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PATHOGENIC GLUT9 MUTATIONS CAUSING RENAL HYPOURICEMIA TYPE 2 (RHUC2)

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□ *Renal hypouricemia (MIM 220150) is an inherited disorder characterized by low serum uric acid levels and has severe complications such as exercise-induced acute renal failure and urolithiasis. We have previously reported that URAT1/SLC22A12 encodes a renal urate-anion exchanger and that its mutations cause renal hypouricemia type 1 (RHUC1). With the large health-examination database of the Japan Maritime Self-Defense Force, we found two missense mutations (R198C and R380W) of GLUT9/SLC2A9 in hypouricemia patients. R198C and R380W occur in highly conserved amino acid motifs in the "sugar transport proteins signatures" that are observed in GLUT family transporters. The corresponding mutations in GLUT1 (R153C and R333W) are known to cause GLUT1 deficiency syndrome because arginine residues in this motif are reportedly important as the determinants of the membrane topology of human GLUT1. Therefore, on the basis of membrane topology, the same may be true of GLUT9. GLUT9 mutants showed markedly reduced urate transport in oocyte expression studies, which would be the result of the loss of positive charges in those conserved amino acid motifs. Together with previous reports on GLUT9 localization, our findings suggest that these GLUT9 mutations cause renal hypouricemia type 2 (RHUC2) by their decreased urate reabsorption on both sides of the renal proximal tubule cells. However, a previously reported GLUT9 mutation, P412R, was unlikely to be pathogenic. These findings also enable us*

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to propose a physiological model of the renal urate reabsorption via GLUT9 and URAT1 and can lead to a promising therapeutic target for gout and related cardiovascular diseases.

Keywords Renal hypouricemia; *GLUT9/SLC2A9*; *GLUT1/SLC2A1*; gout/hyperuricemia; urate reabsorption transporter

INTRODUCTION

Renal hypouricemia is a common inherited disorder that is characterized by low serum uric acid (urate) levels and impaired renal urate transport; it is typically associated with severe complications such as exercise-induced acute renal failure and nephrolithiasis.[1,2] We have previously reported that a causative gene for renal hypouricemia is *URAT1/SLC22A12*.[3] However, the existence of renal hypouricemic patients without *URAT1* mutations[4,5] implies the presence of another urate transporter. Recent genome-wide association studies have revealed that the most significant single-nucleotide polymorphisms (SNPs) are associated with urate concentrations map within *GLUT9/SLC2A9*.[6–8] Therefore, we decided to search an actual human health examination database to genetically identify and investigate human hypouricemia patients with GLUT9 deficiency.

MATERIALS AND METHODS

Clinicogenetic Analysis of Hypouricemia with *GLUT9* Mutations

We used a large human database in our approach, and finally succeeded in identifying the *GLUT9* gene as the novel causative gene for renal hypouricemia. To collect a sufficient number of hypouricemia cases, we used the health examination database for about 50,000 Japan Maritime Self-Defense Force (JMSDF) personnel. We selected 21,260 personnel data sets in which serum urate data were available. Among them, 200 persons showed serum urate levels of $\leq$3.0 mg/dl (178 μM) (0.94%). 50 JMSDF persons who gave written consent and an additional 20 outpatients with hypouricemia (70 hypouricemic cases in sum) participated in this clinicogenetic study. First, we excluded the *URAT1* W258X mutation, the most frequent mutation in Japanese hypouricemia patients. After 47 cases having the *URAT1* W258X mutation were excluded, the remaining 23 hypouricemic cases were analyzed to find mutations in *GLUT9*.

Mutation Analysis and Functional Analysis of GLUT9

For the *GLUT9* sequence determination, we used primers described by Li et al. with slight modifications.[8] Some primer sequences were newly selected according to the genomic structure of the human *GLUT9*. High molecular

weight genomic DNA was extracted from peripheral whole blood cells[9] and was amplified by PCR. The PCR products were sequenced in both directions using a 3130xl Genetic Analyzer (Applied Biosystems). Functional analysis of GLUT9 mutants was performed using the *Xenopus* oocyte expression system, as described elsewhere.[3]

RESULTS

GLUT9 Mutations in Patients with Renal Hypouricemia

The human *GLUT9* gene contains 14 exons (1 noncoding and 13 coding) and is located on chromosome 4p15.3-p16. 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 were identified in the patients with renal hypouricemia. Both are missense mutations from the basic amino acid arginine to neutral amino acids. GLUT9L mutations were R380W and R198C, and GLUT9S mutations were R351W and R169C, which correspond to R380W and R198C in GLUT9L.

Urate Transport Activity in Oocytes

High urate transport activities were observed in oocytes that express each wild-type GLUT9 isoform. In contrast, urate transport activity in oocytes was markedly reduced (4.6%–10.8%) both in GLUT9L mutants (R198C and R380W) (Figure 1A) and in GLUT9S mutants (R169C and R351W) (Figure 1B). The P412R mutation[10] is unlikely to be a pathogenic mutation for renal hypouricemia because neither the P412R mutation in GLUT9L nor the P383R mutation in GLUT9S, which corresponds to P412R in GLUT9L, reduced their urate transport activities at all (Figure 1). The results from the GLUT9L mutants (Figure 1A) are quite similar to those from the GLUT9S mutants (Figure 1B), suggesting the reproducibility and reliability of the results.

Amino Acid Conservation in GLUT Family Transporters

GLUT9 mutations (R198C and R380W) are observed at the well-known conserved motif (D/E-x(2)-G-R-R/K) and another conserved motif (Y-x(2)-E-x(6)-R-G) that is 100% conserved in all GLUT family transporters. These motifs are a part of the consensus patterns 1/2 that are demonstrated in the PROSITE database (http://au.expasy.org/prosite/) as "sugar transport proteins signatures 1/2." The mutation sites in GLUT9 would be key residues in these consensus patterns.

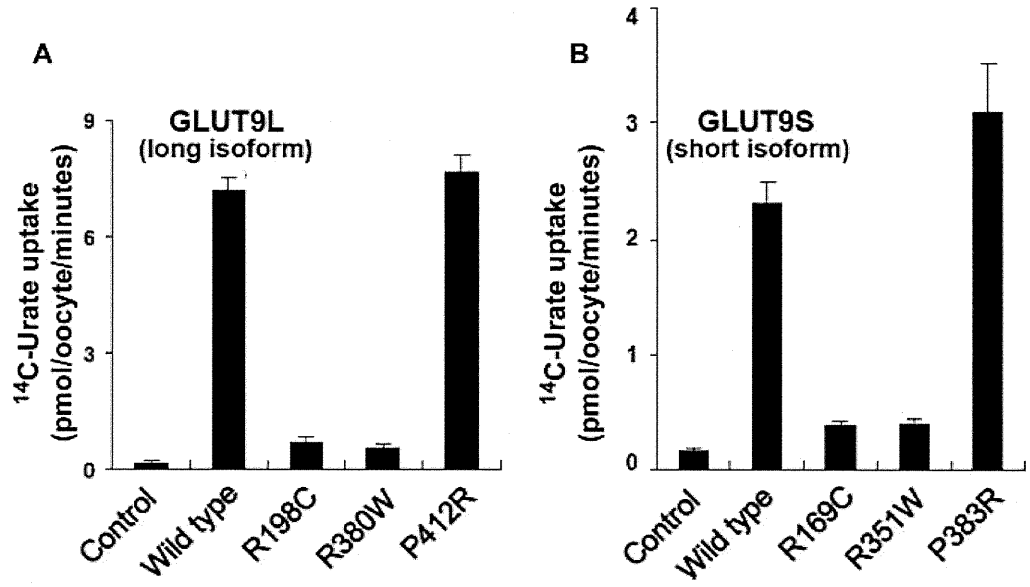

FIGURE 1 Urate transport activity via wild-type and mutant GLUT9 expressed in oocytes. Urate transport activity in oocytes was markedly reduced both (A) in GLUT9L mutants (R380W and R198C) and (B) in GLUT9S mutants (R351W and R169C, which correspond to R380W and R198C in GLUT9L). These figures also show that a P412R mutation in the *GLUT9* gene had less effect on the transport function.

