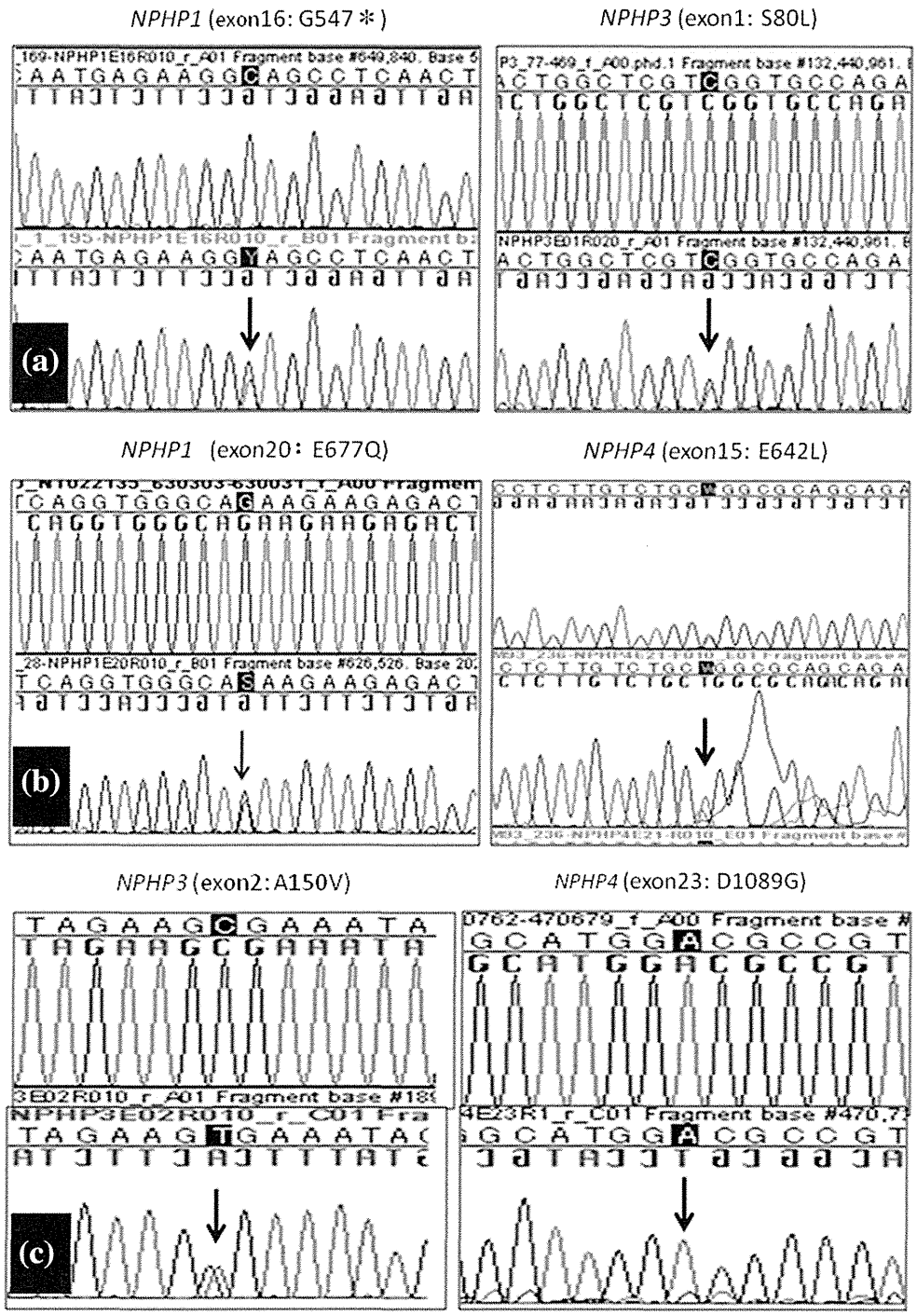

Fig. 4 Compound heterozygotes with heterozygous mutations in different *NPHP* genes. In **a**, a compound heterozygote has one heterozygous mutation involving each of *NPHP1* (G547*) and *NPHP3* (S80L). In **b**, a compound heterozygote has one heterozygous mutation involving each of *NPHP1* (E677Q) and *NPHP4* (E642L). In **c**, a compound heterozygote has one heterozygous mutation involving each of *NPHP3* (A150 V) and *NPHP4* (D1089G)

and the extracellular matrix, intercellular adhesion, cytoskeletal integrity, cell polarity, primary cilia function, and intracellular signal transmission to the nucleus. Structural and functional disorders involving the renal tubular epithelium result.

An *NPHP* gene mutation was detected in about 54 % of all patients, but no mutation was noted within the sequences analyzed in the other 46 %. However, nephronophthisis was suspected clinically and histologically, suggesting possible mutation in some other *NPHP* gene. An *NPHP1* mutation was most frequent among our Japanese patients, most often representing a large deletion rather than a point mutation. Frequency of an *NPHP1* mutation was similar to that reported in Western populations [10].

On the other hand, mutation in the gene responsible for the infantile type, *NPHP2*, a patient in a compound heterozygous stable with another abnormal *NPHP* gene such as that responsible for NPH3 recently has been

