Substantial anti-gout effect conferred by common and rare dysfunctional variants of URAT1/SLC22A12

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Abstract

**Objectives.** Gout, caused by chronic elevation of serum uric acid levels, is the commonest form of inflammatory arthritis. The causative effect of common and rare variants of ATP-binding cassette transporter G2 (ABCG2/BCRP) on gout risk has been studied, but, little attention has been paid to the effect of common (rs121907892, p.W258X) and rare variants of urate transporter 1 (URAT1/SLC22A12) on gout, despite dysfunctional variants of URAT1 having been identified as pathophysiological causes of renal hypouricemia.

**Methods.** To address this important but overlooked issue, we investigated the effects of these URAT1 variants on gout susceptibility, using targeted exon sequencing on 480 clinically-defined gout cases and 480 controls of Japanese male in combination with a series of functional analyses of newly-identified URAT1 variants.

**Results.** Our results show that both common and rare dysfunctional variants of URAT1 markedly decrease the risk of gout (OR 0.0338, reciprocal OR 29.6, p = 7.66 × 10⁻⁸). Interestingly, we also found that the URAT1-related protective effect on gout eclipsed the ABCG2-related causative effect (OR 2.30 - 3.32). Our findings reveal only one dysfunctional variant of URAT1 to have a substantial anti-gout effect, even in the presence of causative variants of ABCG2, a “gout gene”.

**Conclusion.** Our findings provide a better understanding of gout/hyperuricemia and its etiology that is highly relevant to personalized health care. The substantial anti-gout effect of common and rare variants of URAT1 identified in the present study support the genetic concept of a ‘Common Disease, Multiple Common and Rare Variant’ model.

**Keywords:** urate reabsorption transporter; gout susceptibility; genetic risk factor; Common Disease Common Variant hypothesis; Common Disease Multiple Rare Variant hypothesis

**Rheumatology key messages:**
- Common and rare dysfunctional variants of URAT1/SLC22A12 have substantial anti-gout effects.
- The anti-gout effect of URAT1 outweighs the gout-promotive effect of ABCG2, a gout gene.
- We show a new ‘Common Disease, Multiple Common and Rare Variant’ model by two genes.
Introduction

Gout is a form of inflammatory arthritis caused by hyperuricemia. Several genome-wide association studies have been conducted to identify the genetic loci that influence serum uric acid (SUA) levels [1, 2] and/or gout risk [3-7]. These revealed the pathophysiological importance of urate transporters on urate-related diseases [8]. Recent studies have chiefly focused on variations that increase genetic susceptibility: for example, regarding \textit{ATP-binding cassette transporter G2 (ABCG2)}, a urate exporter excreting urate from the body whose dysfunction is well-characterized as a genetic risk factor of hyperuricemia and gout [9-12], not only common but also rare variants of this “gout gene” have been examined [9, 13, 14]. Compared to such cases, there is little information about the relationship between the expected gout-protective effect and the genetic variations in urate handling processes contributing to the decrease of SUA levels.

In humans, in addition to endogenous production of uric acid, renal urate re-absorption makes a significant contribution to the regulation of SUA levels. The latter process in urate handling is mediated by a renal urate re-absorber encoded by \textit{urate transporter 1 (URAT1, also known as SLC22A12)} gene, which is a molecular target of urate-lowering drugs [15]. Its dysfunctional variants are also known as pathophysiological causes of renal hypouricemia (RHUC) [15-17]. However, despite the high prevalence of gout [8], little attention has been paid to the effects of genetic variations of \textit{URAT1} on the risk of gout. We herein address this issue using a clinical genetics approach on gout in combination with functional analyses of the urate transporter URAT1. The findings of this study shed light on the genetic significance of common and rare variants of \textit{URAT1} as anti-gout factors.