DISCUSSION

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.[11] Therefore, it is of great significance to identify the nonfunctional mutations in human *GLUT9* using the large human database. In MDCK cells, GLUT9L and GLUT9S show basolateral and apical localization, respectively.[12] Since nonfunctional mutations of either GLUT9L or GLUT9S dramatically reduced the urate transport activity in our in vitro studies (Figure 1), renal hypouricemia caused by these mutations may be ascribed to the decreased urate reabsorption on both sides of the renal proximal tubules, where GLUT9 expresses.[13] Based on our findings, we propose a physiological model in which GLUT9 mediates renal urate reabsorption. These findings are also supported by Dinour et al., who reported on severe renal hypouricemia patients with GLUT9 homozygous mutations.[14]

GLUT9 Mutations and Perturbation of Membrane Topology

Interestingly, these *GLUT9* mutations (R198C and R380W) correspond to the *GLUT1* pathogenic mutations (R153C and R333W), which cause GLUT1 deficiency syndrome.[15] Sato and Mueckler reported that the loss of positive charges of GLUT1 result in the perturbation of the membrane topology and aberrant "flipping" of the corresponding cytoplasmic loop into the exogenous compartment.[16] This showed that the positive charge

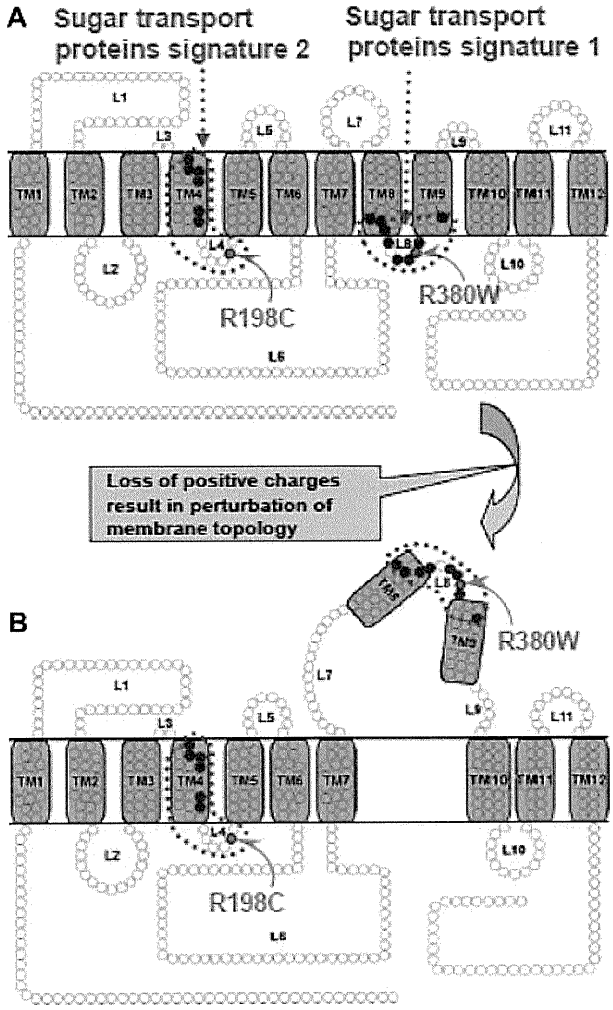

FIGURE 2 Pathogenic GLUT9 mutations causing renal hypouricemia type 2 and possible mechanisms. Both mutations are at equivalent positions within the cytoplasmic loops, which causes a loss of positive charge and results in diminished urate transport function via GLUT9.

of arginine residues in this conserved motif plays a critical role in forming cytoplasmic anchor points that are involved in the membrane topology of human GLUT1. The marked reduction of the urate transport activity in mutated GLUT9 may be ascribed to the loss of cytoplasmic anchor points and the local perturbation of the membrane topology (Figure 2).

GLUT9: Promising Therapeutic Target for Gout/Hyperuricemia

Taken together, we have identified *GLUT9* as a causative gene for renal hypouricemia type 2 (RHUC2) and demonstrated that human GLUT9 physiologically regulates serum urate levels in vivo. Since another urate reabsorption transporter, URAT1, is known to be a therapeutic target of a uricosuric agent benzbromarone, our results suggest that a urate reabsorption transporter GLUT9 can also be a promising therapeutic target for hyperuricemia and gout.

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Significant association of serum uric acid levels with *SLC2A9* rs11722228 among a Japanese population

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ABSTRACT

Genome-wide association studies identified that *SLC2A9* (*GLUT*9) gene polymorphisms were associated with serum uric acid (SUA) levels. Among the Japanese, a C/T polymorphism in intron 8 (rs11722228) was reported to be highly significant, though the function and strength of association were unknown. This study aimed to confirm the association, estimating the means of SUA according to the genotype, as well as OR of the genotype. Subjects were 5024 health checkup examinees (3413 males and 1611 females) aged 35 to 69 years with creatinine <2.0 mg/dL. Since *SLC22A12* 258X allele and *ABCG2* 126X allele are known to influence SUA levels strongly, the subjects with *SLC22A12 258WW* and *ABCG2 126QQ* (3082 males and 1453 females, in total 4535 subjects) were selected. The genotype frequency of *SLC2A9* rs11722228 was 2184 for *CC*, 1947 for *CT*, and 404 for *TT*, being in Hardy–Weinberg equilibrium (p = 0.312). Mean SUA was 6.10 mg/dL for *CC*, 6.25 mg/dL for *CT*, and 6.45 mg/dL for *TT* among males (p = 1.5E-6), and 4.34 mg/dL, 4.59 mg/dL, and 4.87 mg/dL among females (p = 4.6E-11), respectively. Males with SUA less than 5.0 mg/dL were 14.7% for *CC*, 10.6% for *CT*, and 7.8% for *TT* (p = 2.3E-4), and females with SUA less than 4.0 mg/dL were 34.1%, 25.5%, and 15.4% (p = 3.7E-6), respectively. This study was the first report to estimate the impact of *SLC2A9* rs11722228 on SUA levels. Since the allele frequency of rs11722228 is similar among different ethnic groups, the impact remains to be examined in other ethnic groups.

