

***SLC22A12* W258X FREQUENCY ACCORDING TO SERUM URIC ACID LEVEL AMONG JAPANESE HEALTH CHECKUP EXAMINEES**

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ABSTRACT

Although the *SLC22A12* (uric acid transporter 1) 258X allele is known to cause hypouricemia, the genotype frequency according to the serum uric acid (SUA) level has not been reported. This study investigated the *SLC22A12* 258WX frequency according to SUA levels among Japanese health-checkup examinees. In addition, the changes were reported in SUA levels during five years for individuals with 258WX. Subjects were 746 Japanese aged 39–86 years in 2003. Their SUA records were linked during the five years from 2003 to 2007. *SLC22A12* W258X was genotyped using a polymerase chain reaction with confronting two-pair primers. The 258X allele comprised 1.9% (95% CI, 1.3–2.8%) of all the subjects. Among those with SUA <3.0 mg/dL, 258WX was more common in males (66.7%, 95% CI, 22.2–95.7%) than in females (39.3%, 95% CI, 21.5–59.4%). Among subjects with a SUA of 3.0–4.9 mg/dL, those with 258WX totaled 10.7% (95% CI, 4.0–21.9%) and 2.6% (95% CI, 1.1–5.0%), respectively. There were no subjects with 258WX among those with a SUA of 5.0 mg/dL or more. During the five years from 2003 to 2007, the changes in SUA among 23 individuals with 258WX were found to be similar to those among 258WW subjects (n=536). This study indicated that *SLC22A12* 258WX was more common among those with a lower serum uric acid concentration. The observed SUA level changes in individuals with 258WX suggested that lifestyle factors could influence the levels of those with 258WX.

Key Words: Hypouricemia, Urate transporter, *SLC22A12* W258X, Polymerase chain reaction with confronting two-pair primers

INTRODUCTION

Although idiopathic hypouricemia is clinically asymptomatic, it is associated with the risks of acute renal failure,^{1,2)} urolithiasis,^{1,3,4)} and hematuria.⁵⁾ While there is no doubt that lifestyle affects serum uric acid (SUA) levels, genetic traits are the factors that determine SUA levels.

Uric acid transporter 1 (URAT1) encoded by *SLC22A12* is a uric acid anion exchanger that reabsorbs uric acid in renal tubules.^{6,7)} *SLC22A12* genotypes with a reduced function have been reported to cause renal hypouricemia. To date, the 16 polymorphisms so far reported in *SLC22A12* are: 11 missense polymorphisms (R90H, V138M, G164S, T127M, A226V, R228E, E298D,

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Q312L, Q382L, M430T, and R477H), two nonsense polymorphisms (W258X and Q297X), two short deletions (1639-1643delGTCCT, del1313D-333P), and one splicing polymorphism (IVS2+1 G>A).⁸⁻¹¹ The W258X polymorphism, which produces a non-functioning truncated protein that lacks half of the mature protein, is predominant among Japanese patients with hypouricemia; in one study, six (85.7%) out of seven patients had a 258X allele,⁸ while the allele was found in 29 (90.6%) out of 32 in another study.⁹ Among 30 Korean hypouricemic examinees, seven (23.3%) were found to have a 258X allele.¹²

Although the *SLC22A12* W258X frequencies among those with hypouricemia were reported as above, the frequency according to SUA levels in a general population remains unknown. Furthermore, the changes in SUA among those with 258WX have yet to be reported. The present study investigated the frequency of *SLC22A12* W258X polymorphism according to the SUA levels among Japanese health checkup examinees. In addition, the changes in SUA levels of individuals with 258WX were examined to document the possible effects of lifestyle on the SUA.

MATERIALS AND METHODS

Subjects

Subjects were 864 residents in a rural area of Hokkaido, Japan, who attended a health checkup in August, 2003. Among them, 803 subjects (280 males and 523 females) aged 39–86 years agreed to participate in this study along with written informed consent. After excluding 57 examinees who reported being under medication for gout, or with blood urea nitrogen greater than 23 mg/dL, or with creatinine greater than 1.1 mg/dL, the remaining 746 subjects (238 males and 508 females) were deemed eligible for the present analysis. This study was approved by the Ethics Committee of the Nagoya University School of Medicine (approval number 398).