Methods

Patients and genetic analyses

This study was approved by the institutional Ethical Committees (National Defense Medical College and Nagoya University, Japan), and written consent was obtained from all of its participants. All the procedures were performed in accordance with the Declaration of Helsinki. Our cases were 480 Japanese males with gout, recruited from the Ryougoku East Gate Clinic (Tokyo, Japan). All had been clinically diagnosed with primary gout according to the criteria established by the American College of Rheumatology [18]. Fractional excretion of uric acid (FEUA, calculated as urate clearance/creatinine clearance ratio) was investigated for each patient, undergoing no urate lowering therapy, by a few-hours urate collection. As controls, 480 Japanese males without hyperuricemia (serum uric acid [SUA] levels > 7.0 mg/dl) or history of gout were recruited from participants in the Shizuoka area of the Japan Multi-Institutional Collaborative Cohort Study (J-MICC Study) [19, 20]. The clinical characteristics are shown in Table 1. Additionally, the control group (53.0 ± 7.9 years old) was eldered compared with the case group (46.2 ± 9.8 years old). As aging is one of the important factors to increase SUA and/or gout risk, to avoid over-adjustment bias, we did not conduct age standardization in this study.

To explore non-synonymous variants of \textit{URAT1}, we performed targeted exon sequencing of \textit{URAT1}. The methods of target sequencing as well as variant calling and annotation were conducted as described previously [13].
To confirm one common (W258X) and ten rare non-synonymous variants, direct sequencing using a 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) was performed according to our previous study [21].

Materials
Critical materials and resources used in this study are summarized in supplementary Table S1. All the other chemicals used were commercially available and of analytical grade.

Plasmid construction
The full-length human URAT1 wild-type (WT) (NCBI accession; NM_144585.3) open reading frame (ORF) in the pcDNA3.1(+) plasmid was amplified with the Xho I site and the Xba I site attached at the 5′- and 3′-end, respectively, by PCR, and then inserted into the pEGFP-C1 vector plasmid (Clontech Laboratories, Palo Alto, CA, USA) for CMV-driven expression of EGFP-URAT1 in mammalian cells as in our previous study [22]. Using a site-directed mutagenesis technique, eight URAT1 mutants (P79L, R90H, Y180X, A227T, W258X, Q297X, F379L and Q533K) were constructed with a pEGFP-C1 vector in the same way as in our previous study [13].

Introduction of all mutations was confirmed by full sequencing using BigDye® Terminator v3.1 (Applied Biosystems) with Applied Biosystems® 3130 Genetic Analyzer (Applied Biosystems) following the manufacturer’s protocol. Prior to the further experiments described below, all the plasmids were obtained from the same lot.

Cell culture
Human embryonic kidney 293 (HEK293)-derived 293A cells were maintained in Dulbecco’s Modified Eagle’s Medium (Nacalai Tesque, Kyoto, Japan) supplemented with 10% fetal bovine serum (Biowest, Nuaillé, France), and 1% penicillin-streptomycin (Nacalai Tesque), 2 mM L-glutamine (Nacalai Tesque), and 1 × Non-Essential Amino Acid (Life Technologies, Tokyo, Japan) at 37°C in a humidified atmosphere of 5% (v/v) CO₂ in air. Twenty-four hours after the cells had been seeded (0.92 × 10⁵ cells/cm²), each vector plasmid was transfected into 293A cells using polyethyleneimine “MAX” (PEI-MAX) (Polysciences, Warrington, PA, USA) as described previously [23].

Preparation of protein lysates and immunoblotting
After preparation of whole-cell lysates, to examine the N-linked glycosylation status of each URAT1 protein, the whole-cell lysate samples were treated with Peptide N-glycosidase F (PNGase F) (New England Biolabs, Ipswich, MA, USA) as described previously [23]. The protein concentration was determined using the Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, Kanagawa, Japan) with BSA as a standard according to the manufacturer’s protocol. The samples were then separated by SDS-PAGE and transferred to an Immobilon-P PVDF membrane (Millipore, Bedford, MA, USA) by electroblotting at 15 V for 60 min. Using appropriate antibodies (supplementary Table S1), immunoblot detection was performed as described in our previous study [23].