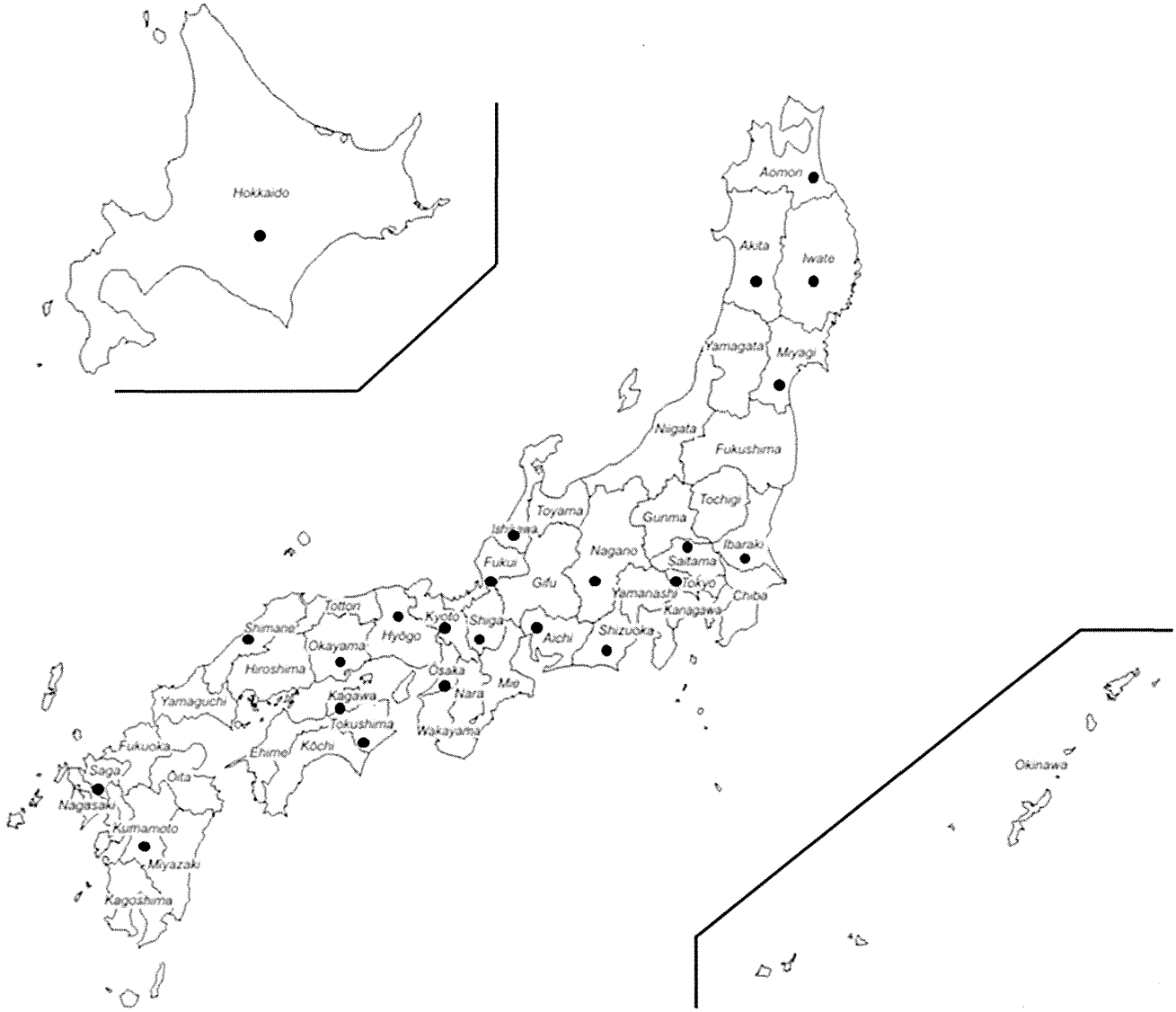

Fig. 5 Demographic features of patients in Japan. Regional distribution of study subjects within Japan, show as a *dot* for each patient

reported [11, 12]. We also found compound heterozygosity across multiple *NPHP* genes in some of our Japanese nephronophthisis patients. In *NPHP4*, L939* (IVS-20T > A) was detected in two geographically distant patients who were not consanguineous. The mutation formed a stop codon by substituting TAG for TTG in exon 21, terminating peptide synthesis. This might prove to be a 'hot spot' among Japanese patients.

We detected in three patients with two mutations in either *NPHP1*, *NPHP3*, or *NPHP4* in this study. As similar to the results of the other studies [13, 14], the age of the initial discovery of this disease and the course of progression to end-stage renal disease were not significantly different from those of the patients having mutation in single *NPHP* gene. An analysis of patient backgrounds revealed that NPH was distributed fairly evenly Japan, including the suspected cases where no causative mutation was identified. Heterozygotes carrying NPHP gene mutations also were rather evenly distributed nationwide. No gender difference was evident from our analysis. Although the median age at time of disease discovery was 12.5 years, individual presentation ranged from infantry to adulthood.

Frequency of disease discovery in mass screening programs, such as school urine tests, was low, as previously reported [15]. Incidental discovery of renal dysfunction during diagnostic workup of possibly unrelated symptoms, or during routine check-ups, accounted for less than 50 % of cases. Often symptoms that led to the discovery of NPH represented extrarenal manifestations such as incomplete physical development reported previously [15]. In particular, currently used urine test strips, intended mainly to detect albuminuria, are insensitive to this disease.

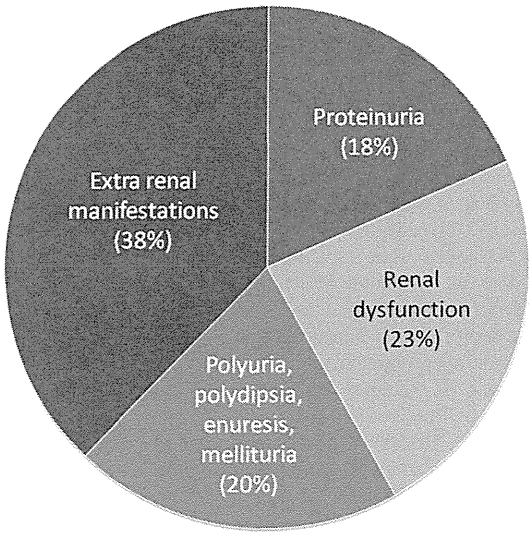

Fig. 6 Clinical suspicion and motivation to discover for NPH. Proteinuria is detected in a urine test at school (18 %), renal dysfunction discovered incidentally (23 %), urinary tract symptoms such as polyuria with or without polydipsia, enuresis, or mellituria (approximately 20 %). Some 38 % were discovered because of either extrarenal manifestations such as lagging physical development, dwarfism, anemia, pallor, hypertension, or visual disturbance arising from pigmentary retinal degeneration

