111334375	*CCDC63*	1.8×10^{-12}	0.60
rs35856328	12	111335360	*CCDC63*	1.8×10^{-12}	0.60
rs11065764	12	111343443	*CCDC63*	1.8×10^{-12}	0.60
rs11065763	12	111343000	*CCDC63*	1.9×10^{-12}	0.60
rs11065762	12	111342722	*CCDC63*	1.9×10^{-12}	0.60
rs2283353	12	111342017	*CCDC63*	1.9×10^{-12}	0.60
rs146280669	12	111335756	*CCDC63*	2.0×10^{-12}	0.60
rs7303257	12	111324923	*CCDC63*	2.1×10^{-12}	0.60
rs11610779	12	111336560	*CCDC63*	2.2×10^{-12}	0.60
rs7305904	12	111337891	*CCDC63*	2.3×10^{-12}	0.60
rs11065756	12	111338794	*CCDC63*	2.4×10^{-12}	0.60
rs11065757	12	111340788	*CCDC63*	2.4×10^{-12}	0.60
rs10774610	12	111340243	*CCDC63*	2.5×10^{-12}	0.60
rs2339717	12	111696528	*CUX2*	2.9×10^{-12}	0.48
rs6490029	12	111698457	*CUX2*	3.1×10^{-12}	0.48
rs916682	12	111699146	*CUX2*	5.1×10^{-12}	0.49
rs11065836	12	111671076	*CUX2*	4.4×10^{-9}	0.23
rs2106410	12	111631946	*CUX2*	1.4×10^{-7}	0.19
rs4766552	12	111631765	*CUX2*	1.4×10^{-7}	0.25
rs4766553	12	111634281	*CUX2*	5.5×10^{-7}	0.19
rs56119815	12	111665521	*CUX2*	1.4×10^{-6}	0.14
rs3809286	12	111664404	*CUX2*	1.7×10^{-6}	0.11
rs4766558	12	111664061	*CUX2*	1.9×10^{-6}	0.11
rs7969686	12	111665388	*CUX2*	2.4×10^{-6}	0.11
rs7962233	12	111655513	*CUX2*	2.7×10^{-6}	0.14
rs10774615	12	111659096	*CUX2*	2.8×10^{-6}	0.11
rs4766556	12	111659074	*CUX2*	2.9×10^{-6}	0.11
rs3825398	12	111651949	*CUX2*	2.9×10^{-6}	0.12
rs10083213	12	111654363	*CUX2*	2.9×10^{-6}	0.11
rs11065832	12	111662984	*CUX2*	3.5×10^{-6}	0.11

rs10082818	12	111663177	*CUX2*	3.5×10^{-6}	0.11
rs7971185	12	111660599	*CUX2*	3.8×10^{-6}	0.13
rs2339715	12	111657745	*CUX2*	4.2×10^{-6}	0.13
rs4766559	12	111666984	*CUX2*	4.6×10^{-6}	0.15
rs3809288	12	111652522	*CUX2*	4.8×10^{-6}	0.11
rs10744769	12	111650780	*CUX2*	5.5×10^{-6}	0.11
rs2339716	12	111680885	*CUX2*	6.5×10^{-6}	0.12
rs3925618	12	111649686	*CUX2*	7.0×10^{-6}	0.14
rs1034603	12	111649344	*CUX2*	7.3×10^{-6}	0.14
rs1034602	12	111649179	*CUX2*	7.4×10^{-6}	0.14
rs4766557	12	111664044	*CUX2*	8.4×10^{-6}	0.12
rs10774614	12	111650339	*CUX2*	8.5×10^{-6}	0.13
rs6490037	12	111713671	*CUX2*	9.9×10^{-6}	0.17

Chr., chromosome.

*dbSNP rs number.

†SNP positions are based on NCBI human genome reference sequence Build 37.4.

‡r^2 indicates the pairwise linkage disequilibrium with rs2188380.