Confocal microscopic observation
Confocal laser scanning microscopic observation was conducted as previously described [23]. In brief, 48 h after transfection, 293A cells were fixed with 4% PFA for 15 min at room temperature. The cells were then treated with a
fluorescent wheat germ agglutinin conjugate (WGA, Alexa Fluor® 594 conjugate; Thermo Fisher Scientific). After 0.02% (w/v) Triton-X100 treatment, the nuclei of the cells were visualized with TO-PRO-3 Iodide (Molecular Probes, Eugene, OR, USA). Stained cells were mounted with VECTASHIELD Mounting Medium (Vector Laboratories, Burlingame, CA, USA), after which fluorescence was detected using an FV10i Confocal Laser Scanning Microscope (Olympus, Tokyo, Japan).

**Urate uptake assay using URAT1-expressing 293A cells**

The urate uptake assay using URAT1-expressing 293A cells was conducted in the same way as in our previous study [23]. In brief, 48 hours after plasmid transfection, the cells were washed twice with Cl⁻-free transport buffer (Buffer T2: 125 mM Na-gluconate, 4.8 mM K-gluconate, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 1.3 mM Ca-gluconate, 25 mM HEPES, 5.6 mM D-glucose and pH 7.4) and pre-incubated in Buffer T2 for 15 min at 37°C. The cells were then further incubated for 20 sec in pre-warmed fresh Buffer T2 containing 5 μM [8-¹⁴C]-urate. Subsequently, the cells were twice washed with ice-cold Buffer T2 and then lysed with 500 μL of 0.2 M NaOH, then neutralized with 100 μL of 1 M HCl. We measured the radioactivity in the lysate using a liquid scintillator (Tri-Carb 3110TR; PerkinElmer, Waltham, MA, USA). The protein concentrations were determined using the Pierce™ BCA Protein Assay Kit as described above.

The urate-transporting activity was calculated as the incorporated clearance (μL/mg protein/min): (incorporated level of urate [DPM/mg protein/min]/urate level in the incubation mixture [DPM/μL]). URAT1-dependent urate-transporting activity was calculated by subtracting the urate-transporting activity of mock cells from that of the URAT1-expressing cells.

**Statistical analysis**

Different statistical tests were used for different experiments as described in the Figure legends which include the numbers of biological replicates (n). All statistical analyses were performed using Excel 2019 (Microsoft, Redmond, WA, USA) in functional analyses. The software R (version 4.0.1) (http://www.r-project.org/) was used for the calculation of Fisher’s exact test. Other regression analyses were performed with SPSS v.22.0J (IBM Japan, Tokyo, Japan). Logistic regression analysis with forward selection (likelihood ratio) was conducted to investigate gout susceptibility associated with dysfunctional variants of URAT1 and ABCG2, and linear regression analyses were performed to evaluate the variants’ effect on FEUA and SUA. Statistical significance is defined in terms of p values of below 0.05 or 0.01.
Results

Common and rare variants of URAT1 detected by targeted exon sequencing

To explore URAT1 variants associated with gout, targeted exon sequencing of URAT1 was performed with 480 clinically-defined gout cases and 480 normouricemic controls of Japanese males. We identified one common (W258X) and 10 rare non-synonymous variants in URAT1 (Table 2). Since three variants (Q382L, L418R and V547fsL) are reportedly nonfunctional [16, 24], we conducted a series of expression, localization, and functional analyses of six uncharacterized rare variants (P79L, Y180X, A227T, Q297X, F379L and Q533K) in addition to the W258X (common) and R90H (rare) variants that are frequently observed in Japanese RHUC patients, as described below.

Functional characterization of URAT1 variants

To examine the effect of the eight variants found in our genetic analysis on the URAT1 function as a urate transporter, we expressed each URAT1 variant tagged with EGFP in 293A cells, a human renal cell line. First, to confirm the protein expression, we performed immunoblot analysis of whole-cell lysate samples using the anti-EGFP antibody (Figure 1A). As expected, URAT1 WT was matured as a glycoprotein evidenced by the presence of PNGase F-sensitive immunoreactive bands at high molecular weight ranges. Our results revealed only three (P79L, A227T and Q533K) variants to be expressed as a matured glycoprotein in addition to WT protein, while the others (R90H, Y180X, W258X, Q297X and F379L) showed little in the way of signals corresponding to the matured form. Next, with confocal laser microscopy, we examined the intracellular localization of each URAT1 variant in 293A cells (Figure 1B). The five variants (R90H, Y180X, W258X, Q297X and F379L), which were not matured as a glycoprotein, hardly localized on the plasma membrane, contrary to the WT.