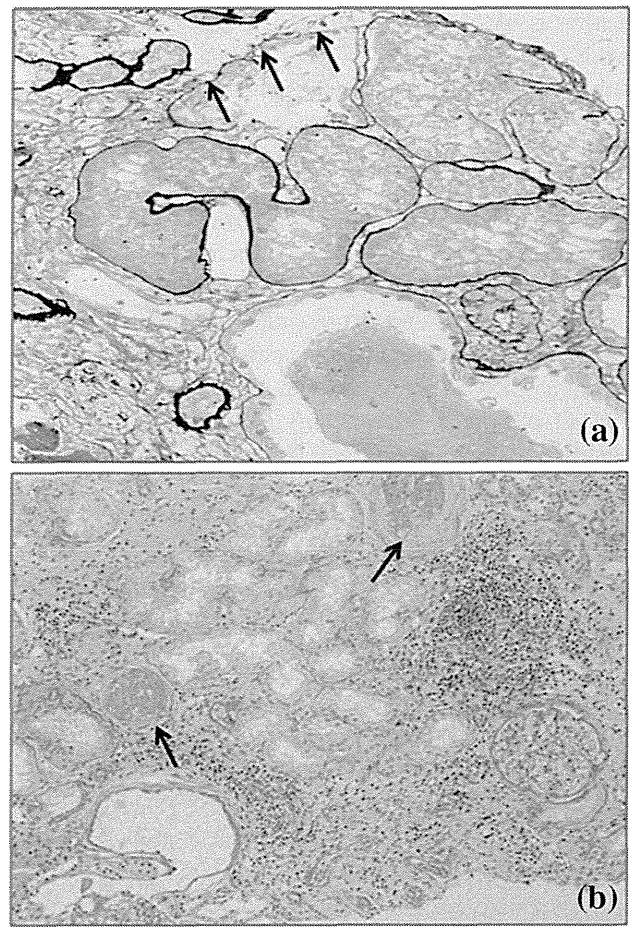

Fig. 7 Pathologic findings in the kidney in nephronophthisis patients. In **a** irregularity (*arrow*) of the renal tubular basement membrane was evident (methenamine silver stain, ×200). In **b**, Inflammatory cell infiltration involved the renal tubular interstitium, and sclerotic glomeruli (*arrow*) were present (periodic acid-Schiff stain, ×100)

Development in siblings was noted in three families, suggesting autosomal recessive inheritance. However, many cases appear to be sporadic. Familial genetic analysis centering on patients, parents is needed.

In contract to albuminuria, urinary findings such as low specific gravity and low-molecular-weight proteinuria are relatively helpful in early discovery. According to the results of this study, we suggest that the findings of the low-molecular weight proteinuria and hypotonic urine reflecting renal tubular disorder coupled with the histologic abnormalities involving cystic dilation of renal tubules and the irregularity of tubular basement membrane could be a convincing diagnostic criterion of this disease. Extrarenal manifestations, such as short stature, delayed physical development, and anemia also were frequent. Unfortunately, these tended to coincide with were progression of renal dysfunction rather than early NPH. Nonetheless, NPH needs to be considered in children with such presentations. Some patients have been reported to show somewhat distinctive extrarenal manifestations [13] such as pigmentary retinal degeneration (Senior-Loken syndrome), ocular dysmetria (Cogan's syndrome), cerebral ataxia, hepatic fibrosis, and skeletal and facial abnormalities [13, 16, 17]. Even the most frequent of these extrarenal manifestations, pigmentary retinal degeneration, was present only in some patients and not in others, even among children showing the same *NPHP1* deletion. Similar lesions also have been reported in Jeune, Joubert, oro-facial-digital (OFD1), and Meckel syndromes [13, 18, 19]. *NPHP1* mRNA is expressed predominantly in a wide range of extrarenal tissues including pituitary gland, spine, testis, lymph nodes, and thyroid [14]. Expression also is high in the central nervous system, which could account for associated cerebellar ataxia. However, associated symptoms may develop in organs with low *NPHP1* expression, such as hepatic fibrosis. The role of nephrocystin in extrarenal manifestations remains poorly understood. The 11 kb interval between the 3′ end of *NPHP1* and an inverted repeat containing the distal deletion breakpoint was found to contain the first exon of a second gene, *MALL* [20]. Although the detail of the *MALL* gene function has not been clarified, recent report suggested the involvement of the age-related macular degeneration (AMD) [21]. Interestingly, associations have also been reported between AMD and chronic kidney disease [22]. Since pigmentary retinal degeneration is the most common extrarenal

manifestation of NPH, similar to AMD, *MALL* gene may involve the pathogenesis of this eye disorder found in NPH patients as the contiguous gene syndrome.

No truly effective treatment currently is available for NPH. Dietary therapy and administration of ion exchange resins and bicarbonate are carried out to manage hyponatremia, hyperkalemia, or metabolic acidosis. Studies possibly relevant to drug therapy have been conducted in various animals, even protozoa [23, 24]. Previous studies reported that renal cyst expression was inhibited by stimulating the G-protein-coupled calcium sensing receptor and elevating Ca^{2+} and cAMP in the renal tubular epithelial cells of pcy mice. Morphology and function of cilia in zebrafish with ciliopathy may be improved by the administration of rapamycin and rescovitine [25, 26]; however, applicability to human NPHP is unknown. Living-donor kidney transplantation was found to have favorable outcome in many reports including the North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS) [27].

Acknowledgments This study was performed after approval by the Ethics Committee of Kinki University Faculty of Medicine. Written informed consent was obtained from the patient's guardian for genetic examination. We thank Ai Itoh for technical support in tissue staining and manuscript preparation.

Compliance with ethical standards

Conflicts of interest This study was partly supported by a Grant-in-Aid for Scientific Research from Morinaga Hoshikai to Tsukasa Takemura (2013–2014) and from Ministry of Health, Labour and Welfare Japan (grant number: 26070201, Representative investigator: Kazumoto Iijima, Pediatrics, Kobe University School of Medicine). The authors declare that they have no competing interests involving this work.

