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# IDENTIFICATION OF A HYPOURICEMIA PATIENT WITH SLC2A9 R380W, A PATHOGENIC MUTATION FOR RENAL HYPOURICEMIA TYPE 2

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□ *Hypouricemia is characterized by low serum uric acid (SUA) levels (≤3.0 mg/dL) with complications such as urolithiasis and exercise-induced acute renal failure. We have previously reported that urate transporter 1* (URAT1/SLC22A12) *and glucose transporter 9* (GLUT9/SLC2A9) *are causative genes for renal hypouricemia type 1 (RHUC1) and renal hypouricemia type 2 (RHUC2), respectively. In the series of experiments, two families have been revealed to have RHUC2 due to* GLUT9 *missense mutations R198C or R380W, respectively. Thus far, however, no studies have reported other RHUC2 families or patients with these pathogenic mutations. This study is aimed to find other cases of RHUC2.*

*We performed mutational analyses of* GLUT9 *exon 6 (for R198C) and exon 10 (for R380W) in 50 Japanese hypouricemia patients. Patients were analyzed out of a collection of more than 2000 samples from the Japan Multi-Institutional Collaborative Cohort Study (J-MICC Study).*

*We identified a novel male patient with heterogeneous RHUC2 mutation R380W. The SUA of this hypouricemia patient was 2.6 mg/dL, which is similar to that of our previous report (SUA: 2.7 mg/dL).*

*This is the second report indicating RHUC2 patient due to GLUT9 mutation R380W. This mutation occurs in highly conserved amino acid motifs and is reported to be an important membrane topology determinant. R380W is a dysfunctional mutation which completely diminishes the urate*

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*transport activities of GLUT9. Our study revealed a second hypouricemia patient with* GLUT9 *R380W, a pathogenic mutation of RHUC2, which may help to expand our understanding of RHUC pathogenesis.*

**Keywords:** SLC transporters; GLUT family; GLUT9L; GLUT9S; renal urate reabsorption

## INTRODUCTION

Renal hypouricemia is characterized by low serum uric acid (SUA) levels (≤3.0 mg/dL), and confers risk of severe complications such as exercise-induced acute renal failure or nephrolithiasis.[1, 2] Renal hypouricemia is mainly caused by impaired renal urate reabsorption. We previously reported that URAT1/SLC22A12[3] and GLUT9/SLC2A9[4] are key regulators of SUA, and play an essential role in urate reabsorption in the human kidney. The dysfunctional mutations of *URAT1* or *GLUT9* cause renal urate hypouricemia, called renal hypouricemia type 1 (RHUC1) and renal urate hypouricemia type 2 (RHUC2), respectively. [5] Previously, two families have been revealed to have RHUC2 due to *GLUT9* missense mutations R198C or R380W, respectively. Thus far, however, no studies have reported other RHUC2 families or patients with these pathogenic mutations. Here, we report another hypouricemia patient with the pathogenic RHUC2 mutation.

## MATERIALS AND METHODS

For the hypouricemia patients, 50 Japanese patients with lower SUA (≤3.0 mg/dl) were identified out of more than 2000 samples from the Japan Multi-Institutional Collaborative Cohort Study (J-MICC Study). We performed mutational analysis of *GLUT9* exon 6 (R198C) and exon 10 (R380W) in these 50 hypouricemia patients.

For the *GLUT9* sequence determination, we used following primers described previously:[4] for exon 6, forward 5′-GTCCTCTGAAATGCACCTCC-3′, and reverse 5′-GCACAGAAGATGCCTAAACAAACACA-3′; for exon 10, forward 5′-GGTGACCATATCCATCCAG-3′, and reverse 5′-GAAGGAG-CACCTTAAGGTTG-3′. High molecular weight genomic DNA was extracted from peripheral whole blood cells,[6] and was amplified by PCR. The PCR products were sequenced in both directions using a 3130xl Genetic Analyzer (Applied Biosystems).[7]

## RESULTS

The human *GLUT9* gene consists of 14 exons (1 noncoding and 13 coding) and the alternative splicing of the *GLUT9* gene results in two main transcripts: GLUT9 isoform 1 (long isoform, GLUT9L) and isoform 2 (short isoform, GLUT9S). Two heterozygous missense mutations of R380W and

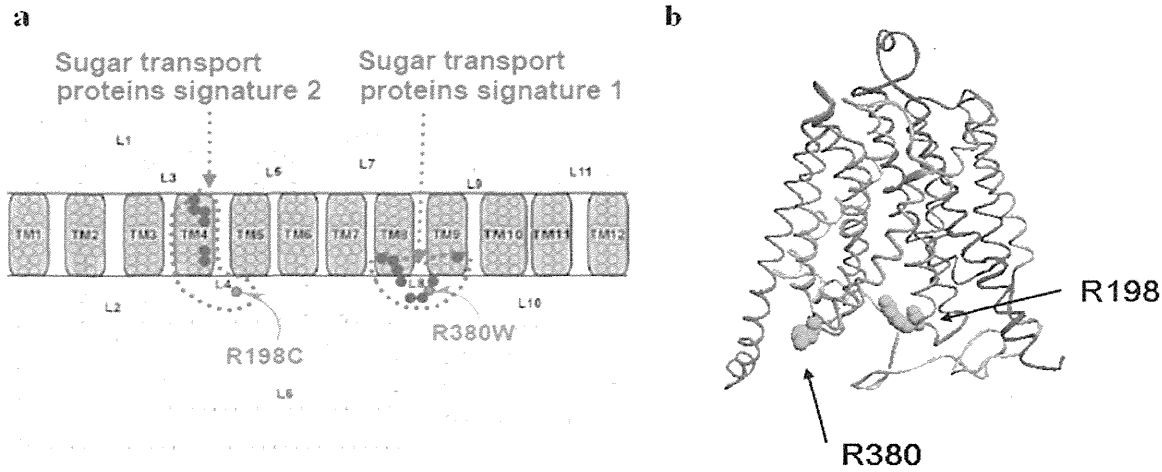


**FIGURE 1** Pathogenic mutation sites of GLUT9 (Color figure available online).

R198C for GLUT9L have been identified in Japanese patients with renal hypouricemia. Both mutations are missense mutations from basic amino acid arginine to neutral amino acids, and are at equivalent positions within the cytoplasmic loops, which cause a loss of positive charge. These pathogenic mutation sites in two-dimensional and three-dimensional models are shown in Figure 1. No hypouricemia patient with the R198C mutation was identified among these 50 patients. However, we identified a novel male patient with heterozygous mutation R380W (Figure 2). SUA of this hypouricemia patient was 2.6 mg/dL (154.6 $\mu$mol/l), which is similar to that of our previous report (SUA: 2.7 mg/dL (160.6 $\mu$mol/l)).