Last, we investigated the urate-transporting activity of each URAT1 variant in the cell-based uptake assay (Figure 1C). As expected, the five immature URAT1 variants (R90H, Y180X, W258X, Q297X and F379L) were functionally null as a urate transporter. The urate transport activities of A227T and Q533K were approximately half that of the URAT1 WT. On the other hand, the P79L mutation hardly at all affected URAT1 function; we therefore excluded this variant from subsequent association analyses.

Lowering effect of dysfunctional URAT1 variants on SUA levels

We next further investigated the pathophysiological impact of identified variants of URAT1 with a focus on renal urate handling in humans (Figure 2). In our cohort, 15 heterozygous carriers and one homozygous carrier of the common dysfunctional variant W258X were found in 480 controls (3.33%); no carriers were found in 480 gout cases. Of the nine dysfunctional variants (R90H, Y180X, A227T, Q297X, F379L, Q382L, L418R, Q533K and V547fsL), 12 control subjects were heterozygous carriers for either of the rare variants (2.50%, 12 heterozygotes among 480 controls). Only one gout patient was a Q533K heterozygous carrier (0.208% of 480 gout cases). Carriers with these dysfunctional variants had significantly higher FE\textsubscript{UA} (Figure 2A) and lower SUA levels (Figure 2B) than those of controls without these variants ($p = 6.18 \times 10^{-11}$ and $7.95 \times 10^{-23}$, respectively). Our results suggest that these common and rare homozygous dysfunctional variants in URAT1 drastically reduce the net amount of reabsorbed
urate from urine into blood, resulting in a marked decrease in SUA level that would provide a substantial gout-protective effect.

Anti-gout effect conferred by common and rare variants of URAT1

Finally, we addressed the effect of the URAT1 variants on gout risk. Stratified association analyses (Table 3) demonstrate the nine rare dysfunctional variants to significantly decrease gout risk (OR 0.0788, reciprocal OR 12.7, $p = 1.47 \times 10^{-3}$). All the common and rare dysfunctional variants of URAT1 drastically decreased the risk of gout (OR 0.0338, reciprocal OR 29.6, $p = 7.66 \times 10^{-8}$). Furthermore, to gain a quantitative insight into the anti-gout effect conferred by the dysfunctional variants of URAT1 against the gout-promotive effect caused by those of ABCG2 (a gout gene), we performed multivariate logistic regression analysis of gout with the dysfunctional variants of URAT1 and ABCG2. The results revealed the dysfunctional variants of URAT1 to have a substantial protective and beneficial effect (OR 0.0339, reciprocal OR 29.5) that outweighed the gout-promotive effect of ABCG2 (OR 2.30 - 3.32) (Table 4). These findings indicate that common and rare variants of URAT1 have substantial anti-gout effect against the common disease of gout.

Discussion

In the present study, using a Japanese cohort of 480 patients with clinically defined gout and the same number of controls without hyperuricemia or gout history, we explored the exonic non-synonymous variants of URAT1 (Table 2). A series of functional assays, together with the previous studies, revealed that all variants, except for the P79L variant found in this study, are functionally null or reduced (Figure 1); such variants of URAT1 have an anti-gout effect that is significant enough to cancel out the gout risk conferred by dysfunctional variants of ABCG2 (Table 4). Additionally, since we addressed the Japanese population, we successfully identified several novel and pathophysiologically-important rare variants of URAT1 that were not found in European and African-American populations in a previous study [25]. A recent study addressing a cohort of gout/hyperuricemia in the Czech Republic also did not find such URAT1 variants [26], which supports the importance of conducting genetic studies in various ethnic populations. To the best of our knowledge, this is the first study to make a quantitative comparison of genetic effects of common and rare variants between URAT1 and ABCG2 in combination with functional validation.