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The effects of *URAT1/SLC22A12* nonfunctional variants, R90H and W258X, on serum uric acid levels and gout/hyperuricemia progression

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Urate transporter 1 (*URAT1/SLC22A12*), a urate transporter gene, is a causative gene for renal hypouricemia type 1. Among several reported nonsynonymous *URAT1* variants, R90H (rs121907896) and W258X (rs121907892) are frequent causative mutations for renal hypouricemia. However, no case-control study has evaluated the relationship between gout and these two variants. Additionally, the effect size of these two variants on serum uric acid (SUA) levels remains to be clarified. Here, 1,993 primary gout patients and 4,902 health examination participants (3,305 males and 1,597 females) were genotyped with R90H and W258X. These *URAT1* variants were not observed in any gout cases, while 174 subjects had the *URAT1* variant in 2,499 health examination participants, respectively ($P = 8.3 \times 10^{-46}$). Moreover, in 4,902 health examination participants, the *URAT1* nonfunctional variants significantly reduce the risk of hyperuricemia ($P = 6.7 \times 10^{-19}$; risk ratio = 0.036 in males). Males, having 1 or 2 nonfunctional variants of *URAT1*, show a marked decrease of 2.19 or 5.42 mg/dl SUA, respectively. Similarly, females, having 1 or 2 nonfunctional variants, also evidence a decrease of 1.08 or 3.89 mg/dl SUA, respectively. We show that *URAT1* nonfunctional variants are protective genetic factors for gout/hyperuricemia, and also demonstrated the sex-dependent effect size of these *URAT1* variants on SUA (P for interaction $= 1.5 \times 10^{-12}$).

Gout (MIM 138900) is one of the most common types of inflammatory arthritis as a consequence of hyperuricemia. Gout and hyperuricemia increase the risk of other common diseases, such as kidney diseases, cerebrovascular diseases, hypertension and cardiovascular diseases[1]. Several transporter genes associated with gout and serum uric acid (SUA) levels were previously reported, such as ATP-binding cassette transporter, subfamily G,

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	Number	R90H			W258X			Number of *URAT1* nonfunctional alleles*			
		G/G	G/A	A/A	G/G	G/A	A/A	0	1	2	*P* value†
Gout	1,993	1,993	0	0	1,993	0	0	1,993	0	0	
Control	2,499	2,477	22	0	2,347	150	2	2,325	172	2	8.3×10^{-46}

Table 1. Genotype distributions of nonfunctional variants in *URAT1/SLC22A12* in gout patients and controls. *The nonfunctional alleles mean A allele of R90H or W258X. †2 × 3 Fisher's exact test.

		Hyperuricemia	Control*	*P* value†	RR (95% CI)	Reciprocal RR (95% CI)
R90H	G/G	806	2,477			
	G/A	0	22			
	A/A	0	0	4.3×10^{-3}	—	—
W258X	G/G	804	2,347			
	G/A	2	150			
	A/A	0	2	3.3×10^{-16}	0.041 (0.010–0.164)‡	24.5 (6.1–98.7)‡
Number of nonfunctional alleles (R90H or W258X)	0	804	2,325			
	1	2	172			
	2	0	2	6.7×10^{-19}	0.036 (0.009–0.143)§	28.1 (7.0–112.8)§

Table 2. Genotype distributions of *URAT1* nonfunctional variants in 3,305 males and risk ratio for hyperuricemia. 3,305 males (806 hyperuricemia and 2,499 controls) are health examination participants of the J-MICC study. Abbreviation: RR = risk ratio; CI = confidence interval. *Control group is comprised of individuals with serum uric acid levels ≤ 7.0 mg/dl, no gout history and no treatments for gout/hyperuricemia. †3 × 2 Fisher's exact test. ‡Dominant model (G/G versus G/A or A/A). §Dominant model (0 versus 1 or 2).

member 2 (*ABCG2/BCRP* [MIM 603756])[2–6], glucose transporter 9 (*GLUT9/SLC2A9* [MIM 606142])[2,7,8], sodium-dependent phosphate cotransporter type 1 (*NPT1/SLC17A1* [MIM 182308])[9], organic anion transporter 4 (*OAT4/SLC22A11* [MIM 607097])[10,11], and urate transporter 1 (*URAT1/SLC22A12* [MIM 607096])[12,13].

Among them, *URAT1*, which is a well-known urate transporter gene, has been identified as a causative gene for renal hypouricemia type 1 (MIM 220150)[14]. Among several reported nonsynonymous variants in *URAT1*, R90H (rs121907896) and W258X (rs121907892) are frequent causative mutations for renal hypouricemia[15]. Previous *in vitro* functional studies showed that R90H variant diminishes the urate transport activity of URAT1[15] as the other common variant, W258X[14]. It has been also reported that nonfunctional variants in *URAT1* were not detected in 77 Spanish gout patients[16], and W258X in *URAT1* suppressed the development of gout[17]. However, to our knowledge, no large-scale case-control study has evaluated the relationship between gout/hyperuricemia and both variants (R90H and W258X). In this study, therefore, we investigated the association between gout and two *URAT1* variants with large-scale Japanese primary gout cases and controls. Moreover, the risk ratio (RR) of these two nonfunctional variants for hyperuricemia was evaluated in approximately 5,000 Japanese health examination participants. Although there is a gender difference in SUA due to sex hormones[18,19], the effect size of these two variants on SUA in each sex remains to be clarified. Furthermore, these *URAT1* variants (R90H and W258X) are frequently observed especially in a Japanese population; thus, it is particularly important to analyze the sex-dependent effect size of these *URAT1* variants on SUA in a general Japanese population. Therefore, we also evaluated the effect size of these *URAT1* variants on SUA in each sex with a large number of Japanese health examination participants.

Results

Case-control study of gout. The genotyping results of *URAT1* nonfunctional variants (R90H and W258X) for 1,993 gout cases and 2,499 controls were shown in Table 1. The two variants were in Hardy-Weinberg equilibrium ($P > 0.05$). The *URAT1* nonfunctional variants (R90H and W258X) were not observed in any gout cases (n = 1,993), while R90H heterozygotes (G/A), W258X heterozygotes (G/A) and W258X homozygotes (A/A) were observed in 22, 150 and 2 subjects, respectively, among 2,499 control subjects ($P = 8.3 \times 10^{-46}$; Table 1). This result is compatible with previous studies[16,17], and indicates that these *URAT1* variants are protective factors of gout.