Supplementary Table S6B Imputation analysis near *GCKR* locus

SNP*	Chr.	Position (bp)†	Gene	p Value	r^{2}‡
rs1260326	2	27730940	*GCKR*	1.7×10^{-7}	-
rs814295	2	27743215	*GCKR*	1.1×10^{-6}	0.48
rs6744393	2	27750139	*GCKR*	1.1×10^{-6}	0.48
rs74928908	2	27744089	*GCKR*	1.2×10^{-6}	0.47
rs780092	2	27743154	*GCKR*	1.2×10^{-6}	0.47
rs78680773	2	27751562	*GCKR*	1.2×10^{-6}	0.45
rs3817588	2	27731212	*GCKR*	1.4×10^{-6}	0.50
rs780093	2	27742603	*GCKR*	2.1×10^{-6}	0.88
rs1313566	2	27748904	*GCKR*	2.3×10^{-6}	0.88
rs182700961	2	27781772	*GCKR-C2orf16*	3.2×10^{-6}	0.22
rs780094	2	27741237	*GCKR*	3.2×10^{-6}	0.92
rs6547692	2	27734972	*GCKR*	3.5×10^{-6}	0.93
rs62131879	2	27755112	*GCKR*	3.5×10^{-6}	0.23
rs10181342	2	27757343	*GCKR*	3.6×10^{-6}	0.23
rs116443177	2	27764122	*GCKR*	3.6×10^{-6}	0.23
rs62131881	2	27764781	*GCKR*	3.7×10^{-6}	0.23
rs62131882	2	27765480	*GCKR*	3.7×10^{-6}	0.23
rs62141278	2	27768381	*GCKR*	3.8×10^{-6}	0.23
rs780096	2	27741072	*GCKR*	3.8×10^{-6}	0.92

rs780095	2	27741105	*GCKR*	3.8×10^{-6}	0.92
rs62141279	2	27771415	*GCKR-C2orf16*	3.9×10^{-6}	0.24
rs62141281	2	27777651	*GCKR-C2orf16*	4.1×10^{-6}	0.23
rs2384627	2	27774449	*GCKR-C2orf16*	4.2×10^{-6}	0.23
rs6751087	2	27778016	*GCKR-C2orf16*	4.2×10^{-6}	0.23
rs1881396	2	27844601	*ZNF512*	4.8×10^{-6}	0.24
rs13405762	2	27834615	*ZNF512*	5.3×10^{-6}	0.23
rs78170284	2	27835082	*ZNF512*	5.4×10^{-6}	0.23
rs1881395	2	27838549	*ZNF512*	5.4×10^{-6}	0.23
rs7571558	2	27839832	*ZNF512*	5.4×10^{-6}	0.23
rs62138971	2	27840754	*ZNF512*	5.4×10^{-6}	0.23
rs6547735	2	27831607	*ZNF512*	5.7×10^{-6}	0.23
rs6719960	2	27830067	*ZNF512*	5.7×10^{-6}	0.23
rs2141372	2	27827196	*ZNF512*	5.7×10^{-6}	0.23
rs62138968	2	27824485	*ZNF512*	5.7×10^{-6}	0.23
rs56725354	2	27824168	*ZNF512*	5.7×10^{-6}	0.23
rs1919129	2	27818721	*ZNF512*	5.8×10^{-6}	0.23
rs62138966	2	27819172	*ZNF512*	5.8×10^{-6}	0.23
rs62138965	2	27816593	*ZNF512*	5.8×10^{-6}	0.23
rs76013440	2	27816315	*ZNF512*	5.8×10^{-6}	0.23
rs1881397	2	27832285	*ZNF512*	5.9×10^{-6}	0.23
rs60854669	2	27786929	*GCKR-C2orf16*	6.1×10^{-6}	0.23
rs965813	2	27789861	*GCKR-C2orf16*	6.2×10^{-6}	0.23
rs59876138	2	27792323	*GCKR-C2orf16*	6.2×10^{-6}	0.23
rs6704596	2	27796927	*GCKR-C2orf16*	6.3×10^{-6}	0.23
rs62138964	2	27814438	*ZNF512*	6.3×10^{-6}	0.23
rs62138963	2	27814374	*ZNF512*	6.3×10^{-6}	0.23
rs3811644	2	27802805	*C2orf16*	6.3×10^{-6}	0.23
rs6734059	2	27808154	*ZNF512*	6.5×10^{-6}	0.23
rs62141291	2	27784034	*GCKR-C2orf16*	6.6×10^{-6}	0.23
rs62141292	2	27784300	*GCKR-C2orf16*	6.6×10^{-6}	0.23
rs62141290	2	27783392	*GCKR-C2orf16*	9.2×10^{-6}	0.22

Chr., chromosome.

*dbSNP rs number.