Data collection

Using a self-administered questionnaire, their health and lifestyle including habits of smoking (never, former, or current) and drinking (never, former, or current) were obtained at the time of their health checkup. The resulting health checkup data, including blood tests, were used for this study. Peripheral blood was drawn in the morning from those fasting overnight, or in the afternoon from those without lunch. Biochemical analysis of the sampled sera was performed using an auto-analyzer (JCA-RX20, Nihon Denshi Co. Ltd.), and SUA levels were measured by the uricase-POD method. Body height and weight were measured during the health checkup, with the body mass index (BMI) calculated from body weight (kg) divided by height (m) squared. The changes in SUA levels were followed for subjects who attended the annual health checkup during the five years from 2003 to 2007.

Genotyping procedure

DNA was extracted from a buffy coat conserved at -80°C using a BioRobot® EZ1 (QIAGEN Group, Tokyo). The *SLC22A12* W258X polymorphism was genotyped by a polymerase chain reaction with confronting two-pair primers (PCR-CTPP).¹³ 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 10x PCR buffer including 15 mM MgCl_2 . The PCR-CTPP was conducted with the initial denaturation at 95°C for 10 minutes, 35 cycles of denaturation at 95°C for 1 minute, annealing at 61.5°C for 1 minute, and extension at 72°C for 1 minute, and with a final extension at 72°C for 5 minutes. The W258X primers were F1: 5'-TCC ATG CAG GCT CCA GG-3', R1: 5'-ACC ACC AGC TGC AGC AGT GTT-3', F2: 5'-TAC GGT GTG CGG

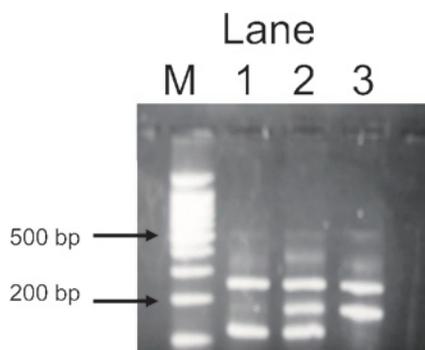


Fig. 1 Representative gel for *SLC22A12* W258X polymorphism. Lane M, a 100-bp ladder; lane 1, a WW homozygote with fragments of 117 bp and 255 bp; lane 2, a WX heterozygote with fragments of 117 bp, 176 bp and 255 bp; lane 3, a XX homozygote with fragments of 176 bp and 255 bp.

GAC TGG-3', and R2: 5'-GGC AGG ATC TCC TCT GAG G-3'. The amplified DNA fragments were 117-base pairs (bp) for the G (258W) allele, 176-bp for the A (258X) allele, and 255-bp for a common band, as illustrated in Fig. 1.

Statistical analyses

Hyperuricemia was defined as SUA level ≥ 7.0 mg/dL, and hypouricemia as SUA level < 3.0 mg/dL. The Hardy-Weinberg equilibrium was examined with a chi-square test. Means between two groups were examined with a *t*-test. The 95% confidence interval (CI) of percentages was calculated based on binomial distribution. Age- and sex-adjusted odds ratios (ORs) and 95% CIs were estimated using an unconditional logistic regression model. Two-sided *p*-values less than 0.05 were considered statistically significant. All statistical analyses were performed using STATA software version 7.0 (STATA, College Station, TX).

RESULTS

Subject characteristics according to sex are summarized in Table 1. The mean and standard deviation (SD) of age was 62.3 ± 10.5 years in males and 60.0 ± 9.9 years in females. Hyperuricemia was found to be 16.4% ($n=39$) in males and 1.8% ($n=9$) in females, while hypouricemia was 2.5% ($n=6$) and 5.5% ($n=28$), respectively. The SUA mean was significantly higher in males than in females (5.7 ± 1.3 mg/dL vs 4.6 ± 1.1 mg/dL, $p < 0.001$).

Among the 746 examinees, 29 individuals (10 males and 19 females) were found with 258WX, and none with 258XX. The 258X allele was 1.9% (95% CI, 1.3–2.8). The genotype distribution was in Hardy-Weinberg equilibrium for males ($p=0.74$), for females ($p=0.67$), and for the combined ($p=0.59$).

Table 2 shows means and SD of age, BMI, blood tests, and blood pressure, according to the *SLC22A12* W258X. A significant difference in SUA was observed between those with 258WW and 258WX; 5.9 ± 1.2 mg/dL and 3.1 ± 1.2 mg/dL for males, and 4.6 ± 1.0 mg/dL and 2.9 ± 0.9 mg/dL for females, respectively. The age- and sex-adjusted OR of the 258WX genotype relative to the 258WW genotype was 45.6 (95% CI, 18.4–113.2) for hypouricemia.