## DISCUSSION

*GLUT9* mutations in renal hypouricemia patients may change its topology.

We have previously identified loss-of-function mutations of *GLUT9* in renal hypouricemic patients having no *URAT1* mutations.[4] Mutation sites in *GLUT9* (R380W and R198C for GLUT9L, corresponding to R351W and R169C for GLUT9S) locate in highly conserved amino acid motifs called "sugar transport proteins signatures," which is observed in GLUT family transporters. The corresponding mutations in *GLUT1* (R333W and R153C) are known to cause *GLUT1* deficiency syndrome.[8] Arginine residues in this motif are reported to be an important determinant of membrane topology of human GLUT1,[9] and the same may be true in GLUT9 on the basis of membrane topology.

### Physiological Importance of GLUT9 in Human Urate Transport

The urate metabolism in humans is quite different from that in mice due to the lack of uricase.[10] In addition, hypouricemia is one of relatively rare

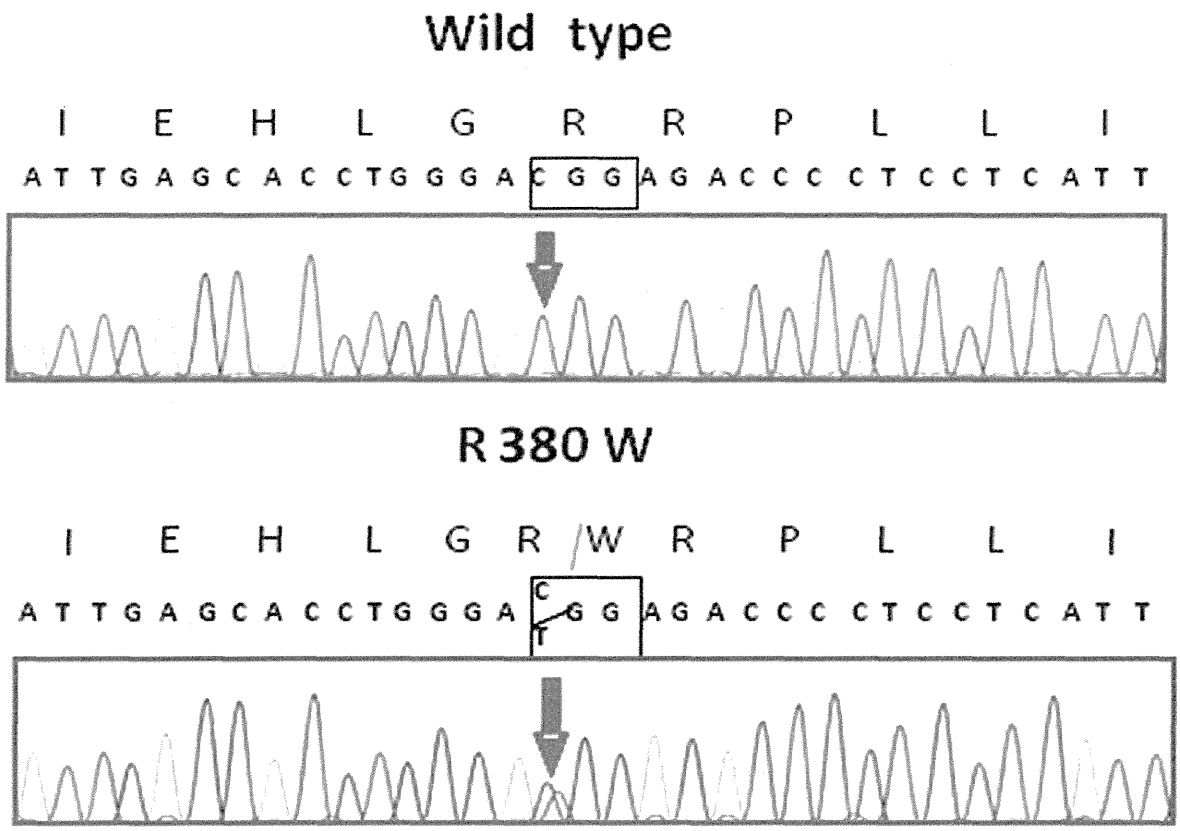


**FIGURE 2** Heterozygous mutation (R380W) in a newly-identified renal hypouricemia patient (Color figure available online).

diseases compared with common diseases including hyperuricemia and gout. Therefore, it is of great significance to identify the dysfunctional *GLUT9* mutations in humans through a large population.

In MDCK cells, GLUT9L and GLUT9S show basolateral and apical localization, respectively. Since dysfunctional mutations of either GLUT9L or GLUT9S dramatically reduced the urate transport activity, renal hypouricemia caused by these mutations could be ascribed to the decreased urate reabsorption on both sides of the renal proximal tubules, where GLUT9 expresses. In the present study, we confirmed the importance of *GLUT9* as a causative gene for renal hypouricemia, which encodes a renal urate reabsorption transporter.

## Identification of a Novel RHUC2 Patient

This is the second report indicating a RHUC2 patient due to *GLUT9* mutation R380W. Screening of large genome cohort samples revealed the second hypouricemia patient with *GLUT9* R380W, a pathogenic mutation of RHUC2. Our results confirm that GLUT9 can be a promising therapeutic target for hyperuricemia, gout, and related cardiovascular diseases. This finding may help to expand the understanding of RHUC pathogenesis.