†SNP positions are based on NCBI human genome reference sequence Build 37.4.

‡r^2 indicates the pairwise linkage disequilibrium with rs1260326.

Supplementary Table S6C Imputation analysis near *CNIH-2* locus

SNP*	Chr.	Position (bp)†	Gene	p Value	$r^{2‡}$
rs524859	11	66041079	*RAB1B*	3.9×10^{-6}	1.00
rs4073582	11	66050712	*CNIH-2*	4.8×10^{-6}	-
rs801742	11	65914766	*PACS1*	6.9×10^{-6}	0.96

Chr., chromosome.

*dbSNP rs number.

†SNP positions are based on NCBI human genome reference sequence Build 37.4.

‡r^2 indicates the pairwise linkage disequilibrium with rs4073582.

Supplementary Table S6D Imputation analysis near *MAP3K11* locus

SNP*	Chr.	Position (bp)†	Gene	p Value	$r^{2‡}$
rs11227230	11	65356674	*EHBP1L1*	1.2×10^{-6}	0.96
rs1151503	11	65357697	*EHBP1L1*	1.3×10^{-6}	0.96
rs4326800	11	65367253	*MAP3K11*	1.9×10^{-6}	1.00
rs10791821	11	65368323	*MAP3K11*	1.9×10^{-6}	-
rs34278912	11	65360243	*KCNK7*	2.0×10^{-6}	0.91

Chr., chromosome.

*dbSNP rs number.

†SNP positions are based on NCBI human genome reference sequence Build 37.4.

‡r^2 indicates the pairwise linkage disequilibrium with rs10791821.

Supplementary Table S7A Univariate and multivariate logistic regression including three SNPs of *ABCG2*

	Univariate		Multivariate	
SNP*	OR (95% CI)	p Value†	OR (95% CI)	p Value†
rs2728125	2.06 (1.84 - 2.30)	2.3×10^{-36}	0.85 (0.68 - 1.08)	0.19
rs72552713	2.18 (1.63 - 2.92)	1.4×10^{-7}	2.95 (2.18 - 3.98)	1.9×10^{-12}
rs2231142	2.35 (2.11 - 2.63)	2.9×10^{-51}	2.83 (2.24 - 3.57)	1.8×10^{-18}

OR, odds ratio; CI, confidence interval.

*dbSNP rs number.

†We analyzed 1993 cases and 1334 controls whose genotype data for rs72552713 and rs223114 were available.

Supplementary Table S7B Association analysis of gout with *ABCG2* dysfunctional missense variants

					Freq.			
SNP*	Chr.	Position (bp)†	Amino Acid change	A1/A2‡	Cases	Controls	OR (95% CI)[d]	p Value§
rs72552713	4	89052957	Gln126Ter	T/C	0.049	0.023	2.86 (2.12 - 3.85)	4.4×10^{-12}
rs2231142	4	89052323	Gln141Lys	A/C	0.46	0.27	2.47 (2.20 - 2.76)	1.6×10^{-55}

Chr., chromosome; Freq., frequency of A1; OR, odds ratio; CI, confidence interval.

*dbSNP rs number.

†SNP positions are based on NCBI human genome reference sequence Build 37.4.

‡A1 is a risk-associated allele and A2 is a non-risk-associated allele.

§We analyzed 1993 cases and 1334 controls whose genotype data for these two SNPs were available. Logistic regression analyses were performed using a multivariate model including two SNPs, which located on different haplotypes.

Supplementary Table S8 Magnitude of gout risk associated with the number of risk alleles

Risk allele No.	Cases	Controls	OR (95% CI)[a]	p Value*
4 or less	45	134	Reference	
5	148	246	1.79 (1.21 - 2.66)	3.5×10^{-3}
6	377	355	3.16 (2.19 - 4.57)	2.3×10^{-10}
7	548	320	5.10 (3.54 - 7.34)	9.7×10^{-21}
8	507	149	10.1 (6.90 - 14.9)	5.3×10^{-39}
9 or more	265	46	18.6 (11.8 - 29.2)	3.6×10^{-45}

OR, odds ratio; CI, confidence interval.

*We analyzed 1937 cases and 1254 controls whose genotype data for the following six SNPs were available: rs3775948 of *SLC2A9*, rs2188380 of *MYL2-CUX2*, rs1260326 of *GCKR*, rs4073582 of *CNIH-2,* rs72552713 and rs2231142 of *ABCG2*.