Risk ratio for hyperuricemia. Next, Table 2 and Supplementary Table S1 show the genotype distributions of *URAT1* nonfunctional variants in 4,902 health examination participants of the Japan Multi-Institutional Collaborative Cohort (J-MICC) study (3,305 males and 1,597 females). Among the 4,902 participants, the nonfunctional allele frequencies of R90H and W258X were 0.28% and 2.24%, respectively. All of the participants were divided into hyperuricemia (SUA > 7.0 mg/dl) or control (SUA ≤ 7.0 mg/dl).

In 3,305 males (Table 2), there were significant differences between hyperuricemia and control in both R90H ($P = 4.3 \times 10^{-3}$) and W258X genotype distributions ($P = 3.3 \times 10^{-16}$). Additionally, the number of R90H or W258X nonfunctional alleles in each group was calculated. Then, the proportion of nonfunctional alleles

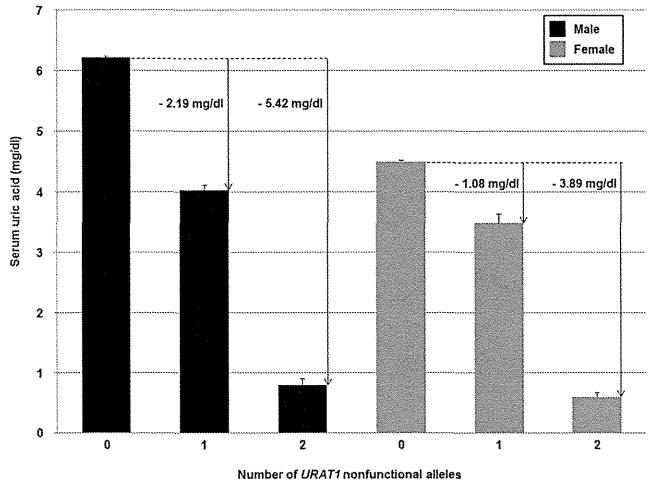

Figure 1. Changes of serum uric acid levels of *URAT1* nonfunctional alleles in health examination participants. 4,753 health examination participants, who received no medication for gout and/or hyperuricemia, were analyzed. Among 3,158 male participants (left, black bars), 0, 1 and 2 nonfunctional alleles (R90H or W258X) were detected in 2,982, 174 and 2 males, respectively. Among 1,595 female participants (right, grey bars), 0, 1 and 2 nonfunctional alleles (R90H or W258X) were detected in 1,529, 63 and 3 females, respectively. Serum uric acid (SUA) levels of participants having 0, 1 and 2 nonfunctional alleles were shown in each sex. The sex-dependent effect size of SUA decrease by nonfunctional alleles (arrow) was also shown. All bars are expressed as means ± SEM.

	Partial regression coefficient	*P* value
b_1	−2.21	8.2×10^{-155}
b_2	−1.72	$<1.0 \times 10^{-323*}$
b_3	1.05	1.5×10^{-12}

Table 3. Multiple regression analysis focused on the interaction between *URAT1* variants and sex. $y = b_0 + b_1x_1 + b_2x_2 + b_3x_1x_2$, where y is SUA level, x_1 is an ordinal variable representing the number of nonfunctional alleles of two *URAT1* variants (R90H and W258X), and x_2 is a dummy variable representing the sex (male = 0 and female = 1). x_1x_2 is an interaction term. *P value was extremely low and the calculation was impossible by the software R.

was more frequent in control than that in hyperuricemia ($P = 6.7 \times 10^{-19}$; RR = 0.036; 95% confidence interval [CI]: 0.009–0.143, Table 2).

In 1,597 females, both R90H and W258X were observed only in controls (Supplementary Table S1). However, because the female hyperuricemia group was comprised of very small sample size (24 individuals), no association analysis was performed.

Effect size of *URAT1* variants on SUA levels. We also investigated SUA for each number of *URAT1* nonfunctional alleles using 4,753 individuals (3,158 males and 1,595 females), who received no medication for gout and/or hyperuricemia among 4,902 health examination participants of the J-MICC Study. The mean SUA levels with standard error of the mean (SEM) of having 0, 1 and 2 nonfunctional alleles were 6.22 ± 0.02, 4.03 ± 0.07 and 0.80 ± 0.10 mg/dl in males, respectively, and were 4.49 ± 0.02, 3.48 ± 0.15 and 0.60 ± 0.06 mg/dl in females, respectively (Fig. 1). Then, the nonfunctional alleles of two *URAT1* variants significantly decreased SUA in both males and females ($P = 2.2 \times 10^{-138}$ and 2.6×10^{-24}, respectively).

Furthermore, a multiple regression analysis, which focused on the statistical significance of the interaction term, revealed that there was an interaction between *URAT1* nonfunctional variants and sex (P for interaction = 1.5×10^{-12}, Table 3).

Discussion

URAT1 has been identified as a urate-anion exchanger which regulates SUA levels by playing an important role in the reabsorption of urate in human kidney[14]. In this study, we performed the genotyping of the two *URAT1* nonfunctional variants (R90H and W258X), and demonstrated the association with gout (Table 1), and the significant effect on hyperuricemia progression (Table 2) and that on SUA (Fig. 1).

Consistent with previous reports (on 77 Spanish[16] and 185 Japanese[17] gout patients, respectively), no *URAT1* nonfunctional variants (R90H or W258X) were found even in our large number of gout patients (n = 1,993). Our results indicate that these *URAT1* variants prevent the development of gout by the large-scale case-control study (case = 1,993 and control = 2,499).