1. Introduction

Serum uric acid (SUA) levels are partly regulated by genetic traits. *ATP-binding cassette subfamily G member 2* (*ABCG2*) gene in chromosome 4q22, coding a uric acid transporter, has a functional polymorphism, Q126X (rs72552713), which was reported to reduce transportation activity, resulting in hyperuricemia [1–3]. Uric acid transporter 1 (URAT1) encoded by *SLC22A12* in chromosome 11q13 is a uric acid anion exchanger, which reabsorbs uric acid in renal tubules [4,5]. *SLC22A12* W258X polymorphism with the reduced function causes renal hypouricemia [6–8]. Glucose transporter 9 (GLUT9) encoded by *SLC2A9* in chromosome 4p16-15.3 is also the molecule to reabsorb uric acid in kidney. The rare mutations of *SLC2A9* were found in hypouricemia patients; R380W and R198C in Japanese [9], L75R in an Israeli-Arab family, exon 7 deletion in Ashkenazi-Jewish [10], and Ile118HisfsX27 (g.27073insC at exon 3, causing Ile118His and stop codon at position 27) in a Czech family [11].

A genome-wide association study (GWAS) on SUA for 28,141 participants of European descent demonstrated the associations with nine genes including *SLC22A12*, *SLC2A9*, and *ABCG2*[12]. The polymorphism of *SLC2A9* selected in the GWAS was rs734553, whose minor allele frequency was reported to be 0.011 in a Japanese population (HapMap-JPT, ss80703). Another GWAS for 1017 African American detected four polymorphisms (rs3775948, rs7663032, rs6856398, and rs6449213) of *SLC2A9* associated with SUA [13]. For 14,700 Japanese, a GWAS identified the associations with *SLC22A12*, *SLC2A9*, and *ABCG2*[14]. In the Japanese GWAS, a C/T polymorphism in intron 8 of *SLC2A9* (rs11722228) was identified as a highly significant polymorphism (p = 7.1E-24).

In this study, we aimed to confirm the association with rs11722228, and further to examine the strength of the association in terms of odds ratio (OR), after eliminating the effects of *SLC22A12*

Abbreviations: ABCG2, ATP-binding cassette subfamily G member 2; bp, base pairs; CI, confidence interval; GLUT9, glucose transporter 9; GWAS, genome-wide association study; OR, odds ratio; PCR-CTPP, polymerase chain reaction with confronting two-pair primers; SUA, serum uric acid; URAT1, uric acid transporter 1.

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258X and *ABCG2* 126X. The present study was approved by the Ethics Committee of Nagoya University School of Medicine (approval number 288).

2. Materials and methods

2.1. Statistical power to detect a significant finding

Under the conditions that a genotype frequency in controls is 0.1, OR is 0.5, two-sided alpha error is 0.05, controls are 1000, and cases are 400, the statistical power is calculated to be 0.8137. As described below, the corresponding numbers of subjects were analyzed for males and females.

2.2. Subjects and data collection

Subjects were 5028 health checkup examinees in a health checkup center in Hamamatsu, Japan, who were the participants of Japan Multi-Institutional Collaborative Cohort Study (J-MICC Study) in 2007–2008 [15,16]. Written informed consent including genotyping was obtained from all participants. Excluding one participant without a blood sample, genotyping was successfully conducted for 5027 participants. Three participants with creatinine of 2.0 mg/dL or over were excluded from the analysis, and 5024 subjects remained.

The health checkup data including blood tests were used for this study. Peripheral blood was drawn in the morning from those with overnight fast. SUA was measured enzymatically (a uricase method) using an auto-analyzer.

2.3. Genotyping procedure

DNA was extracted from buffy coat conserved at −80 °C using a BioRobot® M48 (QIAGEN Group, Tokyo). *SLC2A9* rs11722228 polymorphism was genotyped by a polymerase chain reaction with confronting two-pair primers (PCR-CTPP) [17]. Each 25 μl reaction tube contained 50–80 ng DNA, 0.12 mM dNTP, 12.5 pmol of each primer, 0.5 U AmpliTaq Gold (Perkin-Elmer, Foster City, CA) and 2.5 μl of 10× PCR buffer including 15 mM $MgCl_2$. The PCR-CTPP was conducted with initial denaturation at 95 °C for 10 min, 35 cycles of denaturation at 95 °C for 1 min, annealing at 56 °C for 1 min, and extension at 72 °C for 1 min, and a final extension at 72 °C for 5 min. The primers were F1: 5′-GGA GCT ATC ACT GCT CC-3′, R1: 5′-CAG AGG ACA GTA GAA ATG TTT GG-3′, F2: 5′-GAT CGA GCC CTG ATC ATC T-3′, and R2: 5′-TAG TGA TTC ATC CCT GGT G-3′. The amplified DNA fragments were 124 base pairs (bp) for the *C* allele, 172 bp for the *T* allele, and 255 bp for common band, as illustrated in Fig. 1. *ABCG2* Q126X and *SLC22A12* W258X were genotyped as described in the previous papers [2,18].

2.4. Statistical analysis

Hardy–Weinberg equilibrium was examined with a chi-square test. The percentages among different genotypes were also tested with a chi-square test. Means among three genotype groups were tested with one-way analysis of variance. Adjusted OR and 95% confidence interval (CI) were estimated using an unconditional logistic regression model. All statistical analyses were performed using STATA software version 11 (STATA, College Station, TX).

3. Results

The subjects were derived from 5024 health checkup examinees (3413 males and 1611 females) aged 35 to 69 years with creatinine <2.0 mg/dL. Table 1 shows the characteristics of the examinees. Those with SUA less than 3.0 mg/dL were 0.6% in males and 5.2% in females, while those with SUA of 7.0 mg/dL or over were 23.5% in males and 1.5% in females. The genotype frequency of *SLC2A9* rs11722228 was in Hardy–Weinberg equilibrium (p = 0.179 in males and p = 0.674 in females). The minor allele (*T* allele) frequency was 0.306 in males, 0.304 in females, and 0.305 in both sexes.

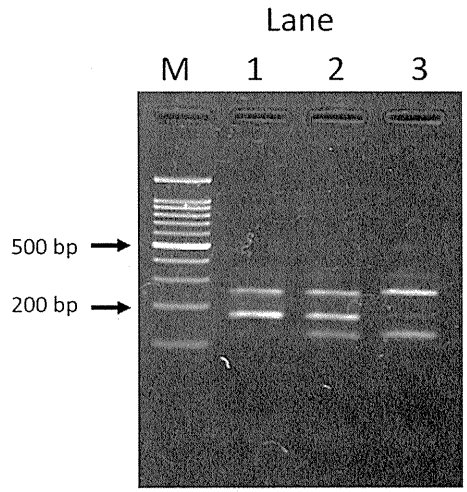

Fig. 1. Representative gel for *SLC2A9* rs11722228 polymorphism. Lane M, a 100-bp ladder; lane 1, a *TT* homozygote with fragments of 172 bp and 255 bp; lane 2, a *CT* heterozygote with fragments of 124 bp, 172 bp and 255 bp; and lane 3, a *CC* with fragments of 124 bp and 255 bp.