Supplementary Table S9 Association analysis between seven SNPs and three uric acid-related parameters

		SUA[†]				FE_{UA}[‡]				UUE[‡]			
			95% CI				95% CI				95% CI		
SNP*	Gene	Coef.	LL	UL	p Value[§]	Coef.	LL	UL	p Value[§]	Coef.	LL	UL	p Value[§]
rs3775948	*SLC2A9*	0.166	0.076	0.256	**3.1×10^{-4}**	-0.260	-0.368	-0.152	**2.6×10^{-6}**	-0.809	-1.482	-0.136	**0.018**
rs2188380	*MYL2-CUX2*	0.074	-0.048	0.196	0.23	-0.141	-0.288	0.006	0.06	0.480	-0.437	1.396	0.30
rs1260326	*GCKR*	0.063	-0.019	0.146	0.13	-0.064	-0.164	0.034	0.20	0.127	-0.490	0.745	0.69
rs4073582	*CNIH-2*	-0.008	-0.187	0.171	0.93	-0.030	-0.245	0.184	0.78	0.224	-1.115	1.562	0.74
rs10791821	*MAP3K11*	0.032	-0.145	0.208	0.72	-0.076	-0.288	0.135	0.48	-0.792	-2.109	0.525	0.24
rs72552713	*ABCG2*	0.402	0.210	0.594	**4.3×10^{-5}**	0.348	0.116	0.579	**3.3×10^{-3}**	2.780	1.338	4.222	**1.6×10^{-4}**
rs2231142	*ABCG2*	0.183	0.098	0.268	**2.6×10^{-5}**	0.244	0.142	0.346	**3.2×10^{-6}**	1.862	1.224	2.500	**1.2×10^{-8}**

SUA, serum uric acid (unit: mg/dl); FE_{UA}, fractional excretion of urate clearance (unit: %); UUE, urinary urate excretion (unit: mg/hr/1.73m^2); Coef., regression coefficient; CI, confidence interval; LL, lower limit; UL, upper limit. We performed multivariate linear regression analyses, in which all 7 SNPs, alcohol drinking and BMI were included in the model.

*dbSNP rs number.

[†]Among 1657 gout patients with records of SUA, 1616 patients with complete information on all the explanatory variables were analyzed.

[‡]Among 1613 gout patients with records of FE_{UA} and UUE, 1574 patients with complete information on all the explanatory variables were analyzed.

[§]p values smaller than 0.05 are shown in bold letters.

Supplementary Table S10 Linkage disequilibrium among rs4073582 of *CNIH-2*, rs10791821 of *MAP3K11*, and rs504915 of *NRXN2*

		r^2		
		rs4073582 (*CNIH-2*)	rs10791821 (*MAP3K11*)	rs504915 (*NRXN2*)
D'	rs4073582 (*CNIH-2*)		0.010	0.006
	rs10791821 (*MAP3K11*)	0.124		0.189
	rs504915 (*NRXN2*)	0.728	0.649	

Supplementary Table S11 Multivariate logistic regression analysis adjusted with rs504915 of *NRXN2*

		Univariate		Multivariate†	
SNP*	Gene	p Value	OR (95% CI)	p Value	OR (95% CI)
rs4073582	*CNIH-2*	1.6×10^{-4}	1.55 (1.23-1.96)	3.3×10^{-4}	1.55 (1.22-1.96)
rs10791821	*MAP3K11*	3.4×10^{-3}	1.41 (1.12-1.77)	2.4×10^{-3}	1.44 (1.14-1.82)

OR, odds ratio; CI, confidence interval.

*dbSNP rs number.

†We performed multivariate logistic regression analyses using replication stage samples. These data were adjusted with rs504915 of *NRXN2*.

Supplementary Methods

Genotyping and quality control

At GWAS stage, the data sets were filtered individually on the basis of single nucleotide polymorphism (SNP) genotype missing call rates (>1%), and the Hardy-Weinberg equilibrium (HWE) in controls ($p<1.0\times10^{-6}$). We confirmed that all the subjects showed high genotype call rates (>98%). Pairwise identity by state was evaluated in order to identify pairs of individuals with cryptic relatedness. We confirmed that there was no pair showing cryptic relatedness greater than expected for second-degree relatives. We performed principal component analysis including our GWAS data set together with HapMap phase II samples.[1 2] As a result, we excluded one gout patient as a population outlier who was presumed to be of mixed origin (East Asian and European) (supplementary figure S2). Finally, 570 442 SNPs passed filters for 945 cases and 1213 controls.