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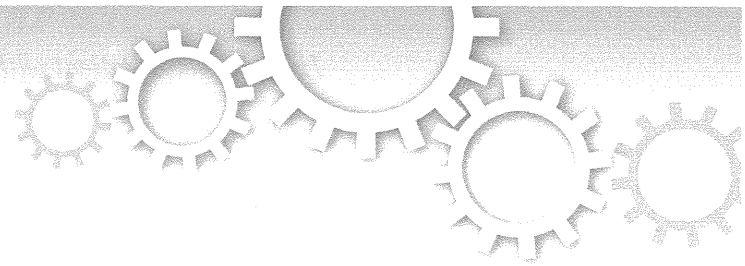

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# ABCG2 dysfunction causes hyperuricemia due to both renal urate underexcretion and renal urate overload

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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[1], GLUT9/SLC2A9[2,3], and ABCG2/BCRP[4–6], 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 gout[4,5]. 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[7]. 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

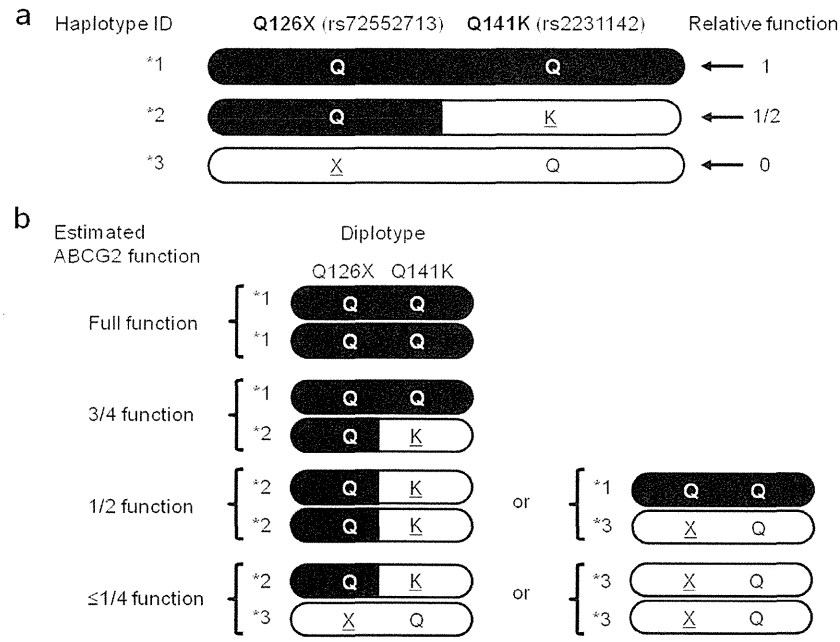


**Figure 1 | Estimation of ABCG2 function from diplotype of Q126X and Q141K alleles.** (a) ABCG2*2 or *3 represents a haplotype with Q141K or Q126X variant, respectively. ABCG2*1 indicates a haplotype with neither Q141K nor Q126X variant. Since Q141K is a half-functional variant and Q126X is a nonfunctional variant, relative function of ABCG2*1, *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.

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 haplotype[5,7], 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. full function (*1/*1), 3/4 function (*1/*2), 1/2 function (*2/*2 or *1/*3), and ≤1/4 function (*2/*3 or *3/*3) (Fig. 1b, Table 1)[5–7]. From the patients' fractional excretion of urate ($FE_{UA}$) and urinary urate excretion (UUE), all cases were then classified into two groups, RUE hyperuricemia and renal overload (ROL) hyperuricemia (Supplementary Fig. S1).

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[7].

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).