We previously reported dysfunctional common and multiple rare variants of ABCG2 to be major contributors to gout as a common disease, supporting the ‘Common Disease, Common Variant (CDCV)’ and ‘Common Disease, Multiple Rare Variant (CDMRV)’ hypothesis. This led us to propose the genetic concept of a ‘Common Disease, Multiple Common and Rare Variant (CDMCRV)’ model [13]. On the other hand, the dysfunctional rare variants of URAT1 have been reported to be the pathophysiological causes of RHUC, a hereditary disorder [27]. Via the stratified association analyses and the multivariate logistic regression analysis of gout susceptibility with dysfunctional variants of URAT1 and ABCG2, the present study revealed that multiple rare dysfunctional variants of URAT1 significantly decrease gout susceptibility, and that their protective effects independently overwhelm the causative effect of common (Q126X and Q141K) and multiple rare variants of ABCG2. In addition to recent studies reporting common protective variants against some common diseases [28, 29], this study is the first to reveal multiple rare protective
variants of \textit{URAT1} against the common disease of gout. Our findings can also strengthen the CDCV and CDMRV hypothesis as well as the CDMCRV model. Furthermore, while our previous study proposed the CDMCRV model for the association between only \textit{ABCG2} and gout [13], the present study shows that multiple common and rare variants of not only \textit{ABCG2} but also \textit{URAT1} are markedly associated with gout, demonstrating a new CDMCRV model by two genes (\textit{ABCG2} and \textit{URAT1}).

Also, the results of this study will deepen the understanding of genetic variations associated with the risk of RHUC. While the frequency of RHUC is relatively high in East Asia, especially in Japan (approximately 0.3%) [27], this hereditary disorder is also reported in Jewish [30] and European Roma [31] populations. Moreover, RHUC patients have been described in a variety of ethnic groups including Arab Israelis, Iraqi Jews, Caucasians, and European Roma and in geographically noncontiguous countries [32]. In addition to RHUC risk, in this context, our findings will also be meaningful to evaluate gout risk in non-Asian populations.

The limitations of this study include the following. To confirm the CDMCRV model more robustly, the identification of more rare variants of \textit{URAT1} will be needed. The expected protective effects of rare dysfunctional variants of \textit{URAT1} against gout should also be assessed in future studies, which will assist with the development of personalized genome medicines for subjects with \textit{URAT1} rare variants against gout.

In conclusion, common and multiple rare dysfunctional variants of \textit{URAT1} have substantial anti-gout effects. Our findings provide significant insights into genetic factors that protectively and substantially influence the risk of gout.
Author contributions
YT, HN, TT, and HM conceived and designed this study. YK, AN, HN, TH, SS, and HM performed genetic and statistical analyses. YT, KM, NU, RS, and TT performed the functional analyses. HO, KW, and HM collected and analyzed the participants’ clinical data. TH, II, HS, KI, and NS provided intellectual input and assisted with the preparation of the manuscripts. YT, YK, AN, TT, and HM wrote the manuscript. YT, YK, and AN contributed equally to this work. TT and HM organized this collaborative study as corresponding authors. All the authors have read and approved the final version of the manuscript.

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Disclosure statement
The authors have declared no conflicts of interest.

Data Availability Statement
Data are available upon reasonable request.

Supplementary data
Supplementary data are available at online.
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Figure legends

Figure 1. Functional characterization of common and rare variants of URAT1 identified in this study.