One hundred twenty-three SNPs passing the significance threshold at $p<1.0\times10^{-5}$ in the GWAS stage were used for the subsequent analyses. For closely located SNPs, we detected top-ranked SNPs and then we examined the pairwise linkage disequilibrium (LD) between SNP showing the most significant association and other SNPs. For each novel risk locus, we confirmed that all of the SNPs showing associations at $p<1.0\times10^{-5}$ were in moderate to strong LD ($r^2>0.3$) with SNP showing the most significant association. Furthermore, these SNPs were no longer significant at $p<1.0\times10^{-5}$ after adjusting for the most significantly associated SNP in each locus by the logistic regression analyses. Finally, we selected 16 SNPs for replication study. We genotyped these SNPs and confirmed that all the 16 SNPs were of high call rate (>98%) and did not show significant deviation from HWE in controls ($p>0.05$).

Statistical analyses for GWAS

We conducted an association analysis using a 2×2 contingency table based on the allele frequency. For each of the filtered SNPs, p value of association was assessed by χ^2 test, and the odds ratio (OR) and 95% confidence interval (95% CI) were calculated. The quantile-quantile plot and the genomic inflation factor (λ) were used to assess the presence of systematic bias in the test statistics due to potential population stratification. After excluding SNPs within 500 kb from the SNPs reaching a genome-wide significance threshold ($p<5.0\times10^{-8}$), the λ was 1.054, indicating a subtle inflation of p values (supplementary figure S3).

Imputation

For the imputation, the 1000 genomes reference panel (the East Asian population) was obtained from the phase 1 release (16 March 2012, http://www.1000genomes.org/announcements/updated-integrated-phase-1-release-calls-2012-03-16), and we ran a logistic regression analysis based on imputation dosages via MACH2DAT.[3] We included all SNPs with estimated $r^2>0.9$ and minor allele frequency ≥ 0.01 for analysis.

Analysis of the two dysfunctional SNPs of *ABCG2*

The genotyping of the two *ABCG2* SNPs (rs72552713 and rs2231142) was performed with an allelic discrimination assay (Custom TaqMan Assay, Applied Biosystems) with a LightCycler 480 (Roche

Diagnostics).[4] All the logistic regression analyses were performed with a software SPSS v.22.0J (IBM Japan Inc.). A software R (version 3.0.2) (http://www.r-project.org/) with GenABEL software package was used for LD analysis.

Estimation of variance explained by identified SNPs

For each variant identified in our GWAS, we calculated percent of the variance explained. We used the liability threshold model in quantitative genetics.[5] In this model, it is assumed that liability of a binary disease trait on unobserved continuous scale is assumed to be normally distributed with a mean of zero and variance of one, and individuals whose liabilities surpass a threshold develop the disease. To calculate the percent variance explained, we assumed that the prevalence of gout was 1.1% based on the estimation in Japanese male population.[4 6] We assumed that SNP effects were additive on the logistic scale. Under the rare disease assumption, we approximated the relative risk by the odds ratio obtained in this study. For the identified SNPs, we used the allele frequencies in the East Asian population in the 1000 Genomes Project.[7]

Subtype analysis

We investigated the magnitude of associations between the identified SNPs and the types of gout by examining type-specific ORs and the case-subtype heterogeneity test. Fractional excretion of urate clearance (FE_{UA}) and urinary urate excretion (UUE) were measured for each patient as described previously,[8] and all cases were classified based on the criteria (supplementary figure S1). To estimate gout type-specific ORs, the frequency of a SNP in each type was compared with the frequency in controls using a logistic regression. To assess whether the estimated type-specific ORs were significantly different, the frequencies of the SNP were compared between types by dichotomous logistic regression (the case-subtype heterogeneity test).[9] For these subtype analyses, the effects of alcohol drinking, body mass index (BMI), and all the identified SNPs were considered in the model. When evaluating the effects of risk allele of SNP on clinical parameters (FE_{UA} and UUE), a linear regression analysis was performed by defining the SNP genotype predictor variable x as the number of risk alleles associated with gout risk. All the logistic and linear regression analyses were performed using the STATA version 11.0.

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