Among them, examinees with *SLC22A12* 258X (230 individuals), *ABCG2* 126X (250 individuals), and/or not genotyped (one for *SLC22A12* W258X and thirteen for *ABCG2* Q126X) were excluded from the analysis, and 4535 subjects (3082 males and 1453 females) with *SLC22A12 258WW* and *ABCG2 126QQ* remained. The genotype frequency was 2184 for *CC*, 1947 for *CT*, and 404 for *TT*, which was in Hardy–Weinberg equilibrium (p = 0.312). Table 2 shows the distribution of SUA levels according to the genotype. Although there was no marked difference in the distribution among three genotypes, males with SUA less than 5.0 mg/dL were 14.7% for *CC*, 10.6% for *CT*, and 7.8% for *TT* (p = 2.3E-4), and females with SUA less than 4.0 mg/dL were

Table 1
Characteristics of participants according to sex.

Characteristics	Males		Females	
	N	(%)	N	(%)
Total number	3413	(100)	1611	(100)
Age (years)				
35–39	265	(7.8)	192	(11.9)
40–49	983	(28.8)	496	(30.8)
50–59	1366	(40.0)	630	(39.1)
60–69	799	(23.4)	293	(18.2)
SUA (mg/dL)				
2.9 and below	21	(0.6)	84	(5.2)
3.0–4.9	522	(15.3)	1074	(66.7)
5.0–6.9	2069	(60.6)	429	(26.6)
7.0 and above	801	(23.5)	24	(1.5)
BMI (kg/m^2)				
18.4 and below	91	(2.7)	160	(9.9)
18.5–24.9	2423	(71.0)	1196	(74.2)
25.0 and above	899	(26.3)	255	(15.8)
Creatinine (mg/dL)				
0.0–0.4	0	(0.0)	33	(2.1)
0.5–0.9	2710	(79.4)	1575	(97.8)
1.0–1.4	701	(20.5)	3	(0.2)
1.5–1.9	2	(0.1)	0	(0.0)
SLC2A9 rs11722228				
CC	1627	(47.7)	785	(48.7)
CT	1483	(43.5)	674	(41.8)
TT	303	(8.9)	152	(9.4)

SUA; serum uric acid, BMI; body mass index, and BUN; blood urea nitrogen.

Table 2
Serum uric acid (SUA) distribution (%) according to *SLC2A9* rs11722228 genotype among Japanese health checkup examinees with *SLC22A12 258WW* and *ABCG 126QQ*.

Genotype	N	SUA (mg/dL)										Mean	95%CI[a]
		0.0–0.9	1.0–1.9	2.0–2.9	3.0–3.9	4.0–4.9	5.0–5.9	6.0–6.9	7.0–7.9	8.0–8.9	9.0 and above		
Males													
CC	1472	0.0	0.0	0.2	1.7	12.8	32.1	31.2	16.2	5.0	0.7	6.10	6.05–6.16
CT	1342	0.0	0.0	0.1	1.3	9.2	32.3	32.0	17.0	7.1	1.0	6.25	6.19–6.31
TT	268	0.0	0.0	0.0	2.2	5.6	24.3	34.7	25.4	6.7	1.1	6.45	6.32–6.59
Total	3082	0.0	0.0	0.1	1.6	10.6	31.5	31.9	17.3	6.1	0.9	6.20	6.16–6.24
Females													
CC	712	0.0	0.0	4.2	29.9	42.0	20.9	2.0	1.0	0.0	0.0	4.34	4.28–4.41
CT	605	0.0	0.2	2.8	22.5	43.5	22.5	6.8	1.3	0.5	0.0	4.59	4.51–4.66
TT	136	0.0	0.0	2.2	13.2	43.4	28.7	10.0	1.5	1.5	0.0	4.87	4.70–5.05
Total	1453	0.0	0.1	3.4	25.3	42.7	22.3	4.7	1.2	0.3	0.0	4.49	4.45–4.52

[a] 95% confidence interval for SUA mean.

34.1%, 25.5%, and 15.4% (p = 3.7E-6), respectively. Mean serum uric acid was 6.10 mg/dL for *CC*, 6.25 mg/dL for *CT*, and 6.45 mg/dL for *TT* among males (p = 1.5E-6), and 4.34 mg/dL, 4.59 mg/dL, and 4.87 mg/dL among females (p = 4.6E-11), respectively. The difference in the mean between *CC* genotype and *TT* genotype was 0.35 mg/dL in males and 0.53 mg/dL in females.

Table 3 shows the mean and standard deviation of SUA for each genotype according to sex and age group. The difference of mean SUA was 0.13 mg/dL for age 35–49 years, 0.45 mg/dL for age 50–59 years, and 0.55 mg/dL for age 60–69 years in males, while the difference in females was 0.54 mg/dL, 0.68 mg/dL, and 0.30 mg/dL, respectively.

The age-adjusted OR and 95% CI of low SUA (<5 mg/dL in males and <4 mg/dL in females) are shown in Table 4. Those with *CT* genotype or *TT* genotype had a significantly lower OR relative to those with *CC* genotype. From the view of hyperuricemia, those with *TT* genotype were found to be less resistant. The age-adjusted OR of SUA 7.0 mg/dL or over was 1.18 (95% CI, 1.00–1.40) for *CT* genotype and 1.62 (95% CI, 1.24–2.12) for *TT* genotype relative to *CC* genotype in males. In females, the OR was 1.99 (95%CI, 0.80–4.92) and 3.23 (95% CI, 0.95–11.1), respectively.

4. Discussion

This study confirmed that mean SUA was significantly higher for *SLC2A9* rs11722228 *TT* genotype than for *CC* genotye among those with *SLC22A12 258WW* and *ABCG2 126QQ*. The difference was larger among females than among males. The frequency of rs11722228 T allele was 0.305 among 5024 subjects, similar to that reported in ss44579198 HapMap-JST (0.289 of 2 N = 90), as well as HapMap-CEU European (0.333 of 2 N = 120), HapMap-HCB Asian (0.284 of 2 N = 88), and HapMap-YRI Sub-Saharan African (0.375 of 2 N = 120). The function of this polymorphism has not been reported, although GLUT9 is known to be an important molecule regulating SUA.

SUA is lower in females than in males. In addition, it is fully documented that age, menopause, meat consumption, alcohol intake, obesity, a sedentary lifestyle, dyslipidemia, insulin resistance, blood pressure, renal function, and drug use for hypertension were associated with SUA levels [19–24]. Accordingly, it is no doubt that SUA levels are determined by lifestyle and physical conditions. Meanwhile, recent studies elucidated that genotypes are also influential factors of SUA.