Moreover, we revealed that the *URAT1* nonfunctional alleles of R90H and W258X markedly reduce the risk of hyperuricemia (RR = 0.036 in males; Table 2) and severely decrease SUA (Fig. 1) using 4,902 health examination participants. Males, having 1 or 2 nonfunctional alleles of *URAT1* exhibit a marked decrease of 2.19 or 5.42 mg/dl SUA, respectively (Fig. 1). Similarly, females, having 1 or 2 nonfunctional alleles of *URAT1* also show a decrease of 1.08 or 3.89 mg/dl SUA, respectively (Fig. 1). Moreover, the interaction between *URAT1* nonfunctional variants and sex was present (P for interaction $= 1.5 \times 10^{-12}$, Table 3). Thus, for the first time, we demonstrated the sex-dependent effect size of SUA by *URAT1* nonfunctional variants, which is also important for understanding the pathogenesis of renal hypouricemia because mild renal hypouricemia (SUA $\leq$ 3.0 mg/dl) could be caused by a heterozygous nonfunctional variant of *URAT1*[20] or *GLUT9*[21]. Our data clearly demonstrated that some individuals with a heterozygous *URAT1* nonfunctional variant exhibit renal hypouricemia.

Interestingly, although the sex difference in SUA is well-known[18,19], SUA of the individuals having 2 nonfunctional alleles is similar between males (0.80 mg/dl) and females (0.60 mg/dl). Moreover, the sex difference in SUA is smaller in the individuals having 1 nonfunctional allele (0.55 mg/dl) than in individuals without nonfunctional alleles (1.73 mg/dl). In other words, our data show that the sex difference of SUA becomes greater as the number of functional alleles (wild-type alleles) of *URAT1* increases, which suggests that the presence of functional URAT1 transporter is strongly related to the sex difference in SUA.

Previously, the sex difference in the expression of URAT1 had been found in a mouse model[22]. In addition, testosterone reportedly enhances the mRNA of Urat1 in a mouse model[23] and increases promoter activity of human *URAT1*[24]. Combined with these previous reports, our data suggest that one of the main causes of the sex difference in SUA is the different expression levels of functional URAT1 transporters between males and females due to sex hormones.

In summary, we demonstrated that the *URAT1* nonfunctional variants are protective genetic factors for gout and hyperuricemia, and showed the sex-dependent effect size of these *URAT1* variants on SUA. These findings provide a better understanding of genetic factors for SUA and gout/hyperuricemia progression.

Methods

Patients and controls. This study was approved by the institutional ethical committee of the National Defense Medical College. All procedures were performed in accordance with the Declaration of Helsinki, and written informed consent was obtained from each subject participating in the present study.

In a case-control study of gout, 1,993 Japanese male patients with primary gout were recruited from the outpatients of Midorigaoka Hospital (Osaka, Japan), Kyoto Industrial Health Association (Kyoto, Japan) and Ryougoku East Gate Clinic (Tokyo, Japan). All of the gout patients were diagnosed according to the criteria established by the American College of Rheumatology[25]. Hyperuricemia was defined as the SUA level that exceeds 7.0 mg/dl (=416.36 mol/l) according to the guideline of the Japanese Society of Gout and Nucleic Acid Metabolism[26]. As the control group, 2,499 male Japanese individuals without hyperuricemia and gout history were selected from participants in the Shizuoka area in the J-MICC Study[27,28].

For evaluation of the influence of two *URAT1* variants on SUA, 4,902 Japanese individuals (3,305 males including above 2,499 controls, and 1,597 females) were also recruited from health examination participants in the J-MICC Study. The details of participants in this study are shown in Supplementary Tables S2 and S3.

Genotyping. Genomic DNA was extracted from whole peripheral blood cells[21]. Genotyping of R90H and W258X variants in *URAT1* was performed by TaqMan method (Life Technologies Corporation, Carlsbad, CA, USA) with a LightCycler 480 (Roche Diagnostics, Mannheim, Germany)[29]. Custom TaqMan assay probes were designed as follows: for R90H in *URAT1*, VIC-CCGCCACTTCCGC and FAM-CGCCGCTTCCGC; for W258X in *URAT1*, VIC-CGGGACTGAACACTG and FAM-CGGGACTGGACACTG. All of R90H heterozygotes (G/A), W258X heterozygotes (G/A) and W258X homozygotes (A/A) were confirmed by direct sequencing with a 3130xl Genetic Analyzer (Life Technologies Corporation)[29] and the following primers: for R90H in *URAT1*, forward 5′-GTTGGAGCCACCCCAAGTGAC-3′ and reverse 5′-GTCTGACCCACCGTGATCCATG-3′; for W258X in *URAT1*, forward 5′-TGATGAACACGGGCACTCTC-3′ and reverse 5′-CTTTCCACTCGCTCCCCTAG-3′.

Data analysis. For all calculations in the statistical analysis, the software R (version 3.1.1) (http://www.r-project.org/) was used[30]. The association analyses were examined with the Fisher's exact tests. RRs were calculated under a dominant model: i.e. G/G versus G/A or A/A in W258X, 0 versus 1 or 2 in the number of nonfunctional alleles, respectively. Linear regression analyses were performed to evaluate the influence of two *URAT1* variants on SUA. Furthermore, we carried out a multiple regression analysis with an interaction term ($x_1 x_2$): $y = b_0 + b_1 x_1 + b_2 x_2 + b_3 x_1 x_2$, where y is SUA level, x_1 is an ordinal variable representing the number f nonfunctional alleles of two *URAT1* variants, and x_2 is a dummy variable representing the sex (male = 0 and female = 1). For the robustness of the statistical test, random re-sampling methods with computer simulation are often applied[31,32]. In this study, the permutation test[32] 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 P values were two-tailed and P value < 0.05 was considered statistically significant.

