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NO ASSOCIATION BETWEEN *MTHFR* C677T AND SERUM URIC ACID LEVELS AMONG JAPANESE WITH *ABCG2 126QQ* AND *SLC22A12 258WW*

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ABSTRACT

Several genome-wide association studies (GWAS) have revealed that single nucleotide polymorphisms (SNPs) of *ABCG2* and *SLC22A12* were strongly associated with serum uric acid (SUA), but those of methylene tetrahydrofolate reductase (*MTHFR*) were not. However, there were several studies indicating the association with *MTHFR* C677T polymorphism. This study examined the association with the polymorphism, taking into account the genotypes of *ABCG2* Q126X and *SLC22A12* W258X. Subjects were 5,028 health checkup examinees of Seirei Preventive Health Care Center (3,416 males and 1,612 females) aged 35 to 69 years, who participated in the Japan Multi-Institutional Collaborative Cohort Study (J-MICC Study). Hyperuricemia was defined as SUA equal to 7 mg/dL or over. The genotype frequency was 35.9% for *CC*, 48.1% for *CT*, and 16.0% for *TT*, being in Hardy-Weinberg equilibrium (p=0.90). Among 4,425 participants with *ABCG2 126QQ* and *SLC22A12 258WW* who were not under medication for hyperuricemia, the mean SUA was 5.6 mg/dL, 5.6 mg/dL, and 5.7 mg/dL, respectively. When 114 participants with *ABCG2 126QQ* and *SLC22A12 258WW* under medication for hyperuricemia were included in hyperuricemia cases, the sex-age adjusted odds ratio (OR) of hyperuricemia was not significant; OR=1.00 (95% confidence interval, 0.89–1.24) for *CT* genotype and OR=0.98 (0.84–1.32) for *TT* genotype, relative to *CC* genotype. The present study indicated no association between SUA and *MTHFR* C677T genotype, after the influences of *ABCG2* Q126X and *SLC22A12* W258X were removed.

Key Words: Serum uric acid, Urate transporter polymorphisms, *MTHFR* C677T

INTRODUCTION

It is well known that serum uric acid (SUA) levels are associated with various factors such as sex, age, body mass index (BMI), dietary habit and drinking habit.[1-3] In addition, there is evidence that genetic traits influence SUA concentrations; the heritability was estimated to be up to 73%.[4] A recent genome-wide association study performed in Japan showed strong associations of SUA with genetic polymorphisms of *SLC22A12* coding uric acid transporter 1 (URAT1),

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SLC2A9 coding glucose transporter 9 (GLUT9), and *ABCG2* coding ATP-binding cassette subfamily G member 2 (ABCG2),[5] which were also reported to have associations in European ancestry.[6] Among the polymorphisms, *SLC22A12* W258X,[7,8] *SLC2A9* R380W and R198C,[9] and *ABCG2* Q126X and Q141K[10,11] were confirmed to have associations with SUA, although the association of *ABCG2* Q141K was relatively weak. *SLC22A12* 258X and *ABCG2* 126X and 141K are found in a general Japanese population, whereas *SLC2A9* R380W and R198C are not because of the extremely rare allele frequency.

Although not detected in the genome-wide association study, *methylenetetrahydrofolate reductase* (*MTHFR*) C677T was reported to have associations with SUA.[12-14] A recent meta-analysis on the association with six studies (two from Iran, two from China, one from Korea, and one from Japan) demonstrated that the summary odds ratio (OR) was 1.879 (95% confidence interval (CI), 1.596–2.213).[15] The present study investigated the association of *MTHFR* C677T with SUA levels among Japanese health checkup examinees, after taking into account the genotypes of *SLC22A12* W258X and *ABCG2* Q126X.

MATERIALS AND METHODS

Subjects

Subjects were 5,028 participants of the Shizuoka Study, a part of the Japan Multi-Institutional Collaborative Cohort Study (J-MICC Study), aged 35–69 years, whose registered address was the west half of Shizuoka Prefecture including 12 cities (Shizuoka, Fujieda, Yaizu, Makinohara, Kikukawa, Omaezaki, Kakegawa, Shimada, Fukuroi, Iwata, Hamamatsu, and Kosai) and 6 towns (Okabe, Oigawa, Yoshida, Kawane, Kawanehon, and Arai).[16] After two individuals without blood samples were excluded, 5,026 participants (3,414 males and 1,612 females) were used for analysis.

Data collection

Written informed consent was obtained from all subjects. The contents of the agreement included 1) permission to use information on lifestyle, disease history, and family history collected with a self-administered questionnaire, 2) permission to use laboratory data obtained through the health checkup, and 3) the donation of blood and urine specimens, as well as the follow-up until 2025 for deaths from any cause, and diagnoses of cancer, cardiovascular, and cerebrovascular diseases.

The self-administered questionnaire used in the Shizuoka Study included questions on employment, eating habits, stress, dental health, and forest-air bathing and walking, as well as questions common to the J-MICC Study.[16] The administered questionnaire was examined by a study staff in an isolated room, who asked participants to respond to all unanswered questions except those the participant had intentionally refused to answer.