SLC22A12 258X allele and *ABCG2 126X* allele are actually very influential, but their alleles were relatively rare; 0.023 for *SLC22A12* 258X and 0.025 for *ABCG2* 126X in this dataset. The removal of those with either of the two alleles was thought to be essential to evaluate the impact of *SLC2A9* rs11722228. *ABCG2 141KK* genotype was also reported to elevate SUA levels, which was equivalent to *126WX*[2]. When 445 subjects with *141KK* genotype in the present subjects were excluded from the analysis, quite similar findings were observed. The mean SUA was 6.07 mg/dL for *CC*, 6.22 mg/dL for *CT*, and 6.42 mg/dL for *TT* among 2790 males, and 4.34 mg/dL, 4.58 mg/dL, and 4.76 mg/dL among 1324 females, respectively. To date, several polymorphisms

Table 3
Mean and standard deviation of serum uric acid (mg/dL) for *SLC2A9* rs11722228 genotype according to sex and age group among Japanese health checkup examinees with *SLC22A12 258WW* and *ABCG 126QQ*.

Genotype	Males (N = 3082)			Females (N = 1453)		
	35–49 years (N)	50–59 years (N)	60–69 years (N)	35–49 years (N)	50–59 years (N)	60–69 years (N)
CC	6.20 ± 1.15 (552)	6.02 ± 1.10 (580)	6.09 ± 1.04 (340)	4.12 ± 0.78 (292)	4.43 ± 0.84 (272)	4.62 ± 0.93 (148)
CT	6.32 ± 1.05 (485)	6.16 ± 1.19 (526)	6.28 ± 1.13 (331)	4.30 ± 0.82 (270)	4.70 ± 0.98 (240)	5.11 ± 1.07 (95)
TT	6.33 ± 1.07 (97)	6.47 ± 1.13 (119)	6.64 ± 1.18 (52)	4.66 ± 0.90 (62)	5.11 ± 1.13 (55)	4.92 ± 1.04 (19)
Whole	6.26 ± 1.10 (1134)	6.12 ± 1.15 (1225)	6.22 ± 1.10 (723)	4.25 ± 0.82 (624)	4.61 ± 0.95 (567)	4.82 ± 1.02 (262)

Table 4
Age-adjusted odds ratio (OR) and 95% confidence interval (95% CI) of low serum uric acid for *SLC2A9* rs11722228 genotype among Japanese health checkup examinees with *SLC22A12 258WW* and *ABCG 126QQ*.

Genotype	Males (N = 3082)						Females (N = 1453)					
	<5 mg/dL[a]		≥5 mg/dL[a]		OR	(95% CI)	<4 mg/dL[a]		≥4 mg/dL[a]		OR	(95% CI)
	N	(%)	N	(%)			N	(%)	N	(%)		
CC	217	(57.1)	1255	(46.4)	1	(Reference)	243	(58.1)	469	(45.3)	1	(Reference)
CT	142	(37.4)	1200	(44.4)	0.68	(0.54–0.85)	154	(36.8)	451	(43.6)	0.62	(0.49–0.80)
TT	21	(5.5)	247	(9.1)	0.49	(0.31–0.79)	21	(5.0)	115	(11.1)	0.32	(0.20–0.53)
Total	380	(100)	2702	(100)			418	(100)	1035	(100)		

[a] Serum uric acid levels.

including *MTHFR* C677T have been reported to have an association with SUA [25,26], but the impact was limited. This study indicated that *SLC2A9* rs11722228 had a substantial influence on SUA, though it was less marked than that of *SLC22A12* 258X and *ABCG2* 126X.

GLUT9 plays a role to reabsorb uric acid in kidney. The reduced activity is considered to lower SUA levels. The reported mutations (R380W and R198C in Japanese [9], L75R in an Israeli-Arab family, exon 7 deletion in Ashkenazi-Jewish [10], and Ile118HisfsX27 in a Czech family [11]) cause hypouricemia. Since the *CC* genotype of *SLC2A9* rs11722228 showed a lower mean SUA than the *TT* genotype, it indicated that the function of the *CC* genotype may be lower than that of the *TT* genotype, though there was no biological evidence. A recent study demonstrated that *SLC2A9* rs1014290 in intron 4 had a similar size of differences in the mean SUA among the genotypes, but the biological characteristics of the polymorphism were also not fully described [27].

The effect of *SLC2A9* rs11722228 on mean SUA was larger for females than for males in this study. The finding was consistent with the past studies [12,28–30]. Although it is known that estrogens reduce SUA levels possibly through more efficient renal clearance of SUA [31], the biological mechanism in connection with the GLUT9 function has not been clarified.

One study reported that the difference in mean SUA among the genotypes was larger for older groups in females, but not in males [29]. The present study found that the difference was larger in older groups in males, and not in females. The reason for the inconsistency was not clear.

One of the limitations in the present study was that the medication for hyperuricemia was not taken into account. Since many with hyperuricemia were treated to lower the SUA, it would tend to weaken the association of SUA with the genotype. Even under this situation, the highly significant moderate associations were observed in this study. Another limitation was no adjustment for the lifestyle factors associated with SUA. Generally speaking, genotypes are not associated with lifestyle, so that the confounding by lifestyle may be limited, if any, in this study. At least, sex, age, body mass index, and creatinine levels were not associated with the genotype.

5. Conclusions

SLC2A9 rs11722228 *TT* genotype was associated with higher SUA levels among those with *SLC22A12 258WW* and *ABCG2 126QQ*. The exclusion of those with *SLC22A12* 258X and *ABCG2* 126X made a clear comparison among the different genotypes of *SLC2A9* rs11722228. Since the T allele frequency of rs11722228 is similar among different ethnic groups, the impact remains to be examined in the other ethnic groups. In addition, the function of the polymorphism and linked polymorphisms needs to be examined.

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Two cases of nephrotic syndrome (NS)-induced acute kidney injury (AKI) associated with renal hypouricemia

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Key words
AKI – minimal change nephrotic syndrome – renal hypouricemia

Abstract. Renal hypouricemia is a clinical disorder attributed to an increased renal urate excretion rate and is well known to involve a high risk of urolithiasis and exercise-induced acute kidney injury (AKI). This report concerns two interesting cases of nephrotic syndrome (NS)-induced AKI associated with renal hypouricemia. A 64-year-old female (Case 1) and a 37-year-old male (Case 2) were hospitalized because of AKI (serum creatinine: 2.07 mg/dl and 3.3 mg/dl, respectively), oliguria and NS. They were treated with prednisolone and temporary hemodialysis. Renal function improved, but hypouricemia persisted during hospitalization. Histological findings in both cases led to a diagnosis of minimal change nephrotic syndrome and identification of the diuretic phase of tubulointerstitial damage because of findings such as acute tubular necrosis. Furthermore, distal tubules of Case 2 showed an amorphous mass, possibly a uric acid crystal. Analysis of the two cases with the URAT1 gene, encoded by SLC22A12, found a homozygous mutation in exon 4 (W258stop) of each one. Our cases show that patients with renal hypouricemia may be susceptible to AKI without involvement of exercise if they possess some facilitators. Renal hypouricemic patients should therefore be carefully examined for all complications from renal hypouricemia because of high risk of AKI.

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Introduction

Renal hypouricemia is a clinical disorder attributed to an increased renal excretion rate of urate. Although hypouricemia is mostly asymptomatic, it is well known to involve a high risk of urolithiasis and exercise-induced acute kidney injury (AKI) [1].

