ABCG2 dysfunction causes hyperuricemia due to both renal urate underexcretion and renal urate overload

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Gout is a common disease which results from hyperuricemia. We have reported that the dysfunction of urate exporter ABCG2 is the major cause of renal overload (ROL) hyperuricemia, but its involvement in renal underexcretion (RUE) hyperuricemia, the most prevalent subtype, is not clearly explained so far. In this study, the association analysis with 644 hyperuricemia patients and 1,623 controls in male Japanese revealed that ABCG2 dysfunction significantly increased the risk of RUE hyperuricemia as well as overall and ROL hyperuricemia, according to the severity of impairment. ABCG2 dysfunction caused renal urate underexcretion and induced hyperuricemia even if the renal urate overload was not remarkable. These results show that ABCG2 plays physiologically important roles in both renal and extra-renal urate excretion mechanisms. Our findings indicate the importance of ABCG2 as a promising therapeutic and screening target of hyperuricemia and gout.

Gout is a common disease which causes severe acute arthritis, and results from persistent hyperuricemia. Hyperuricemia shows elevated serum uric acid (SUA) levels and most of them are asymptomatic. So far, three urate transporters, URAT1/SLC22A12, GLUT9/SLC2A93, and ABCG2/BCRP4–6, have been reported to play important roles in the regulation of SUA, and their dysfunctions cause urate transport disorders. Among them, common dysfunction of ABCG2 exporter has proved to be a major cause of hyperuricemia and gout4–6. Recently, we have provided a new mechanism for hyperuricemia that the decrease in extra-renal (intestinal) urate excretion by ABCG2 dysfunction induces renal urate overload, thereby causing hyperuricemia. This mechanism, however, does not give a sufficient explanation for all ABCG2 dysfunction cases as a major cause of hyperuricemia and gout because the most prevalent type of hyperuricemia is not renal urate overload but renal
urate underexcretion (Supplementary Fig. S1). In this study, we first focused on the involvement of ABCG2 dysfunction in renal underexcretion (RUE) hyperuricemia.

**Results**

Genotyping was performed for 2,267 Japanese male participants, who consisted of 644 hyperuricemia cases (SUA > 7.0 mg/dl) and 1,623 controls. Their functional ABCG2 activities were estimated from their genotype combinations of its two dysfunctional missense variants, Q126X (rs72552713) and Q141K (rs2231142). Because there is no simultaneous presence of the minor alleles of non-functional variant Q126X and half-functional variant Q141K in one haplotype5,7, we defined three haplotype IDs as *1, *2, and *3, as shown in Figure 1a. Thus, all participants were divided into four functional groups; i.e., ≤1/4 function, 1/2 function, 3/4 function, and full function.

The association analysis revealed that ABCG2 dysfunction increased the risk of overall hyperuricemia according to the severity of its impairment (Fig. 2a, Supplementary Table S1); the odds ratios (ORs) in 3/4, 1/2 and ≤1/4 function were 2.64, 4.11 and 6.81, respectively. In RUE hyperuricemia that represents the dysfunction of renal urate excretion, the ORs also increased as the ABCG2 dysfunction became more severe; the ORs in 3/4, 1/2 and ≤1/4 function were 2.05, 2.66 and 4.53, respectively (Fig. 2b, Supplementary Table S1). In ROL hyperuricemia in which extra-renal (mainly intestinal) urate excretion plays an important role, contributions of ABCG2 dysfunction to the increase of ORs were more obvious; the ORs in 3/4, 1/2 and ≤1/4 function were 3.60, 6.83 and 16.0, respectively (Fig. 2b, Supplementary Table S1). Furthermore, Q126X homozygote signifying complete deficiency of ABCG2 was identified in one case with gout in the ROL hyperuricemia group. This fact is consistent with our previous report on the homozygous Abcg2 knockout mice having characteristics of ROL hyperuricemia.

When hyperuricemia was divided into three distinct types (i.e., RUE type, combined type, and ROL type as shown in Supplementary Fig. S1), severe ABCG2 dysfunction (≤1/4 function) significantly raised the risk of combined and ROL types but not that of RUE type.

| Table 1 | ABCG2 functions of participants |
|---------|----------------------------------|
| **Estimated transport activity** | **Diplotype of Q126X (rs72552713) and Q141K (rs2231142) alleles** | **Case** | **Control** |
|        |                                  | N     | %     | N  | %     |
| ≤1/4 function | *3/*3 or *2/*3 | 29 (26) | 4.5 (4.7) | 22 | 1.3 |
| 1/2 function  | *1/*3 or *2/*2  | 151 (135) | 23.4 (23.5) | 190 | 11.7 |
| 3/4 function  | *1/*2           | 307 (277) | 47.7 (48.2) | 600 | 37.0 |
| Full function | *1/*1           | 157 (136) | 24.4 (23.7) | 811 | 50.0 |
| Total        |                  | 644 (575) | 100.0 (100.0) | 1,887 | 100.0 |

**Haplotypes “Q-Q”, “Q-K”, and “X-Q” of two SNPs [Q126X and Q141K] are referred to as *1, *2, and *3, respectively. Risk alleles are X for Q126X, and K for Q141K. The relative functional activities of these haplotypes are 1, 1/2, and 0, respectively, and visualized as Figure 1.**

**The numbers in parentheses show the numbers and percentages of gout cases only (cases without asymptomatic hyperuricemia).**

Figure 1 | Estimation of ABCG2 function from diplotype of Q126X and Q141K alleles. (a) ABCG2*2 or *3 represents a haplotype with Q141K or Q126X variant, respectively. ABCG2*1 indicates a haplotype with neither Q141K nor Q126X variant. Since Q141K is a half-functional variant and Q126X is a nonfunctional variant, relative function of ABCG2*2*, *2, and *3 is 1, 1/2, and 0, respectively, which is visualized by black-indicated areas. Substituted residues are underlined. (b) Each participant’s function of urate exporter ABCG2 can be estimated from the diplotype, and can be also divided into four functional groups; i.e., ≤1/4 function, 1/2 function, 3/4 function, and full function.
Figure 2 | Risk of hyperuricemia by ABCG2 dysfunction. The risk of hyperuricemia is calculated based on the estimated ABCG2 dysfunction, i.e., 3/4 function (mild dysfunction), 1/2 function (moderate dysfunction), and ≤1/4 function (severe dysfunction). All bars show odds ratio (OR) ≥ 95% confidence interval (CI).