Venous blood was drawn into a 7 ml of vacuum tube including serum separation, and a 7 ml EDTA-Na added vacuum tube on the day of the health checkup. Eight tubes with 300 µl serum, 8 tubes with 300 µl plasma, and 2 tubes with 300 µl buffy coat were separated. All tubes were stored at –80°C at Nagoya University Graduate School of Medicine.

Genotyping procedure

DNA was extracted from the buffy coat fraction of the 7 ml EDTA-Na added vacuum tube. *MTHFR* C677T 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'-AGC CTC TCC TGA CTG TCA TCC-3', R1: 5'-TGC GTG ATG ATG AAA TCG G-3', F2: 5'-GAG AAG GTG TCT GCG GGA GT-3', and R2: 5'-CAT GTC GGT GCA TGC CTT-3'. The amplified DNA fragments were 128 bp for the *C* allele, 93 bp for the *T* allele, and 183 bp for the common band.[14)]

Statistical analysis

Hyperuricemia was defined as SUA level ≥ 7.0 mg/dL, and hypouricemia as SUA level < 3.0 mg/dL. Hardy-Weinberg equilibrium was examined with a chi-square test. Means between two groups were tested with a *t*-test. The 95% CI of percentage was calculated based on a binomial distribution. Age- and sex-adjusted OR and 95% CI were estimated using an unconditional logistic model. Two-sided p-values less than 0.05 were considered to be statistically significant. All statistical analyses were performed using SAS Enterprise Guide software version 4.1.

RESULTS

Subject characteristics according to sex are summarized in Table 1. The mean and standard deviation (SD) of age was 50.7±8.6 years in males and 49.2±8.7 years in females. Hyperuricemia was found to be 23.5% (n=801) in males and 1.5% (n=24) in females, while hypouricemia was 0.6% (n=21) and 5.2% (n=84), respectively. The SUA mean was significantly higher in males than in females (6.1±1.2 mg/dL vs 4.4±1.0 mg/dL, $p < 0.001$).

The genotype frequency of *MTHFR* C677T among 5,026 subjects was 35.9% for *CC*, 48.1% for *CT*, and 16.0% for *TT*, with 0.40 for the *T* allele. The distribution was in Hardy-Weinberg equilibrium (P=0.90). Among 5,026 examinees, 4,539 subjects with *ABCG2 126QQ* and *SLC22A12 258WW* remained after excluding those with *ABCG2 126X* allele or *SLC22A12 258X* allele. From them, 114 subjects under medication for hyperuremia were further removed. The genotypes frequencies of the *MTHFR* accrding to SUA level among the remaining 4,425 subjects (2,972 males and 1,453 females) are shown in Table 2. In both sexes, the genotype frequencies were similar across the different SUA levels; p=0.31 in males and p=0.83 in females from a 4 by 3 chi-square test. The mean SUA was also similar among the three different genotypes. When those under medication for hyperuremia were added, no differences were observed.

Adjusted OR and 95% CI of hyperuricemia (SUA ≥ 7.0 mg/dL and/or medication for hyperuricemia) for *MTHFR* C677T are shown in Table 3. The OR adjusted for sex, age, BMI, and creatinine was 1.00 (95% CI, 0.89–1.24) for *CT* genotype and 0.98 (95% CI, 0.84–1.32) for *TT* genotype, relative to CC genotype. When the analysis was conducted for males and females separately, the ORs were not statistically significant.

DISCUSSION

In the present study with 4,425 subjects aged 35–69 years with *SLC22A12 258WW* and *ABCG2 126QQ*, there were no differences in mean SUA among those with different *MTHFR* C677T genotypes. The distribution of the genotype was also similar across those with different SUA levels. The findings were inconsistent with those from the previous studies for Japanese[12,14,18)]

Table 1 Characteristics of participants according to sex

Characteristics	Males (n=3,416)		Females (n=1,612)	
	N	(%)	N	(%)
Age (years)				
35–40	265	(7.8)	192	(12.0)
40–49	984	(28.8)	496	(30.8)
50–59	1,367	(40.0)	631	(39.1)
60–69	800	(23.4)	293	(18.2)
BMI (kg/m^2)				
<18.5	92	(2.7)	162	(10.0)
18.5–24.9	2,424	(71.0)	1,195	(74.1)
≧25	900	(26.3)	255	(15.8)
SUA (mg/dL)				
<3.0	21	(0.6)	84	(5.2)
3.0–4.9	524	(15.4)	1,074	(66.6)
5.0–6.9	2,070	(60.6)	430	(26.7)
≧7.0	801	(23.4)	24	(1.5)
Creatinine (mg/dL)				
<0.5	0	(0.0)	33	(2.0)
0.5–0.7	640	(18.7)	1,476	(91.6)
0.8–1.0	2,070	(60.6)	100	(6.2)
1.0–	706	(20.7)	3	(0.2)
BUN (mg/dL)				
<8.0	11	(0.3)	26	(1.6)
8.0–19.9	3,173	(92.9)	1,520	(94.3)
20–22.9	232	(6.8)	66	(4.1)
Medication for hyperuricemia				
Yes	131	(3.8)	0	(0.0)
No	3,285	(96.2)	1,612	(100.0)