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Author Contributions

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

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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 ($p=2.0\times10^{-12}$;

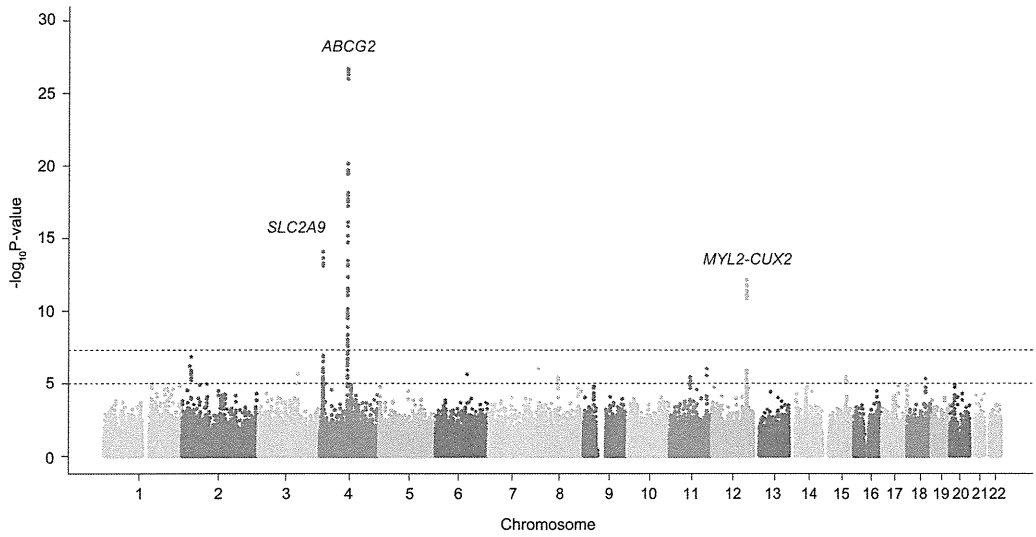

Figure 1 Manhattan plot of a genome-wide association analysis of gout. X-axis shows chromosomal positions. Y-axis shows -$\log_{10}$ p values. The upper and lower dotted lines indicate the genome-wide significance threshold ($p=5.0\times10^{-8}$) and the cut-off level for selecting single-nucleotide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

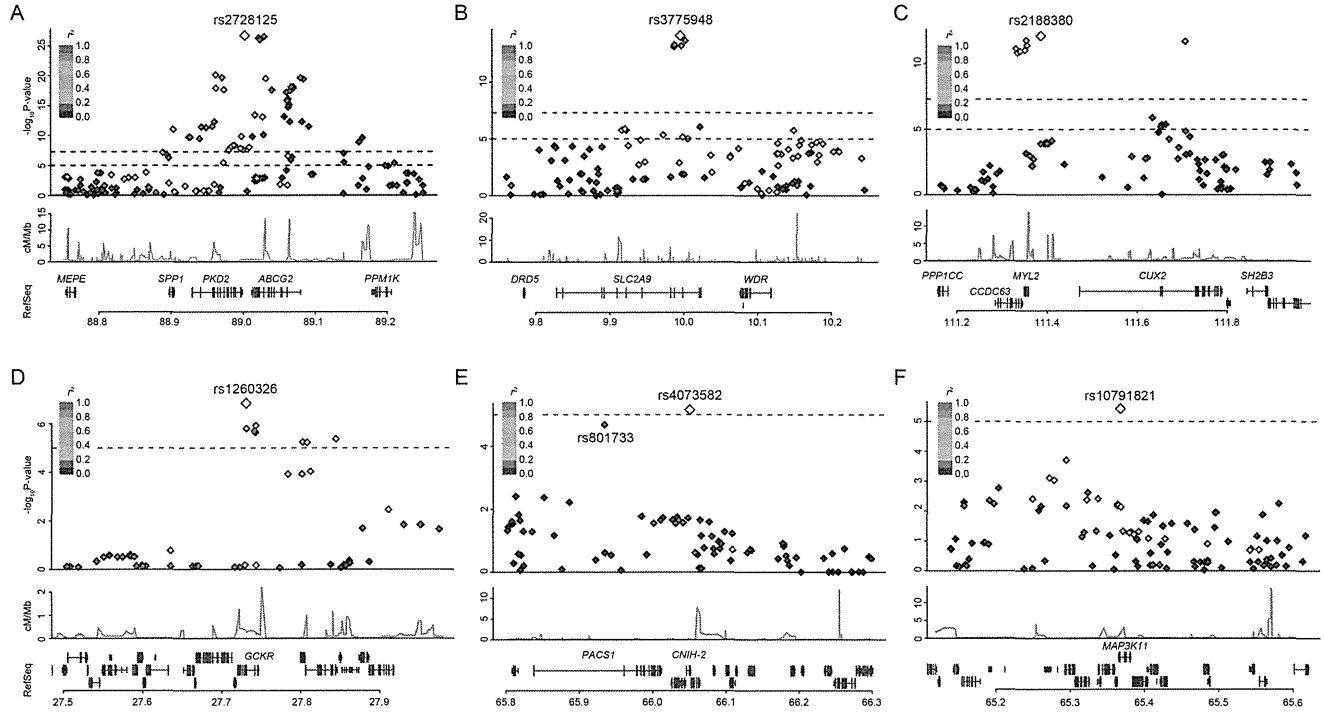

Figure 2 Regional association plots for six discovered loci of gout. Five regions exceeding the genome-wide significance level (A–E) and one region showing a suggestive association (F). The highest association signal in each panel is located on *ABCG2* (A), *SLC2A9* (B), *MYL2-CUX2* (C), *GCKR* (D), *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.				Freq.					
					Cases	Controls	OR (95% CI)	p Value	Cases	Controls	OR (95% CI)	p Value	OR (95% CI)	p Value
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

		Freq.		ROL type vs controls*		RUE type vs controls*		Case–subtype heterogeneity test	
SNP†	Gene	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