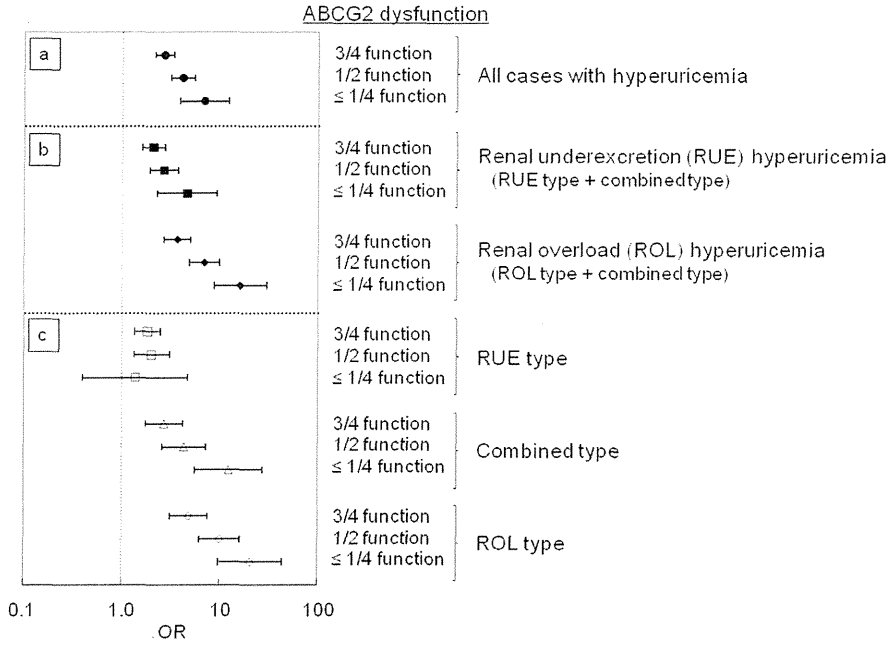


**Figure 2 | Risk of hyperuricemia by ABCG2 dysfunction.** The risk of hyperuricemia is calculated based on the estimated ABCG2 dysfunction, i.e., 3/4 function (mild dysfunction), 1/2 function (moderate dysfunction), and ≤1/4 function (severe dysfunction). All bars show odds ratio (OR) ± 95% confidence 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[7]. ROL hyperuricemia consists of urate overproduction and extra-renal underexcretion, while most ROL hyperuricemia is supposed to be induced by extra-renal underexcretion due to ABCG2 dysfunction[7] (Supplementary Fig. S1). However, about two-thirds of uric acid is known to be excreted from kidney in humans[8–10], and RUE hyperuricemia consists of approximately 70–90% of all hyperuricemia cases[10–12]. 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[13,14]. Severe ABCG2 dysfunction did not increase the risk of RUE type (Fig. 2c), and this type involved only a very small number of patients (n=3) (Supplementary Table S1). This result indicates that severe ABCG2 dysfunction (≤1/4 function) causes either ROL type or combined type rather than RUE type because of renal urate overload. Furthermore, our data show that moderate and mild ABCG2 dysfunction (1/2 and 3/4 function) significantly increase the risk of RUE type (Fig. 2c). These findings support our idea that 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).

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[15], which suggests the intestinal expression of MCT9 and its association with intestinal urate excretion. Likewise, common variants in *URAT1/SLC22A12* and *GLUT9/SLC2A9* are reported to have an association with SUA[16,17]. We previously showed that *URAT1/SLC22A12* and *GLUT9/SLC2A9* are causative genes of renal hyporucemia 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 expression levels of Urat1 are markedly decreased in *Abcg2* knockout mice which represent ROL hyperuricemia[7], 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 (Fig. 3). Besides our previous reports[6,7], the present study showed that *ABCG2* genotyping in combination with $FE_{UA}$ and UUE tests is sufficient for screening high-risk individuals with hyperuricemia and gout. Our findings will therefore serve to build up the health of people predisposed to hyperuricemia and gout.

## Methods

All procedures involved in this study were performed in accordance with the Declaration of Helsinki and were approved by the institutional ethical committees

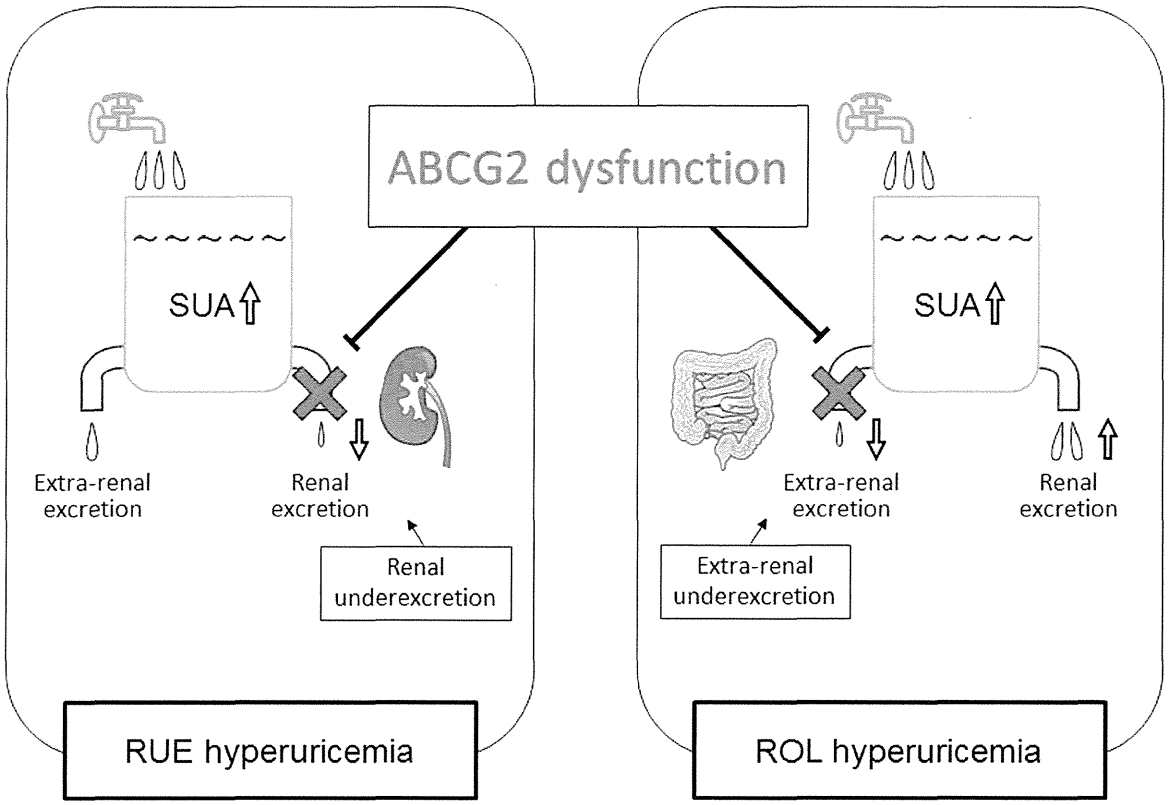


**Figure 3 | Pathophysiology of hyperuricemia due to ABCG2 dysfunction.** The dysfunction of urate exporter ABCG2 is revealed to cause RUE hyperuricemia as well as ROL hyperuricemia due to blockade of urate excretion from the kidney and intestine, respectively. Abbreviation: SUA, serum uric acid. RUE, renal underexcretion. ROL, renal overload. (This figure, and the images contained therein, were produced by the authors).