The frequency of the 258WX genotype according to the SUA is shown in Table 3. Among those with hypouricemia, 258WX was more common in males (66.7%, 95% CI, 22.2–95.7%)

Table 1 Characteristics of participants according to sex

Characteristics	Males (n=238)		Females (n=508)	
	N	(%)	N	(%)
Age (years)				
-49	31	(13.0)	76	(15.0)
50-59	56	(23.5)	169	(33.3)
60-69	89	(37.5)	175	(34.4)
70-	62	(26.1)	88	(17.3)
BMI (kg/m ²)				
<18.4	5	(2.1)	14	(2.8)
18.5-24.9	152	(63.9)	307	(60.4)
25.0-	81	(34.0)	187	(36.8)
SUA (mg/dL)				
<0.9	1	(0.4)	0	(0.0)
1.0-1.9	0	(0.0)	3	(0.6)
2.0-2.9	5	(2.1)	25	(4.9)
3.0-3.9	13	(5.5)	113	(22.2)
4.0-4.9	43	(18.1)	200	(39.4)
5.0-6.9	137	(57.6)	158	(31.1)
7.0-	39	(16.4)	9	(1.8)
Creatinine (mg/dL)				
<0.4	0	(0.0)	20	(3.9)
0.5-0.7	88	(37.0)	447	(88.0)
0.8-1.0	150	(63.0)	41	(8.1)
BUN (mg/dL)				
<7.9	1	(0.4)	11	(2.2)
8.0-19.9	210	(88.2)	477	(93.9)
20.0-22.9	27	(11.3)	20	(3.9)
Smoking				
Never	47	(19.7)	407	(80.1)
Former	116	(48.7)	51	(10.0)
Current	75	(31.5)	47	(9.3)
Unknown	0	(0.0)	3	(0.6)
Drinking				
Never	83	(34.9)	429	(84.4)
Former	17	(7.1)	11	(2.2)
Current	138	(58.0)	66	(13.0)
Unknown	0	(0.0)	2	(0.4)

BMI: body mass index; SUA: serum uric acid; BUN: blood urea nitrogen

SLC22A12 W258X ACCORDING TO SERUM URATE

Table 2 Means and standard deviations (SD) of age, body mass index (BMI), blood tests, and blood pressure according to *SLC22A12* W258X genotype

Characteristics	Males			Females		
	WW (n=228)	WX (n=10)	p value	WW (n=489)	WX (n=19)	p value
	Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD	
Age (years)	62.4 ± 10.6	59.9 ± 7.4	0.462	59.9 ± 9.9	63.4 ± 9.6	0.123
BMI (kg/m ²)	24.1 ± 3.1	23.0 ± 1.9	0.293	24.0 ± 3.4	24.6 ± 2.9	0.431
Uric acid (mg/dL)	5.9 ± 1.2	3.1 ± 1.2	<0.001	4.6 ± 1.0	2.9 ± 0.9	<0.001
Triglyceride (mg/dL)	113.1 ± 76.4	80.9 ± 20.2	0.186	92.0 ± 47.4	115.3 ± 85.6	<0.05
Total cholesterol (mg/dL)	207.4 ± 31.5	206.3 ± 31.9	0.911	217.6 ± 33.2	218.3 ± 34.2	0.925
HDL (mg/dL)	54.0 ± 12.7	55.6 ± 6.9	0.697	62.1 ± 13.7	57.9 ± 11.2	0.191
Creatinine (mg/dL)	0.79 ± 0.12	0.74 ± 0.11	0.189	0.61 ± 0.10	0.62 ± 0.12	0.635
BUN (mg/dL)	15.2 ± 3.2	16.2 ± 3.5	0.352	13.9 ± 3.2	14.4 ± 3.7	0.515
Hematocrit (%)	44.3 ± 3.4	43.8 ± 3.6	0.603	39.7 ± 3.1	39.1 ± 3.8	0.378
ALP (IU/L)	155.5 ± 43.8	160.1 ± 48.5	0.748	158.6 ± 48.8	171.2 ± 73.0	0.280
Total protein (g/dL)	7.5 ± 0.42	7.3 ± 0.46	0.236	7.6 ± 0.41	7.60 ± 0.29	0.716
C reactive protein (mg/L)	0.17 ± 0.46	0.05 ± 0.04	0.409	0.10 ± 0.29	0.14 ± 0.19	0.510
Systolic blood pressure (mm Hg)	138.3 ± 19.2	144.2 ± 24.0	0.349	135.0 ± 19.4	133.1 ± 16.9	0.660
Diastolic blood pressure (mmHg)	88.2 ± 10.7	92.4 ± 13.7	0.230	84.4 ± 10.5	82.9 ± 9.3	0.564