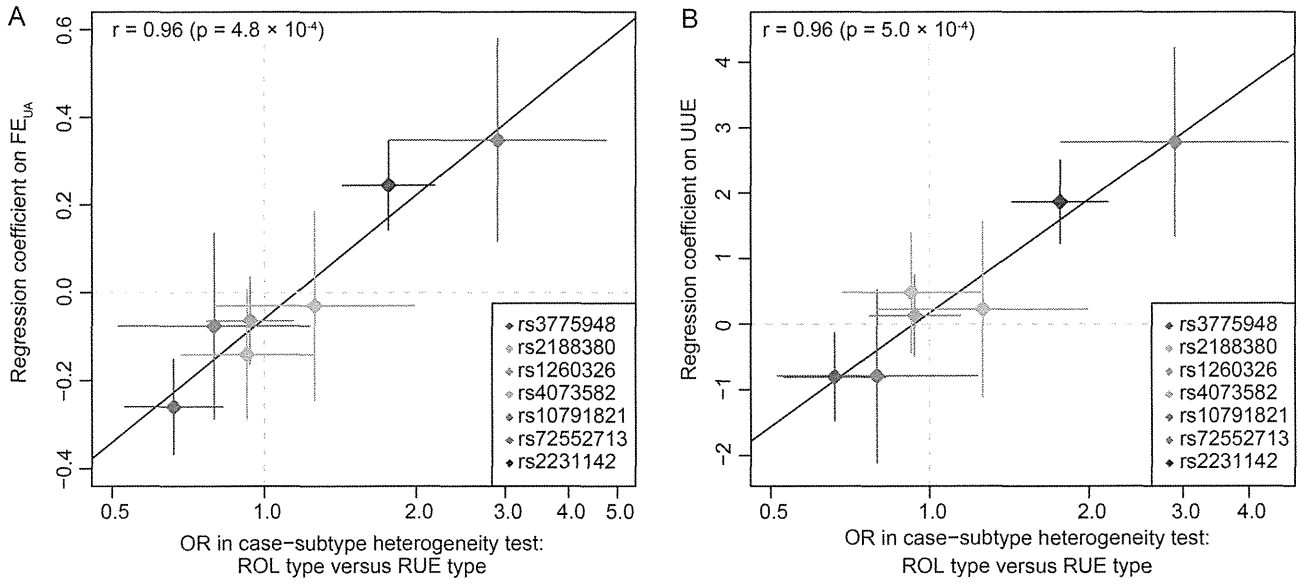

Figure 3 Relationships between effects of risk alleles on clinical parameters and ORs in case–subtype heterogeneity test. (A) FE_{UA} and (B) urinary urate excretion (UUE). The seven single-nucleotide polymorphisms (SNPs) listed in table 2 were examined. OR in case–subtype heterogeneity test is an estimate 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.

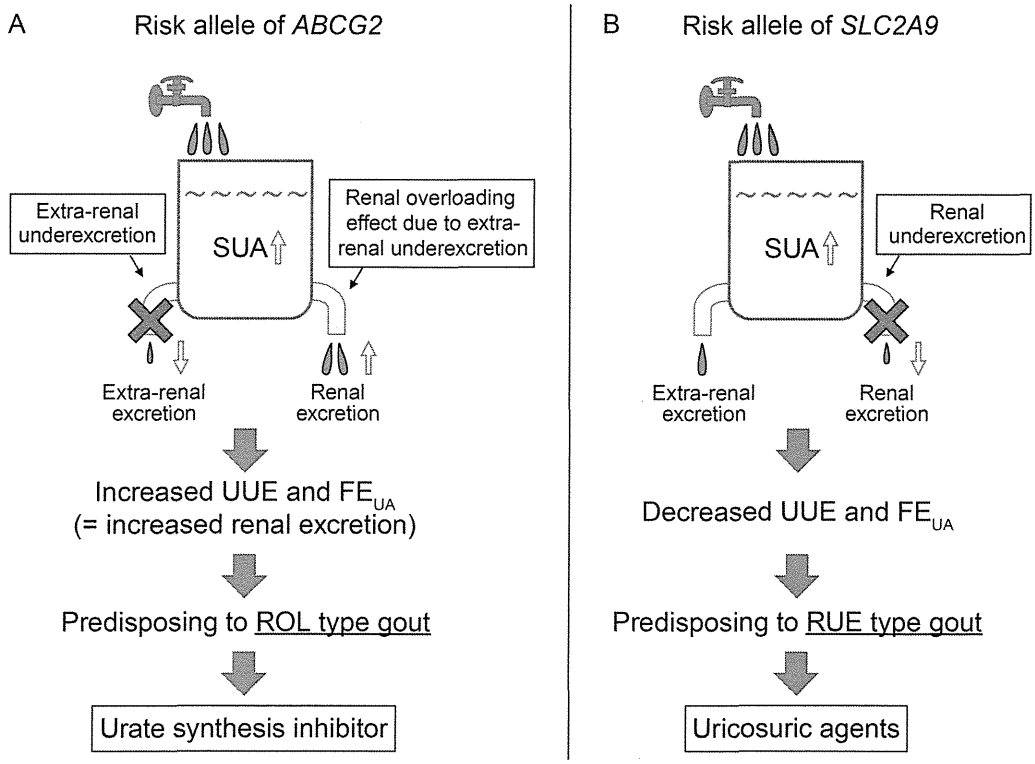

Figure 4 Differential effects by risk allele on clinical parameters and gout. (A) The risk alleles of *ABCG2* increase UUE and FE_{UA}, which leads to the overloading effect on renal urate excretion and increases the risk of the ROL-type gout. Therefore, patients with risk alleles for the ROL-type gout 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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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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ORIGINAL ARTICLE

Transition of adolescent and young adult patients with childhood-onset chronic kidney disease from pediatric to adult renal services: a nationwide survey in Japan

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Abstract

Background Transition of adolescent and young adult (AYA) patients with childhood-onset chronic kidney diseases (C-CKD) from pediatric to adult renal services has received increasing attention. However, information on transition of Japanese patients with C-CKD is limited.

Methods The Transition Medicine Working Group, in collaboration with the Japanese Society for Nephrology, the Japanese Society for Pediatric Nephrology and the Japanese Society of Pediatric Urology, conducted a retrospective cross-sectional study in 2014 on issues concerning the transition of Japanese patients with C-CKD.

Results Few institutions in Japan had transition programs and/or transition coordinators for patients with C-CKD. Refusal to transfer by patients or their families, lack of concern about transition and inability to decide on transfer were common reasons for non-transfer of patients still followed by pediatric renal services. Around 25 % of patients who had ended or interrupted follow-up by pediatric renal services presented to adult renal services because of symptoms associated with C-CKD. Patients with various types of childhood-onset nephrourological diseases were transferred from pediatric to adult renal services. IgA nephropathy, minimal change nephrotic syndrome and congenital anomalies of the kidney and urinary tract were the most frequent primary kidney diseases in adult patients with C-CKD.

Conclusion These survey results indicate the need for introduction of transitional care for Japanese AYA patients with C-CKD. Consensus guidelines for the optimal clinical management of AYA patients with C-CKD are required to ensure the continuity of care from child to adult renal services.

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