(National Defense Medical College and Jikei University School of Medicine). Written informed consent was obtained from all subjects participating in this study. 644 male outpatients with hyperuricemia (SUA>7.0 mg/dl) including 575 gout patients were registered at the gout clinics of either Jikei University Hospital (Tokyo, Japan) or Midorigaoka Hospital (Osaka, Japan) as previously described[7]. As a control group, 1,623 male individuals with normal SUA (≤7.0 mg/dl) were collected from the Japan Multi-Institutional Collaborative Cohort Study (J-MICC Study)[18]. Genotyping of *ABCG2* Q126X (rs72552713) and Q141K (rs2231142) was performed by high-resolution melting analysis with a LightCycler 480 (Roche Diagnostics)[19]. From the haplotype analyses reported in the previous studies[5,7], there is no simultaneous presence of the minor alleles (risk alleles) of non-functional variant Q126X and half-functional variant Q141K in one haplotype. In this study, their haplotype IDs, *1, *2, and *3, were defined as Figure 1a; the combination of wild-type Q126X and Q141K alleles ("Q-Q") was designated as ABCG2*1, which corresponds to the cDNA sequence of GenBank (accession number NM_004827). "Q-K" and "X-Q" were also named as ABCG2*2 and *3, respectively. Based on the diplotype of Q126X and Q141K alleles (Fig. 1b)[5,7], ABCG2 function was estimated and divided into four groups[5–7]; i.e., full function, 3/4 function, 1/2 function, and ≤1/4 function (Table 1). As previously described[7], $FE_{UA}$ and UUE were measured and used as markers for renal and extra-renal urate excretion function, respectively. Hyperuricemia patients were then classified into two groups, RUE hyperuricemia and ROL hyperuricemia; the former was characterized by low $FE_{UA}$ (< 5.5%) and the latter was defined by high UUE (> 25 mg/hr/1.73 $m^2$) (Supplementary Fig. S1)[7]. RUE type, the combined type, and ROL type, were also defined as shown in Supplementary Fig. S1. Association analysis with $\chi^2$ test was performed by SPSS software (version 17.0J) to estimate the risk of each type of hyperuricemia.


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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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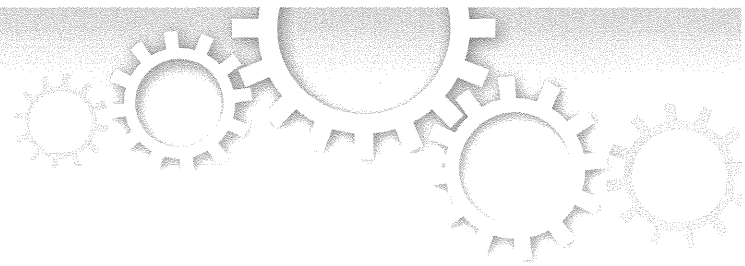

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# Common dysfunctional variants in *ABCG2* are a major cause of early-onset gout

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**Gout is a common disease which mostly occurs after middle age, but more people nowadays develop it before the age of thirty. We investigated whether common dysfunction of ABCG2, a high-capacity urate transporter which regulates serum uric acid levels, causes early-onset gout. 705 Japanese male gout cases with onset age data and 1,887 male controls were genotyped, and the ABCG2 functions which are estimated by its genotype combination were determined. The onset age was 6.5 years earlier with severe ABCG2 dysfunction than with normal ABCG2 function ($P = 6.14 \times 10^{-3}$). Patients with mild to severe ABCG2 dysfunction accounted for 88.2% of early-onset cases (twenties or younger). Severe ABCG2 dysfunction particularly increased the risk of early-onset gout (odds ratio 22.2, $P = 4.66 \times 10^{-6}$). Our finding that common dysfunction of ABCG2 is a major cause of early-onset gout will serve to improve earlier prevention and therapy for high-risk individuals.**

Gout is a common disease which causes acute arthritis as a consequence of hyperuricemia[1]. Gout and hyperuricemia are reportedly associated with other common diseases[1], such as hypertension[2,3], coronary artery diseases[4], cerebrovascular diseases[5], and kidney diseases[6]. Although gout mostly occurs after middle age[7], the number of patients experiencing its onset at a younger age is now increasing[8,9]. While gout with an earlier onset has a heritable component[10], its common genetic causes are still unclear.

ATP-binding cassette (ABC) transporter, subfamily G, member 2 gene *ABCG2/BCRP* locates in a gout-susceptible locus (MIM 138900) on chromosome 4q[11], which was earlier demonstrated by a genome-wide linkage

Table 1 | ABCG2 functions of participants

| Estimated Function | Genotype Combination | | Number (%) | |
|---|---|---|---|---|
| | Q126X* (rs72552713) | Q141K* (rs2231142) | Gout | Control |
| ≤1/4 function | **T**/**T** | C/C | 37 (5.2) | 22 (1.2) |
| | **T**/C | C/**A** | | |
| 1/2 function | **T**/C | C/C | 169 (24.0) | 219 (11.6) |
| | C/C | **A**/**A** | | |
| 3/4 function | C/C | C/**A** | 331 (47.0) | 699 (37.0) |
| Full function | C/C | C/C | 168 (23.8) | 947 (50.2) |
| Total | | | 705 (100.0) | 1,887 (100.0) |

*Risk alleles (T for Q126X, A for Q141K) are indicated in bold type at four locations, respectively.

study of gout[11]. Genome-wide association studies (GWAS) of serum uric acid (SUA) also identified several transporter genes including *ABCG2*[12–14]. Recently, Woodward *et al.*[15] and the present authors[16] independently showed that ABCG2 regulates SUA as a urate transporter, which mediates urate excretion. We also showed that genotyping of only two dysfunctional variants, Q126X (rs72552713) and Q141K (rs2231142), is sufficient to estimate the severity of ABCG2 dysfunction; i.e. full function, 3/4 function (mild dysfunction), 1/2 function (moderate dysfunction), and ≤ 1/4 function (severe dysfunction). This dysfunction increases gout risk markedly, conferring an OR of more than 3.0[16]. Furthermore, our human genetic analysis and animal model studies demonstrated that ABCG2 dysfunction plays an important role in the pathogenesis of hyperuricemia[17]. Because the dysfunctional *ABCG2* genotype combinations are very common in gout/hyperuricemia patients[15,16,18,19], ABCG2 dysfunction is a possible major cause of early-onset gout. In this study, we investigated the estimated ABCG2 function in 705 gout cases with onset age data and 1,887 controls to determine whether or not common dysfunction of ABCG2 causes early-onset gout.