BMI: body mass index; HDL: high-density lipoprotein cholesterol; BUN: blood urea nitrogen; ALP: alkaline phosphatase

Table 3 *SLC22A12* 258WX genotype frequency (%) and 95% confidence interval (95% CI) according to serum uric acid (SUA)

	SUA (mg/dL)						Total
	0.0–0.9	1.0–1.9	2.0–2.9	3.0–3.9	4.0–4.9	5.0–	
Males							
258WX	1	0	3	4	2	0	10
%	100.0	–	60.0	30.8	4.7	0.0	4.2
(95%CI)	(2.5–100)	(–)	(14.7–94.7)	(9.1–61.4)	(0.6–15.8)	(0–2.1)	(2.0–7.6)
Females							
258WX	0	2	9	4	4	0	19
%	–	66.7	36.0	3.5	2.0	0.0	3.7
(95% CI)	(–)	(9.4–99.2)	(18.0–57.5)	(1.0–8.8)	(0.5–5.0)	(0–2.2)	(2.3–5.8)

than in females (39.3%, 95% CI, 21.5–59.4%). Among subjects with an SUA of 3.0–4.9 mg/dL, those with 258WX were 10.7% (95% CI, 4.0–21.9%) and 2.6% (95% CI, 1.1–5.0%), respectively. There were no individuals with 258WX among those with a SUA equal to 5.0 mg/dL or more. The 258X allele was 1.9% (95% CI, 1.3–2.8%) of all the subjects.

Among the subjects, 8 males and 15 females participated in a health checkup twice or more between 2003 and 2007, the period available for a record linkage. Fig. 2 demonstrates changes in the SUA of 23 participants; (A) for 8 males, (B) for 8 females with BMI<25, and (C) for 7 females with BMI>25; the respective differences from 2003 to 2007 were 0.3–1.1 mg/dL, 0.4–1.0 mg/dL, and 0.2–2.4 mg/dL. In one female depicted in Fig. 2 (C), a marked reduction in SUA was observed between 2005 and 2006. She was a 71-year-old participant who never smoked and had no drinking habit. The available data showed no obvious reasons; her BMI was 26.9 in 2005 and 25.7 in 2006, with a serum creatinine of 0.5 mg/dL in both years. The change in