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

Table 2 Genotype frequencies (%) of *MTHFR* C677T according to serum uric acid (SUA)

SUA (mg/dL)	n	Genotype		
		CC	*CT*	*TT*
Total		n=1,573	n=2,147	n=705
Males		n=1,061	n=1,435	n=476
<3.0	3	0.0	100.0	0.0
3.0–4.9	367	36.8	50.4	12.8
5.0–6.9	1,902	35.5	47.6	16.8
≥7.0	700	35.7	48.3	15.6
Whole	2,972	35.7	48.3	16.0
Mean ± SD		6.2±1.1	6.2±1.2	6.2±1.0
Females		n=512	n=712	n=229
<3.0	51	33.3	52.9	13.7
3.0–4.9	988	34.6	50.0	15.4
5.0–6.9	392	37.2	45.7	17.1
≥7.0	22	31.8	54.5	13.6
Whole	1,453	35.2	49.0	15.8
Mean ± SD		4.5±1.0	4.5±1.0	4.5±1.0

Table 3 Adjusted odd ratio (OR) and 95% confidence interval (CI) of hyperuricemia (serum uric acid ≥ 7.0 mg/dL and/or medication for hyperuricemia) for *MTHFR* C677T among those with *ABCG2 126QQ* and *SLC22A12 258WW*

Genotype		OR		95% CI
All subjects			(n = 4,539)	
MTHFR C677T	*CC*	1		(Reference)
	CT	1.00		0.89–1.24
	TT	0.98		0.84–1.32
Males			(n = 3,086)	
MTHFR C677T	*CC*	1		(Reference)
	CT	1.00		0.88–1.25
	TT	0.97		0.83–1.35
Females			(n= 1,453)	
MTHFR C677T	*CC*	1		(Reference)
	CT	1.02		0.32–2.07
	TT	0.69		0.27–4.08

Adjusted for (sex), age, BMI and creatinine

and for other ethnic groups.[13,19)]

There may be several reasons for the inconsistent reports on the association between the *MTHFR* polymorphism and SUA. The other relatively common genetic traits, such as the polymorphisms of *ABCG2* and *SLC22A12*, would conceal the moderate influence of *MTHFR* C677T. Few studies have examined the effect after removing the effects of such influential genotypes. This study examined the association among those with *SLC22A12 258WW* and *ABCG2 126QQ*. Another reason masking the effect of the *MTHFR* polymorphism may be the medications for hyperuricemia. In this study, the medications for hyperuricemia were taken into account.

The frequency of *T* allele varies among different ethnic groups; it was reported in a study in Hawaii that the frequency was 0.41 in Japanese-Americans, 0.36 in Caucasians, 0.41 in Latino, 0.13 in African-American, and 0.22 in Native Hawaiians.[20)] In the present study, the frequency was 0.40, which was close to the Japanese average (0.391 among 10,854 Japanese).[21)] The subjects seemed to reflect the Japanese general population.

Although the mechanism of the relationship between the *MTHFR* polymorphism and SUA is still unknown, there are several studies which assume that the *MTHFR* polymorphism could affect the mechanisms such as the de novo synthesis of purines via 10-formyl tetrahydrofolate with consequent overproduction of UA by the substrate of the *MTHFR* reaction.[12,21,22)] It is evident that the *TT* genotype of *MTHFR* is more closely related to the rise of plasma homocysteine in patients with a low folic acid level[18,23-26)] and high plasma homocysteine is lowered by folic acid fortification.[27)] A direct relation between plasma homocysteine levels and UA levels was also reported.[12,19,28-31)] These suggest that in a group with low folic acid intake, the effects of *MTHFR* gene polymorphism on SUA levels are more likely to be marked, and vice versa. In this study, all of the data were collected in one healthcare center located in Shizuoka Prefecture. Shizuoka is well known for its green tea, which contains folic acid. The yearly amount of expenditure on green tea was the largest in Shizuoka, almost three times higher than the average in Japan in 2011.[32)] This factor could affect the results of this study.

One of the limitations of our study is the remaining unadjusted potential confounding factors associated with SUA, such as alcohol consumption, physical activity, animal protein intake, folate, and serum vitamin B12 intake, although they seemed to be independent of the *MTHFR* genotype. A systematic review showed that the ethnicity may affect the relationship between the *MTHFR* mutation and SUA levels.[33)]

In conclusion, though some limitations remain, the present study indicated no association between SUA and *MTHFR* C677T genotype among Japanese, after the influences of *ABCG2* Q126X and *SLC22A12* W258X were removed.

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