## Results

**Onset age and ABCG2 function.** Table 1 shows the genotype and estimated function of ABCG2 in 2,592 male Japanese (705 gout cases and 1,887 controls). Among them, in 705 gout cases, the less activity the ABCG2 function showed the younger the onset age of gout became (Fig. 1). The onset age of patients with severe ABCG2 dysfunction (≤ 1/4 function) was 6.5 years younger than those with full function. Cox regression analysis also showed that ABCG2 dysfunction significantly hastened the onset age ($P = 6.14 \times 10^{-3}$).

**Association analysis of gout.** The logistic regression analysis of ABCG2 dysfunction demonstrated the increased risk of gout in each dysfunctional group with 705 cases and 1,887 controls. The odds ratio (OR) was 2.74 (95% CI 2.21–3.39; $P = 3.98 \times 10^{-20}$) with mild dysfunction (3/4 function), and was markedly increased to 9.98 (95% CI 5.63–17.7; $P = 3.62 \times 10^{-15}$) with severe dysfunction (≤ 1/4 function) (Fig. 2).

The subsequent logistic regression analysis was performed to evaluate the association between ABCG2 dysfunction and early-onset gout (twenties or younger), as ABCG2 dysfunction accounted for as much as 88.2% of the early-onset gout cases. Compared with full function, severe ABCG2 dysfunction especially increased the risk of early-onset gout, conferring an adjusted OR of 22.2 (95% CI 5.89–83.7; $P = 4.66 \times 10^{-6}$). In addition, moderate and mild dysfunction of ABCG2 markedly increased the risk of early-onset gout, conferring an adjusted OR of 15.3 (95% CI 7.53–30.9; $P = 4.08 \times 10^{-14}$) and 6.47 (95% CI 3.31–12.7; $P = 4.89 \times 10^{-8}$), respectively (Supplementary Fig. S1). In fact, any dysfunction of ABCG2 significantly increased the risk of gout in all onset-age groups (Fig. 2).

## Discussion

Our findings make it clear for the first time that any ABCG2 dysfunction causes early-onset gout. Dysfunctional ABCG2 accounts for approximately 90% of early-onset gout patients and accelerated early onset significantly in the present study. Moreover, the risk of early-onset gout is markedly increased by severe ABCG2 dysfunction, conferring an adjusted OR of 22.2. Thus, ABCG2 dysfunction is indeed a major cause of early-onset gout. To our knowledge, this is the first report on a common genetic cause of an early-onset gout that occurs in the twenties or earlier.

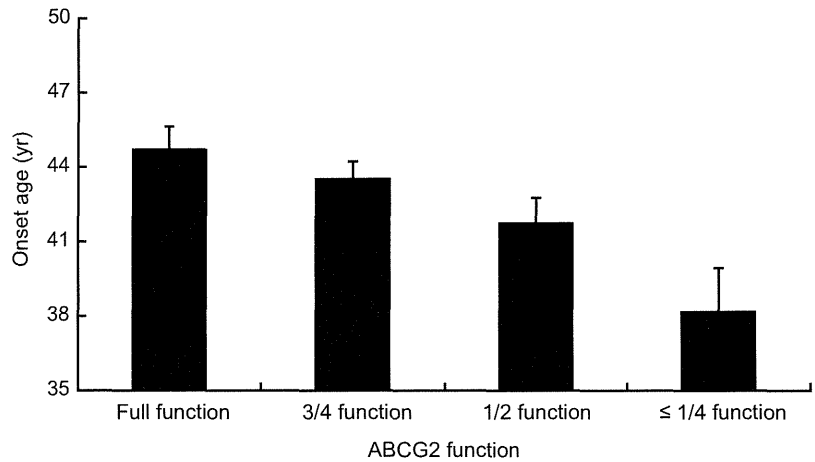


**Figure 1 | Onset age of gout for each ABCG2 function.** The onset age of cases with 1/4 function or less was 38.2 years old, whereas that with full function was 44.7 years old, a difference of 6.5 years. All bars show mean ± s.e.m.

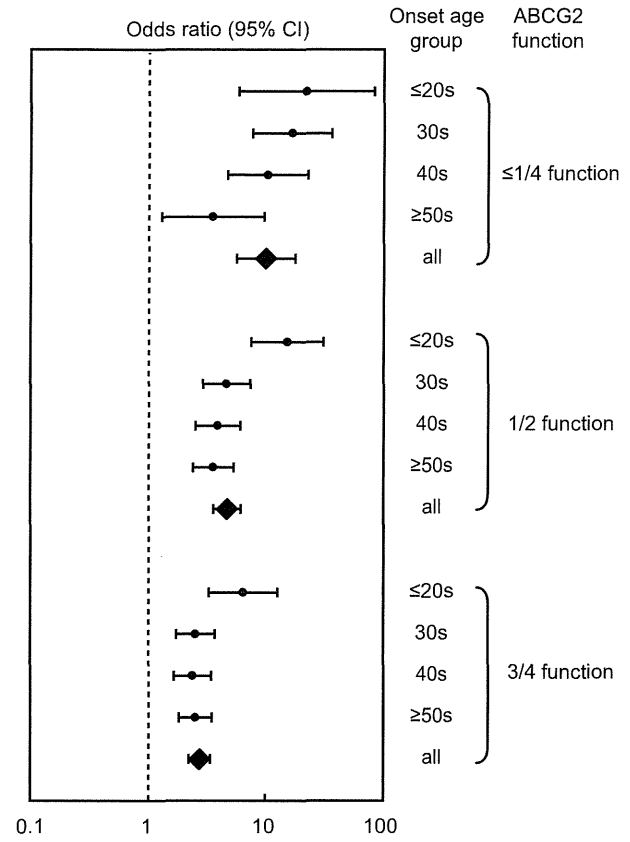


**Figure 2 | Odds ratios for ABCG2 dysfunctions among gout patients in each onset age group.** Shown are the odds ratios (ORs) on a $\log_{10}$ scale of the gout risks for each onset age group and ABCG2 dysfunction. ORs and 95% confidence intervals (CIs) for each ABCG2 dysfunction were obtained by comparing with full function and adjusted for body mass index with logistic regression analysis. Circles and diamonds with horizontal lines indicate ORs with 95% CIs of each onset age groups. All ABCG2 dysfunction levels significantly increased the risk of gout (OR > 2.38) in all onset-age groups. Severe ABCG2 dysfunction especially increased the risk of early-onset gout, conferring an adjusted OR of 22.2.