Some studies have dealt with the association of exercise-induced AKI with renal hypouricemia [2, 3], but no studies appear to have dealt with cases complicated with nephrotic syndrome (NS) and AKI associated with renal hypouricemia. Here we report two interesting cases of NS-induced AKI associated with renal hypouricemia.

Case reports

Case 1

A 64-year-old female experienced leg and facial edema in late May 2005. She had been treated with nonsteroidal antiinflammatory drugs (NSAIDs) because of lumbago since she was 30. When she visited a local clinic on June 1, her body weight had increased 3 kg. Laboratory findings showed a slight elevation of serum creatinine (s-Cr: 1.08 mg/dl). Two days later she visited the same clinic again and was diagnosed with AKI and NS because of elevated s-Cr (2.07 mg/dl), and diminished total protein (TP: 5.2 g/dl) and albumin (Alb: 2.3 g/dl), and proteinuria (3+). She was admitted to a general hospital on June 3.

After admission, she showed oliguria (urine volume: 186 ml/day), and general fatigue. Because her s-Cr level was elevated even more, she underwent hemodialysis (HD) for 3 days. Four days after admission, she was transferred to Kobe University Hospital on June 7.

On admission, her arterial blood pressure was 110/60 mmHg and pulse rate 72 beats/min.

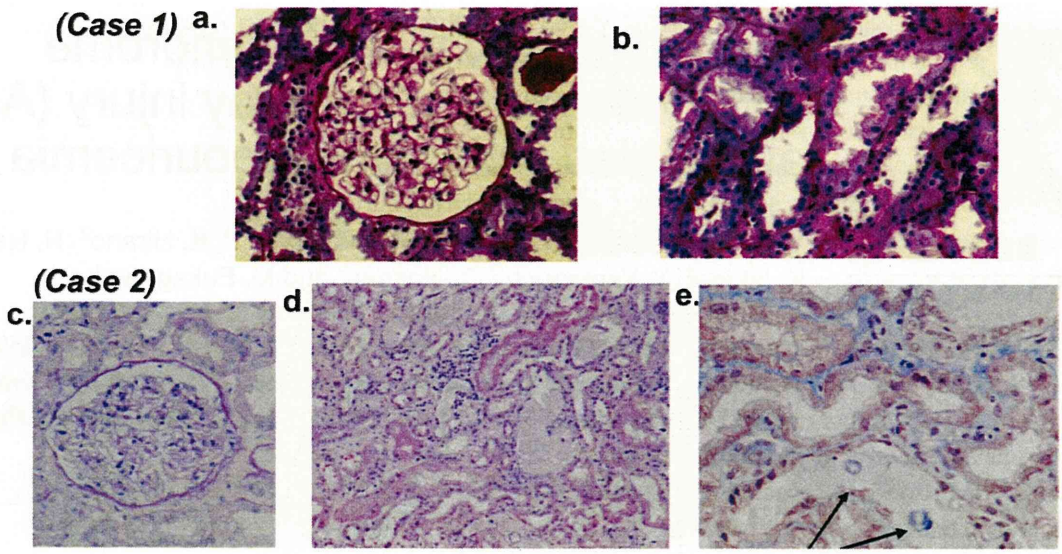

Figure 1. Renal pathology. a: Minor glomerular abnormalities (Periodic acid-Shiff (PAS) staining, × 400). b: Proximal and distal tubular cells had partial increase of nuclear density and swelling of nuclei. Tubular regenerative change of epithelial cells was focally observed. (PAS staining, × 400). c: Minor glomerular abnormalities (PAS staining, × 400). d: Vacuolization, desquamation, and regenerative changes of tubular epithelial cells was observed. Tubular regenerative changes in the proximal tubular epithelial cells, which indicate moderate patchy tubular injury was observed. (PAS staining, × 400). e: Crystalloid body (Urate salt?) was partly observed within hyaline cast(↑). (Masson Tricrome staining, × 400).

She weighed 51.3 kg and was 153 cm tall. She showed pitting edema in the pretibial area.

Laboratory evaluation showed hemoglobin (Hb) of 10.9 g/dl (109 g/l); platelet count (Plt): 230 × 10^3/μl(230 × 10^9/l); white blood cells (WBC): 6.5 × 10^3/μl (6.5 × 10^9/l); blood urea nitrogen (BUN): 21 mg/dl (7.5 mmol/l); S-Cr: 3.18 mg/dl (281.1 μmol/l); uric acid (UA): 4.0 mg/dl (normal: 3.8 ~ 5.1 mg/dl) (238 μmol/l); TP: 4.6 g/dl (46 g/l); Alb 1.7 g/dl (17 g/l); creatine kinase (CK): 132 U/l; total-cho 384 mg/dl. Urinalysis found protein 4+ (5.4 g/day), occult blood –, sediments; RBC 1 ~ 4/HPF, WBC 0 ~ 1/HPF, granular cast +, waxy cast +. Abdominal computed tomography (CT) scan, chest X-ray and electrocardiogram all appeared normal.

Renal pathology

Kidney biopsy was performed on July 20 (Day 41 of steroid therapy).

Light microscopy with Periodic Acid-Shiff (PAS) staining showed 23 glomeruli without any remarkable changes. Focal tubulointerstitial damage was observed. Proximal and distal tubular cells showed partial increase in nuclear density and swelling of nuclei, while tubular regenerative change of epithelial cells was focal (Figure 1).

Immunofluorescence demonstrated no staining for immunoglobulins complements.

Electron microscopy demonstrated mild foot process effacement. There were no electron dense deposits.

Our diagnosis of minimal change nephrotic syndrome (MCNS) was based on evidence of NS, minimal glomerular abnormalities, and a good response to steroid therapy. Renal biopsy was indicated for the diuretic phase of tubulo-interstitial damage such as acute tubular necrosis (ATN).

Clinical course

Because the clinical history indicated suspected NS-induced AKI, treatment with prednisolone (PSL) 50 mg/day was initiated after steroid pulse (methylprednisolone 1 g/day × 3 days). However, because oliguria and renal dysfunction continued (June 13: BUN 70 mg/dl; Cr 7.25 mg/dl; UA 6.8 mg/dl), the patient underwent HD 5 times in total.

Renal function improved to creatinine clearance (CCr) of 77.7 ml/min at hospital discharge, and proteinuria, which was 5.4 g/day, disappeared after steroid therapy.

Hypouricemia persisted during hospitalization (UA: 0.6 ~ 1.0 mg/dl), although a kid-

ney biopsy showed only slight tubular damage and the patient was already in recovery. Moreover, fractional excretion of uric acid (FEUA) ranged from 61.3 to 100% (normal: < 10%), suggesting an increase in the renal excretion rate of urate and renal hypouricemia. In consideration of the effect of AKI, probenecid and pyrazinamide loading tests were performed one year after discharge. Probenecid caused FEUA to increase from 61.3% to 66.4%, while pyrazinamide did not reduce FEUA to less than 53.0%. These test findings prompted us to classify this case as presecretory reabsorption defect of UA.