(A) Immunoblot detection. Arrowhead, matured URAT1 as a glycoprotein; α-tubulin, loading control. Three variants characterized by an acquired stop codon (Y180X, W258X and Q297X) resulted in the production of truncated proteins; two variants (R90H and F379L) were not matured; wild-type (WT) and three variants (P79L, A227T and Q533K) were expressed as matured forms. (B) Confocal microscopy. Nuclei were stained with TO-PRO-3 iodide (gray); plasma membrane (PM) was labeled with Alexa Fluor® 594-conjugated WGA (red). Bars, 5 μm. The five variants (R90H, Y180X, W258X, Q297X and F379L) were only barely localized on the PM. (C) Functional assay. Incorporation of radiolabeled urate into the cells expressing each URAT1 variant was measured and URAT1-dependent urate transport activities were calculated. Data are shown as % of WT and expressed as mean ± S.D., n = 3. ††, p < 0.01; †, p < 0.05 vs. WT control (one sample t-test).

Figure 2. Physiological impact of common and rare variants of URAT1 identified in this study.

The dysfunctional variants of URAT1 significantly increased FEUA (A) and decreased SUA (B). *p < 0.001. Bars show means ± S.E.
### Table 1. Characteristics of the participants

|                      | Case     | Control  |
|----------------------|----------|----------|
| Number               | 480      | 480      |
| Call rate (%)        | 100      | 99.8     |
| Age (years)          | 46.2 ± 9.8 | 53.0 ± 7.9 |
| Body-mass index (kg/m²) | 25.3 ± 3.7 | 23.2 ± 2.6 |

480 gout cases and 480 normouricemic controls of Japanese male were analyzed. Plus-minus values are means ± standard deviation.
Table 2. All common and rare non-synonymous variants of *URAT1* identified in the present study and their urate transport function

| Type of variant | rs number   | Position* | DNA sequence change† | AA change | Case (n) | MAF in case (%) | Control (n) | MAF in control (%) | Transport function (%) | MAF in Japanese§ |
|-----------------|-------------|-----------|----------------------|-----------|----------|----------------|-------------|---------------------|----------------------|-----------------|
| Common variant  | rs121907892 | 64361219  | G774A                | W258X     | 0        | 0              | 16          | 1.77                | 0                    | 2.28            |
| Rare variant    | rs757926758 | 64359264  | C236T                | P79L      | 1        | 0.104          | 0           | 0                   | 108.8               | NA              |
|                 | rs121907896 | 64359297  | G269A                | R90H      | 0        | 0              | 2           | 0.208               | 0                    | 0.339           |
|                 | rs1286450383| 64360910  | C540A                | Y180X     | 0        | 0              | 2           | 0.208               | 0                    | NA              |
|                 | rs201136391 | 64361124  | G679A                | A227T     | 0        | 0              | 2           | 0.208               | 48.3                | 0.248           |
|                 | NA          | 64366046  | C889T                | Q297X     | 0        | 0              | 1           | 0.104               | 0**                 | 0.0558          |
|                 | NA          | 64367214  | C1137G               | F379L     | 0        | 0              | 1           | 0.104               | 3.5                 | NA              |
|                 | rs765990518 | 64367222  | A1145T               | Q382L     | 0        | 0              | 2           | 0.208               | 3.8††               | NA              |
|                 | rs121907895 | 64367330  | T1253G               | L418R     | 0        | 0              | 1           | 0.104               | 1.9††               | 0.167           |
|                 | NA          | 64368409  | C1597A               | Q533K     | 1        | 0.104          | 0           | 0                   | 57.2                | 0.0418          |
|                 | NA          | 64368997  | 1639_1643del         | V547fsL   | 0        | 0              | 1           | 0.104               | 5.7††               | NA              |

Total of rare variant carriers¶ 2 12
Total number of participants 480 480

*SNP positions are based on the GRCh37 assembly.

†Nucleotide numbering is based on the DNA reference sequence from GenBank (accession code NM_144585).

‡Summary count of homozygous and heterozygous carrier participants. Except for one W258X homozygous participant, only heterozygous or wild-type carriers were observed. There were no individuals with more than one rare variant.

§MAF in Japanese refers to the Human Genetic Variation Database (ver. 2.30) [33].

*Count of rare variant carriers of *URAT1*. Only P79L was excluded from dysfunctional variants in the subsequent analyses (see Table 3).

**Values were calculated as under 0.

††Values are from Wakida *et al.*, [16].