Generally, SUA levels in humans are higher than in most other mammals including mice, because humans lack the uric acid-degrading enzyme uricase[20]. Most uric acid mobilization is mediated by urate transporters in human kidneys. Therefore, human genetic studies have an advantage over rodent models in analyzing the urate transporters in humans. Indeed, in addition to ABCG2, our human genetic studies demonstrated that a urate transporter 1 (*URAT1/SLC22A12*) encodes renal urate reabsorption transporter and that its loss-of-function mutant causes renal hypouricemia type 1 (MIM 220150)[21]. After GWAS identified an association between SUA and glucose transporter 9 (*GLUT9/SLC2A9*) gene[22], we also demonstrated that *GLUT9* encodes another renal urate reabsorption transporter and is a causative gene for renal hypouricemia type 2 (MIM 612076)[23].

Recent genetic studies also revealed that various genes have associations with common diseases, such as coronary artery diseases[24–26], stroke[27], diabetes mellitus[26,28], and Alzheimer's disease[29]. The ORs to assess the risk of onset in these studies were, however, likely to fall in the 1.2 to 1.3 range or lower[30]. To date, there have been few genes to explain major genetic causes of common diseases. The same holds true for early-onset common diseases[31,32]. In the case of early-onset gout, the genetic causes have not been identified except for very rare Mendelian disorders[33] such as hypoxanthine guanine phosphoribosyltransferase (HPRT) deficiency including Lesch-Nyhan syndrome (MIM 300322)[34], phosphoribosylpyrophosphate synthetase (PRPS) superactivity (MIM 300661)[35], and familial juvenile hyperuricemic nephropathy (FJHN [MIM 162000])[36,37].

In the present study, Cox regression analysis of 705 gout patients revealed that ABCG2 dysfunction significantly decreases onset age ($P = 6.14 \times 10^{-3}$). The onset age was 6.5 years earlier with severe ABCG2 dysfunction. The gout risk is markedly increased in the younger generation having ABCG2 dysfunction. The ORs in the youngest onset-age group (onset age ≤ twenties) with severe, moderate and mild dysfunction were 22.2, 15.3 and 6.47, respectively (Fig. 2). These risks were considerably higher than those of all gout patients, conferring ORs of 9.98, 4.71 and 2.74, respectively (Fig. 2). Thus, ABCG2 dysfunction remarkably increases the risk of gout, especially for younger age-onset groups. In addition, mild to severe ABCG2 dysfunction was detected in up to 88.2% of early-onset gout patients, against 49.8% in controls. Our overall results clearly show that common dysfunction of ABCG2 is a major cause of early-onset gout.

Because early-onset gout will compromise patients' quality of life (QOL) for a long time and require huge life-long medical costs[38], early screening for ABCG2 dysfunction and appropriate interventions will greatly benefit high-risk individuals. Moreover, risk assessment by genotyping of only two SNPs will provide a very cost-effective method for screening and personalized medicine including adequate prevention and effective therapy. Therefore, our findings will serve to improve the QOL of high-risk individuals and reduce health-care costs, which also promote public health and preventive medicine.

## Methods

**Study participants.** All procedures were carried out in accordance with the standards of the institutional ethical committees involved in this project and the Declaration of Helsinki. Informed consent in writing was obtained from each subject participating in this study. Genotyping was performed in 2,592 male Japanese (705 gout cases and 1,887 controls). All cases were clinically diagnosed as primary gout according to the criteria established by the American College of Rheumatology[39] at the gout clinics of either Jikei University Hospital (Tokyo, Japan) or Midorigaoka Hospital (Osaka, Japan). Patients with inherited metabolism disorders including Lesch-Nyhan syndrome were excluded beforehand, and onset age data were available in all cases. As control, 1,887 individuals were assigned from Japanese male health examinees with normal SUA (≤ 7.0 mg/dl) and no gout history.

**Genetic analysis.** Genomic DNA was extracted from whole peripheral blood cells[40]. Genotyping of Q126X (rs72552713) and Q141K (rs2231142) in *ABCG2* gene by high-resolution melting (HRM) analysis was performed with a LightCycler 480 (Roche Diagnostics)[41]. To confirm their genotypes, more than one hundred samples including all genotype combinations identified by HRM were subjected to direct sequencing. DNA sequencing analysis was performed with a 3130xl Genetic Analyzer (Applied Biosystems)[23]. *ABCG2* genotype combinations were divided into four functional groups on the basis of the estimated ABCG2 transport functions[16]; i.e. full function, 3/4 function (mild dysfunction), 1/2 function (moderate dysfunction) and ≤ 1/4 function (severe dysfunction) as shown in Table 1.

**Statistical analysis.** For all calculations in the statistical analysis, the software SPSS v. 16.0J (IBM Japan Inc., Tokyo, Japan) and JMP 10.0.0 (SAS Institute Japan Inc., Tokyo, Japan) were used. Logistic regression analysis was performed to estimate adjusted genetic effects. Cox regression analysis was conducted to obtain adjusted *P* value for onset age. These regression analyses were corrected by body-mass index (BMI).


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## Author contributions

H.M., K.I., T.T., A.N., M.H., H.S., Y.K. and N.S. designed the experiment. H.M., K.I., A.N., T.H. and T.S. carried out patient analysis. H.M., K.I., A.N., Y.K., Y.T., K.Y., H.I., Y.O., C.O., S.S., M.S., T.C., H.O., K.N. and N.S. performed genetic analysis. H.M., A.N., M.N., A.H., K.W., A.M. and N.H. collected samples. H.N., T.N. and Y. S. performed statistical analysis. H.M., K.I., T.T., A.N. and N.S. wrote the paper. H.M., K.I., T.T. and A.N. contributed equally to this work.