(P=0.62) (Fig. 2c, Supplementary Table S1). Nevertheless, moderate and mild dysfunction (3/4 and 1/2 functions) still contributed to increase the risk of RUE type hyperuricemia, conferring ORs of 1.80 and 2.00, respectively. These data imply that ABCG2 dysfunction under certain conditions causes renal urate underexcretion and leads to hyperuricemia even without renal urate overload.

Discussion
We previously reported a new mechanism by which ABCG2 dysfunction leads to the blockade of intestinal urate excretion (extra-renal underexcretion, Supplementary Fig. S1), thereby inducing hyperuricemia with renal urate overload (i.e., ROL hyperuricemia) and its overflow into the kidney. ROL hyperuricemia consists of hyperuricemia with renal urate overload (i.e., ROL hyperuricemia) and its dysfunctional mutations are involved in all types of hyperuricemia cases. Therefore, the elucidation of ABCG2 involvement in the pathogenesis of RUE hyperuricemia is of great importance.

The present study showed that ABCG2 dysfunction also had a great influence on renal urate underexcretion, and thus strongly involved in the pathogenesis of two hyperuricemia groups, RUE and ROL hyperuricemia, through two different mechanisms; i.e., one is retention of urate in the blood stream because of the blockade of urate excretion from the kidney, and the other is renal urate overload because of the blockade of urate excretion from the intestine (Fig. 3). Our results are consistent with the fact that urate exporter ABCG2 expresses in both kidney and intestine in humans, and RUE hyperuricemia is supposed to be induced by extra-renal underexcretion due to ABCG2 dysfunction (Supplementary Fig. S1). However, about two-thirds of uric acid is known to be excreted from kidney in humans, and RUE hyperuricemia consists of approximately 70–90% of all hyperuricemia cases. Therefore, the elucidation of ABCG2 involvement in the pathogenesis of RUE hyperuricemia is of great importance.

We wish to emphasize here that the present study was performed as a subtype analysis based on participants’ clinical information of SUA-related parameters. This approach could be applicable for other research on common diseases; i.e., the results of genetic analysis also indicate both the molecular function and localization of their gene products. For instance, we have reported that a common variant of transporter gene MCT9 (also known as SLC16A9) increases the risk of ROL gout, which suggests the intestinal expression of MCT9 and its association with intestinal urate excretion. Likewise, common variants in URAT1 (URAT1/SLC22A12 and GLUT9/SLC2A9) are reported to have an association with SUA. We previously showed that URAT1/SLC22A12 and GLUT9/SLC2A9 are causative genes of renal hyperuricemia type 1 and type 2, respectively, and encode renal urate reabsorption transporters. Thus, it is probable that changes in the function of these two transporters associate with RUE hyperuricemia. Because our previous study showed that renal urate reabsorption transporter URAT1/SLC22A12 also should be involved in the pathogenesis of ROL hyperuricemia by ABCG2 dysfunction.

Taken together, we first indicated that ABCG2 physiologically mediates renal urate excretion as well as extra-renal (intestinal) urate excretion, and its dysfunctional mutations are involved in all types of hyperuricemia as their major genetic causes. Furthermore, ABCG2 dysfunction caused renal urate underexcretion and induced hyperuricemia even without renal urate overload. Importantly, the present study is the first to show that mild to severe ABCG2 dysfunction also causes RUE hyperuricemia (Fig. 2b), suggesting its pathophysiological involvement in decreased renal urate excretion (Fig. 3).

Methods
All procedures involved in this study were performed in accordance with the Declaration of Helsinki and were approved by the institutional ethical committees.
Figure 3 | Pathophysiology of hyperuricemia due to ABCG2 dysfunction. The dysfunction of urate exporter ABCG2 is revealed to cause RUE hyperuricemia as well as ROL hyperuricemia due to blockade of urate excretion from the kidney and intestine, respectively. Abbreviation: SUA, serum uric acid. RUE, renal underexcretion. ROL, renal overload. (This figure, and the images contained therein, were produced by the authors).

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**Author contributions**

H.M., A.N. and N.S. designed the experiment. H.M., A.N., M.S., T.C., S.S., K.W., S.K., Y.G., H. Nakagawa, T.H., K.I. and T.S. collected samples and analyzed clinical data. H.M., A.N., M.S., T.C., S.S., Y.K., Y.T., Y.O., J.A., H.I., K.N., K.Y. and K.I. performed genetic analysis. H. Nakashima, T.N., H. Nakaoka and Y.S. performed statistical analysis. M.S., T.T., H. Nakaoka, T.I., K.Y., H.S., K.I., T.S. and N.S. provided intellectual input and assisted with the preparation of the manuscript. H.M., A.N. and N.S. wrote the paper. H.M. and A.N. contributed equally to this work.

**Additional information**

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

Competing financial interests: H.M., T.T. and N.S. have a patent pending based on the work reported in this paper. The other authors declare no competing financial interests.

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