AA, amino acid; CI, confidence interval; OR, odds ratio; MAF, minor allele frequency; NA, not assigned.
Table 3. Common and rare dysfunctional variants of *URAT1/SLC22A12* strongly decrease gout susceptibility

| Dysfunctional variants of *URAT1* | Case | Control | p-value | OR (95%CI) | Reciprocal OR (95%CI) |
|----------------------------------|------|---------|---------|------------|----------------------|
| All rare variants                | 480  | 1       | 0.208   | 464        | 12                   | 2.59                 |
|                                  |      |         |         |            |                      | 1.47 × 10⁻³          |
|                                  |      |         |         |            |                      | 0.0788 (0.00184 – 0.536) |
|                                  |      |         |         |            |                      | 12.7 (1.86 – 543.4)  |
| All common and rare variants     | 480  | 1       | 0.208   | 480        | 28                   | 5.83                 |
|                                  |      |         |         |            |                      | 7.66 × 10⁻⁸          |
|                                  |      |         |         |            |                      | 0.0338 (0.000827 – 0.206) |
|                                  |      |         |         |            |                      | 29.6 (4.85 – 1209.0) |

One common variant W258X and nine rare variants (R90H, Y180X, A227T, Q297X, F379L, Q382L, L418R, Q533K and V547fsL) were identified as dysfunctional variants of *URAT1/SLC22A12* (details are in Table 2). A non-synonymous variant P79L of *URAT1* is excluded here due to its non-altered function. Fisher’s exact test was used for the calculation for the *p*-values.

*The number of carriers with dysfunctional non-synonymous variants in *URAT1*.

†The percentage of carriers in case or control populations.

Freq: frequency, OR: odds ratio, CI: confidence interval
### Table 4. Multivariate logistic regression analysis of gout susceptibility with dysfunctional variants of *URAT1* and *ABCG2*

| Variables                          | β     | OR (95% CI)          | p-value       |
|-----------------------------------|-------|----------------------|---------------|
| Dysfunctional variants of *URAT1* | −3.39 | 0.0339* (0.00454 – 0.253) | 9.68 × 10⁻⁴  |
| Q126X (Common variants of *ABCG2*)| 1.20  | 3.32 (1.97 – 5.59)   | 6.20 × 10⁻⁶   |
| Q141K (Common variants of *ABCG2*)| 0.83  | 2.30 (1.88 – 2.82)   | 1.26 × 10⁻¹⁵ |
| Rare variants of *ABCG2*          | 0.90  | 2.47 (1.29 – 4.73)   | 6.62 × 10⁻³   |

*The reciprocal OR (odds ratio) of common and rare dysfunctional variants of *URAT1* is 29.5.

CI, confidence interval. β is for per copy of the allele.
Figure 1. Functional characterization of common and rare variants of URAT1 identified in this study.

(A) Immunoblot detection. Arrowhead, matured URAT1 as a glycoprotein; α-tubulin, loading control. Three variants characterized by an acquired stop codon (Y180X, W258X and Q297X) resulted in the production of truncated proteins; two variants (R90H and F379L) were not matured; wild-type (WT) and three variants (P79L, A227T and Q533K) were expressed as matured forms. (B) Confocal microscopy. Nuclei were stained with TO-PRO-3 iodide (gray); plasma membrane (PM) was labeled with Alexa Fluor® 594-conjugated WGA (red). Bars, 5 μm. The five variants (R90H, Y180X, W258X, Q297X and F379L) were only barely localized on the PM. (C) Functional assay. Incorporation of radiolabeled urate into the cells expressing each URAT1 variant was measured and URAT1-dependent urate transport activities were calculated. Data are shown as % of WT and expressed as mean ± S.D., n = 3. ††, p < 0.01; †, p < 0.05 vs. WT control (one sample t-test).
Figure 2. Physiological impact of common and rare variants of URAT1 identified in this study. The dysfunctional variants of URAT1 significantly increased $\text{FE}_{\text{UA}}$ (A) and decreased SUA (B). *$p < 0.001$. Bars show means ± S.E.