## Additional information

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

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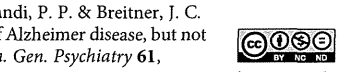

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VI 嚢胞性腎疾患

# ネフロン癆

Nephronophthisis

Key words：腎髄質，末期腎不全，嚢胞性腎疾患，nephrocystin，尿細管間質性腎炎

竹村　司

## 1. 概念・定義

ネフロン癆(nephronophthisis: NPH)は，腎髄質に嚢胞形成を認める疾患の代表であり，組織学的には，進行性の硬化，硝子化糸球体を伴う尿細管間質性腎炎像を呈する．遺伝形式は，主として常染色体劣性遺伝を示すが，孤発例もある[1]．末期腎不全(ESRD)に至る時期により，3つのサブタイプに分類される．それらは，3-5歳頃までにESRDとなる乳児ネフロン癆(NPH2)，幼少期から学童期までの比較的若年期に発症し，平均年齢13-14歳でESRDに移行する若年性ネフロン癆(NPH1)，平均年齢19歳頃にESRDに至る思春期ネフロン癆(NPH3)であり，なかでも最も頻度が高いものが若年性ネフロン癆である．現在，ネフロン癆には，*NPHP1*～*NPHP11*までの責任遺伝子が同定されているが，これらのいずれの遺伝子にも異常を見いだせないものも少なからず存在する．本症に対する特殊な治療法はなく，ESRDは避けられない状況にある．

## 2. 疫　　学

小児期の慢性腎不全の原因疾患として，我が国では3-4%，欧米では10-15%を占める．しかし，我が国では遺伝子解析がまだ十分に浸透しておらず，臨床学的，組織学的に評価されたものが大部分を占めるため，正確な発症頻度は不明な部分も多い．

## 3. 病因と病態

*NPHP1*は若年性ネフロン癆の責任遺伝子であり，染色体2q12-13上に存在し，nephrocystin-1分子をコードする[2]．nephrocystin-1は，腎では尿細管上皮細胞のprimary ciliaのtransition zoneに存在する．nephrocystinは，腎docking proteinとして，細胞対細胞，細胞対細胞外マトリックスのシグナル伝達に重要な役割を有し，またN-cadherin，catenin，β-cateninと協調して細胞接着にも関与する[3](図1)．また，β-tubulinとともに，actin cytoskeleton構造に影響を与え，細胞骨格の維持や細胞極性の変化にも寄与している．細胞内シグナル伝達の役割については，nephrocystinは，Crk-associated substrate(CAS)と複合体を形成し，Pyk2依存性経路を介して細胞内情報を核内まで伝達する．また最近の研究では，primary cilia上で，α-tubulinとともにその機能維持に寄与するだけでなく，細胞内小器官におけるシグナル伝達，すなわちcilia-sensoryにかかわる役割も明らかにされている．したがって，nephrocystin分子に異常を生じると，細胞と細胞外マトリックスとのシグナル伝達，細胞間接着，細胞骨格，細胞極性やciliaの機能，細胞内情報の核内への移行に障害が生じ，腎尿細管上皮の構造的・機能的障害を引き起こすことが推察される．

乳児ネフロン癆の責任遺伝子(*NPHP2*)は，9q22-31上に存在する．*NPHP2*は，*INVS*遺伝子，すなわちinversinと呼ばれる分子をコードする遺伝子を含み，*INVS*の異常は，ネフロン癆に類似した嚢胞形成を伴う腫大した腎とともに，内臓逆転位，膵臓におけるislet cellの異形成，心血管の欠損や形態異常，肝・胆管系障害など，様々な異常をきたし，その結果，乳児期

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本症の初期症状としては，多飲，多尿，尿最大濃縮能の低下，また2次性の遺尿や成長発育障害などがある．これらの症状は，4-6歳頃より出現する．主要な病態は，尿細管や集合管からのNaの喪失である．そのため，低Na血症，高K血症が認められるが，著明な浮腫や血尿，タンパク尿などの腎症状は通常初期には認められず，血圧も正常である．腎機能障害が進行した段階で，初めて尿量の減少とともに，Naの貯留や高血圧，貧血を呈する．したがって，低身長などの身体発育障害にて受診する小児については，本症をも念頭に置いた検査やフォローが必要である．また，原因不明の貧血を診た場合にも，本症も念頭に置き精査する．また，本症には，腎外症状を有する例もある．特に，網膜色素変性症(Senior-Loken症候群)，眼球運動の失調(Cogan症候群)，小脳失調症，肝線維症，骨格や顔貌の異常なども発見のためのポイントとなる[5]．

## 5. 治療と予後

現時点では特別有効な治療法はなく，低Na血症や高K血症あるいは代謝性アシドーシスに対して，食事療法，イオン吸着樹脂と重炭酸塩の投与を行う．また，腎機能の低下が進行する場合には，ESRDに準じた治療が行われる．また，本症の出生前診断は，現在のところ不可能であるため，家族に対する遺伝相談も重要である．本症の最大の問題点は，初期にはタンパク尿が陰性で，かなり病態が進展してからでないとタンパク尿が検出されない点である．タンパク尿の主体は，β2-microglobulin(MG)やα1-MGなどの低分子タンパク尿であるため，アルブミンを中心に検出する通常の試験紙法では検出されにくい．このような理由から，学校検尿をはじめとするマススクリーニング検査でも見逃される例が多い．すなわち，タンパク尿を検出したときには，既にESRD状態ということも珍しくない．また，β2-MGなどの低分子タンパク尿の検出も全例ではなく，しかもある程度病期が進展した状態になってから初めて検出されるということもある．すなわち，早期発見のための明らかな手がかりが少ないのが現状である．しかし，この疾患の概念を知っているか知っていないかで，発見時期に差が生じる可能性がある．予後は，各病型ともに時期は異なるが，ESRDに至ることは避けられない．適切な身体管理の下，生体腎移植を目指す．




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