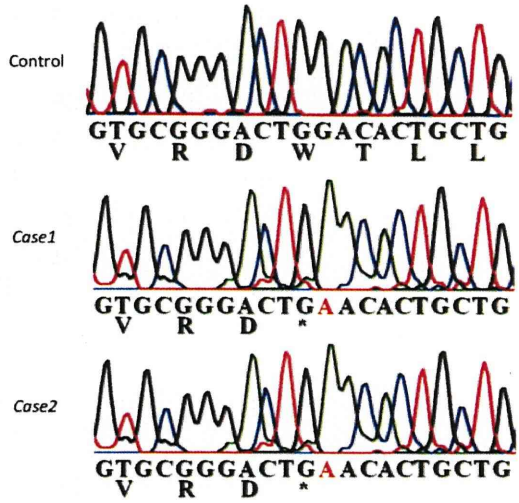

Figure 2. Mutation analysis of *SLC22A12*.

Case 2

A 37-year-old male experienced leg edema in late February 1999. After a checkup in April found proteinuria (4+), he presented with oliguria on May 10 and his body weight increased about 9 kg. When he visited the Jikei University Aoto hospital, laboratory findings showed elevation of s-Cr (3.3 mg/dl) and BUN (87.2 mg/dl). He was diagnosed with AKI and NS, and was hospitalized on May 15.

On admission, arterial blood pressure was 132/76 mmHg and pulse rate 76 beats/min. He weighed 91.2 kg and was 171.8 cm tall. He presented with general pitting edema.

Laboratory evaluation showed Hb of 15.3 g/dl (153 g/l); Plt 328 × 10^3/μl (328 × 10^9/l); WBC 9.1 × 10^3/μl (9.1 × 10^9/l); blood BUN 87.2 mg/dl (31.13 mmol/l); S-Cr 3.3 mg/dl (291.72 μmol/l); UA 3.3 mg/dl (196.3 μmol/l); TP 4.0 g/dl (40 g/l); Alb 2.1 g/dl (21 g/l); CK 98 U/l. Urinalysis found protein 4+ (17.38 g/day) and occult blood 2+, sediments; RBC 5 ~ 10/HPF, WBC 10 ~ 19/HPF, granular cast 3+, waxy cast +, fatty cast +. Abdominal CT scan, chest X-ray and electrocardiogram all appeared normal.

Renal pathology

Kidney biopsy was performed on July 1 (Day 47 of steroid therapy).

Light microscopy with PAS staining showed global sclerosis in 2 of 18 glomeruli with minimal abnormalities.

Tubular regenerative changes were also observed in the proximal tubular cells, indicating patchy tubular injury. Furthermore, distal tubules were filled with hyaline casts which had in part escaped to the interstitium. Crystalloid bodies (Urate salt?) were found in part of the hyaline casts, suggesting that obstructive nephropathy might be involved (Figure 1).

Immunofluorescence demonstrated mild insudative deposits of IgM and C1q in glomerular mesangial areas.

The diagnosis of MCNS was evident. Renal biopsy was indicated for the recovery phase of tubulo-interstitial damage such as acute tubular necrosis.

Clinical course

Because the clinical history indicated suspected NS-induced AKI, treatment with PSL 50 mg/day was initiated. But because oliguria and renal dysfunction continued (May 27: BUN 131 mg/dl; Cr 4.7 mg/dl; UA 5.3 mg/dl), HD was administered 10 times in total.

Renal function improved to CCr 64.5 ml/min, and proteinuria decreased from 17.38 g/day (urinary quantity: 500 ml/day) to 2.86 g/day at discharge.

Hypouricemia persisted during hospitalization (UA: 0.8 ~ 1.1 mg/dl) and FEUA was 64%, suggesting an increase in the renal excretion rate of urate. Probenecid and pyrazinamide loading were not administered.

Two cases sequence analysis of the URAT1 Gene

Mutation in URAT1 is known as a cause of renal hypouricemia [4]. We analyzed two cases with the URAT1, encoded by SLC22A12 and reported by Ichida et al [1], and found a homozygous mutation in exon 4 (W258stop) in each one (Figure 2).

Discussion

Renal hypouricemia is a common hereditary disease in Japanese and possibly in the non-Ashkenazi Jewish ethnic group [4]. It is accompanied by urolithiasis and exercise-induced AKI in 10% of patients [1, 2]. In 2002, Ishikawa reviewed exercise-induced AKI including 49 reported cases of exercise-induced AKI with renal hypouricemia and clarified the characteristics [5]. In the same year, Enomoto et al. identified a urate transporter, URAT1, responsible for renal hypouricemia [6].

Renal hypouricemia leads to low antioxidant capability because of less UA, a scavenger of free radicals. Exercise-induced AKI with renal hypouricemia is suggested to result from increased free radicals caused by exercise, which induces renal reperfusion injury due to renal vasoconstriction.

Interestingly, our cases had no exercise before the manifestation of AKI. We do not know whether renal hypouricemia plays a causal role in the development of NS (MCNS)-induced AKI, because there have been no reports on cases like ours.

Recent reports note about the importance of reactive oxygen species (ROS) in kidney disease [7, 8, 9]. ROS have been considered to have roles in the pathogenesis of glomerular disease such as NS, postischemic or toxic AKI. Cellular defense mechanisms against ROS include enzyme systems that directly remove some species (superoxide dismutase, catalase and glutathione peroxidase) and nonenzymatic scavengers that are either endogenous molecules (albumin, glutathione, and UA) or derived from the diet (vitamin C, vitamin E, carotenoids, selenium and zinc) [10].

This accounts for the increase in oxidative stress in renal hypouricemia patients with NS because of hypoalbuminemia and low intake of certain antioxidant components due to appetite loss resulting from NS. These results indicate that renal hypouricemia with NS is likely to lead to kidney injury under increased oxidative stress even without exercise that exceeds the antioxidant capacity of the human body. It is therefore a matter of concern that our cases with NS and renal hypouricemia also had high oxidative stress, since this can lead to AKI just as exercise can.

As for the frequency of cases needing dialysis due to AKI with MCNS, Shibasaki et al. in 1990 [11] reported that 12 patients showed AKI followed by primary NS among 420 cases, and 3 cases (2 cases of MCNS, 1 case of FGS) needed continuous and transient dialysis. In addition, Kawai et al. in 1994 [12] reported a case of renal hypouricemia with NS and idiopatic hypercalcemia, but their case did not lead to AKI. Our cases can be considered rare in the sense that the incidence of AKI due to MCNS and renal hypouricemia needed transient HD.

Furthermore, Case 1 had been taking NSAIDs. According to the review by Ishikawa [5], about 37.9% of patients with exercise-induced AKI had taken analgesics including NSAIDs. Therefore, NSAIDs might facilitate exercise-induced AKI.

The renal biopsy of Case 2 showed an amorphous mass, possibly a UA crystal. Renal hypouricemia is well known to lead to urolithiasis, and renal tubular obstruction with UA crystals resulting from explosive UA loading to tubules is another causative candidate.

In our cases AKI was induced not only by the reduction in effective circulatory volume related to MCNS and administration of NSAIDs but also by renal hypouricemia, which features obstruction with UA and antioxidant fragility, and also oxidative stress related to NS.