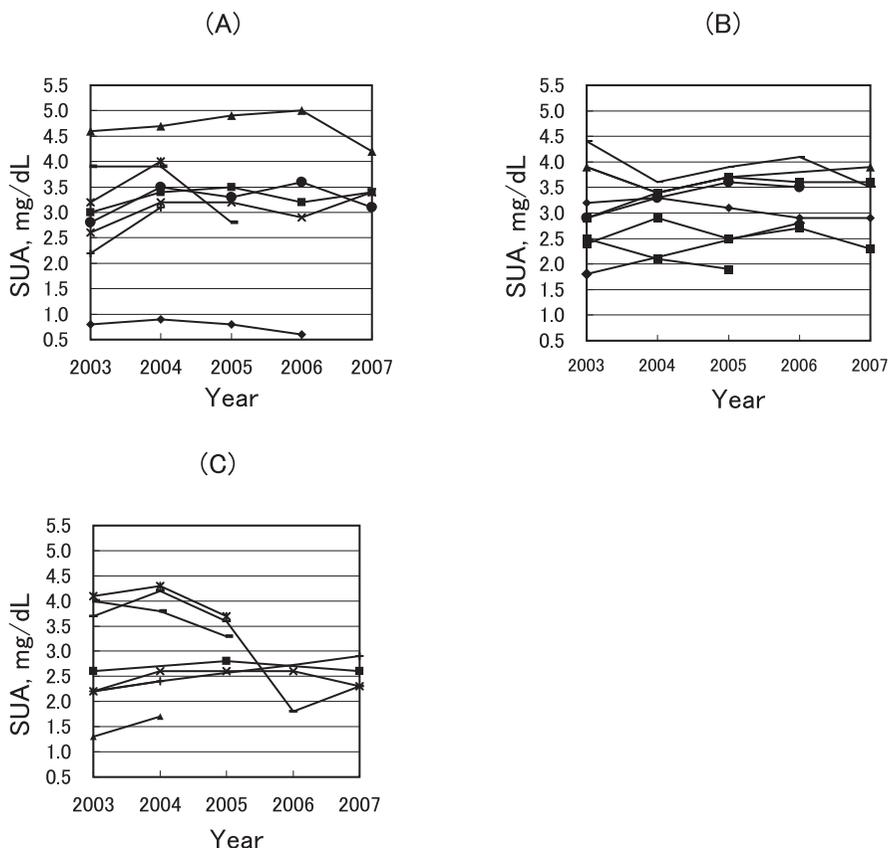


Fig. 2 SUA levels of individuals with *SLC22A12* 258WX who participated in the health checkup twice or more during 2003 to 2007. (A) 8 males; 5 times for 4 males, 4 times for 1 male, 3 times for 1 male, and twice for 2 males, (B) 8 females with body mass index (BMI)<25; 5 times for 4 females, 4 times for 2 females, 3 times for 1 female, and twice for 1 female. (C) 7 females with BMI≥25; 5 times for 2 females, 3 times for 4 females, and twice for 2 females.

the percentage (difference between the maximum and minimum divided by the average of all obtained measurements $\times 100$) ranged from 7.5% to 76.9% among 258WX subjects ($n=23$), whereas it ranged from 0.0% to 112.2% among 258WW subjects ($n=536$).

DISCUSSION

This study demonstrated that the mean SUA was significantly lower for those with *SLC22A12* 258WX than for those with 258WW. The 258X allele frequency was 1.9% (95% CI, 1.3–2.8) when 57 subjects were excluded due to gout, high blood urea nitrogen or high creatinine. Those with at least one 258X allele were 66.7% of 6 males and 39.3% of 28 females with SUA < 3.0 mg/dL, and 10.7% of 56 males and 2.6% of 313 females with SUA 3.0–4.9 mg/dL. This was the first study reporting the allele frequency according to sex and SUA levels among Japanese. It was also the first that the SUA of those with a 258WX allele changed similarly to those with the 258WW genotype during the entire 5-year observation period.

The frequency of the 258X allele in a Japanese population was estimated to be 2.4% among 1,875 participants (83 heterozygotes and 3 homozygotes) in a cohort study,¹⁰ and 2.3% among 980 controls in a case-control study,¹⁴ both of which were slightly higher than the estimate in this study. When the 57 examinees under medication for gout, or with high serum levels of urea nitrogen or creatinine were included (n=803), the frequency in the present study was 1.8% (95% CI, 1.2–2.6). While the 258X allele was found in 3 of 5 hypouricemia patients in Korea,¹⁵ the frequency in a general Korean population was 1.1%.¹² Since this allele was not reported among other ethnic groups, its origin was thought to be in East Asia.¹⁶

Interestingly, the 258X allele was found in those with SUA 4.0–4.9 mg/dL in the present study. Such a finding indicates that there may be other genetic traits and/or lifestyle factors compensating for the effect of a 258X allele on SUA levels. It is well known that SUA is lower in females than males. In this study, the SUA mean was 5.7 ± 1.3 mg/dL in males and 4.6 ± 1.1 mg/dL in females ($p < 0.001$). Age, menopause, meat consumption, alcohol intake, obesity, sedentary lifestyle, dyslipidemia, insulin resistance, blood pressure, renal function, and drugs for hypertension were the factors associated with SUA levels.^{17–22} Since there were only 29 participants with 258WX, associations with the above factors among them could not be examined; even between males and females, the difference was small (3.1 mg/dL in males and 2.9 mg/dL in females).

Among those with 258WX, the changes in SUA ranged from 7.5% to 76.9% of the average. Those changes were similar to those of the examinees with 258WW, indicating that lifestyle factors were similarly influential on SUA. Since hypouricemia increases the risk of acute renal failure, urolithiasis, and hematuria, the elevation of SUA to an appropriate level through lifestyle changes might benefit those with hypouricemia.

The present observational study has several limitations. The first was the limited number of subjects. Because of this limitation, the factors possibly associated with the SUA among those with 258WX could not be examined. Second, the follow-up period for the changes in SUA levels was only five years, so that lifestyle changes potentially affecting the SUA could not be examined for the long-term changes in the SUA. Lastly, genotypes other than *SLC22A12* W258X were not examined.

In conclusion, 746 Japanese health checkup examinees were genotyped for *SLC22A12* W258X. Although a strong association was found between the *SLC22A12* 258X allele and low SUA, some participants with 258X allele showed a normal SUA. The allele among those with hypouricemia was 66.7% in 6 males and 39.3% in 28 females. The SUA levels changed among those with 258WX, as they also did among those with 258WW, indicating that lifestyles could similarly influence SUA for those with 258WX.

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