When a patient suffering from AKI with renal hypouricemia is first seen, it is generally difficult to diagnose renal hypouricemia because of the increasing level of UA. Since Case 1 was admitted after two dialysis sessions, it is difficult to determine whether the level of UA was low or high. The UA level of Case 2 was within normal range, but could be low in view of AKI. For this reason, careful attention should be paid to any signs of hypouricemia during the recovery phase of AKI.

Renal involvement with renal hypouricemia is focused on exercised-induced AKI

only. But our cases show that patients with renal hypouricemia may be susceptible to AKI without involvement of exercise if they possess some facilitators. Renal hypouricemic patients with NS as in our cases should therefore be carefully examined in consideration of the possibility of causing AKI depending on antioxidant stress.

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DIAGNOSTIC TESTS FOR PRIMARY RENAL HYPOURICEMIA

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□ *Primary renal hypouricemia is a genetic disorder characterized by defective renal uric acid (UA) reabsorption with complications such as nephrolithiasis and exercise-induced acute renal failure. The known causes are: defects in the SLC22A12 gene, encoding the human urate transporter 1 (hURAT1), and also impairment of voltage urate transporter (URATv1), encoded by SLC2A9 (GLUT9) gene. Diagnosis is based on hypouricemia (<119 μmol/L) and increased fractional excretion of UA (>10%). To date, the cases with mutations in hURAT1 gene have been reported in East Asia only. More than 100 Japanese patients have been described. Hypouricemia is sometimes overlooked; therefore, we have set up the flowchart for this disorder. The patients were selected for molecular analysis from 620 Czech hypouricemic patients. Secondary causes of hyperuricosuric hypouricemia were excluded. The estimations of (1) serum UA, (2) excretion fraction of UA, and (3) analysis of hURAT1 and URATv1 genes follow. Three transitions and one deletion (four times) in SLC22A12 gene and one nucleotide insertion in SLC2A9 gene in seven Czech patients were found. Three patients had acute renal failure and urate nephrolithiasis. In addition, five nonsynonymous sequence variants and three nonsynonymous sequence variants in SLC2A9 gene were found in two UK patients suffering from acute renal failure. Our finding of the defects in SLC22A12 and SLC2A9 genes gives further evidence of the causative genes of primary renal hypouricemia and supports their important role in regulation of serum urate levels in humans.*

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INTRODUCTION

Primary hereditary renal hypouricemia (OMIM: 220150) is an inborn error of renal membrane transport of uric acid, with an autosomal recessive mode of inheritance.[1] This genetic disorder is characterized by defective renal uric acid reabsorption. The known major causes are defects in the *SLC22A12* gene,[2] encoding the human urate transporter 1 (hURAT1), and also impairment of the voltage urate transporter (URATv1), encoded by *SLC2A9* (GLUT9) gene.[3] The hURAT1 mainly acts as an influx transporter for urate at apical membrane at the proximal renal tubule.[2] URATv1 (GLUT9) is an efflux transporter of intracellular urate from the tubular cell to the interstitium/blood space.[3] The majority of patients are asymptomatic, but some may experience urolithiasis and/or be predisposed to exercise-induced acute renal failure. Some patients developed hematuria only. Diagnosis is based on hypouricemia (<119 μmol/L) and an increased fractional excretion of UA (>10%). Confirmation of the diagnosis is accomplished by molecular analysis of the *SCL22A12* and *SLC2A9* genes. Therapy is based on alkalization of urine, drinking of plenty of water, and avoidance of strenuous exercise.[2–4] To date, the cases with mutations in *hURAT1* gene have been reported only in East Asia, with more than 100 Japanese patients described.[5,6]

Although the measurement of uric acid in serum and urine is a useful diagnostic tool for many inborn errors of purine metabolism, asymptomatic hypouricemia is sometimes overlooked. We therefore suggest a flowchart for the diagnosis of primary hereditary renal hypouricemia.

MATERIALS AND METHODS

The patients (with hypouricemia and increased fractional excretion of uric acid) were selected for molecular analysis of *SLC22A12* and *SLC2A9* genes from 620 Czech hypouricemic patients. Those hypouricemic individuals were found from 3600 blood and urine samples. Serum and urinary uric acid and creatinine were determined. Uric acid in serum and urine was measured by a specific enzymatic method. Creatinine in plasma and urine was measured by the Jaffé reaction adapted for an autoanalyzer. The fraction of uric acid excreted was evaluated as uric acid clearance factored by creatinine clearance × 100. Sequence analysis by automated DNA sequencer (Applied Biosystems 3100) of the coding region of *SLC22A12* and *SLC2A9* genes was performed after informed consent.

(1) Estimation of uric acid (UA) in serum---if less than 119 μmol/L
↓
(2) Estimation of excretion fraction of UA---if more than 10%
↓
(3) Exclusion of other secondary causes of hyperuricosuric hypouricemia
↓
(4) Molecular analysis of *SLC22A12* and *SLC2A9* genes

FIGURE 1 Protocol for the diagnosis of hereditary renal hypouricemia.

RESULTS

Figure 1 shows our established protocol for the diagnosis of primary hereditary renal hypouricemia. The first step is measurement of uric acid in serum. When a patient with hypouricemia is identified, estimation of the fraction excretion of uric acid follows. The exclusion of secondary causes of hyperuricosuric hypouricemia (such as Wilson disease and Fanconi syndrome, etc.) is important before molecular analysis of the *SLC22A12* and *SLC2A9* genes.

Using this flowchart, we were able to diagnose eight new patients with hypouricemia and an increased fractional excretion of uric acid due to primary hereditary renal hypouricemia (Table 1). All the patients fulfilled the criteria for idiopathic renal hypouricemia, which consist of (1) hypouricemia (serum uric acid concentration of less than 119 μmol/L), (2) fractional excretion of uric acid more than 10%, and (3) no other secondary causes of hyperuricosuric hypouricemia such as Fanconi syndrome, Wilson disease, or drug-induced tubulopathy were found.

We have found three transitions and deletion in the *SLC22A12* gene. One nucleotide insertion in the *SLC2A9* gene was found.[7] Three patients

TABLE 1 Clinical features and mutations (patients 1–6 in *SLC22A12* gene and patients 7–8 in *SLC2A9* gene)

Case	Sex	Age (yrs)	UA (μmol/L)	FEUA (%)	ARF	Uro-lithiasis	Mutation
1.	F	73	124	52.4	+	–	g. 82948302del
2.	F	39	58	53.4	+	–	g. 82948302del/g.9184C/T
3.	F	53	78	60.3	–	–	g. 82948302del/g.9184C/T
4.	M	35	63	43.0	–	–	g. 8145G/Cg.9214G/A
5.	F	15	35	55.2	–	–	g. 8294-8302del g.9184C/T
6.	M	5	95	52.6	–	+	1242-1250delGCTGGCAGG
7.	F	18	11	240.0	–	–	g. 43412_43413insC
8.	M	23	10	220.0	–	–	g. 43412_43413insC

UA—serum uric acid; FEUA—fractional excretion of uric acid; ARF—exercise-induced acute renal failure.