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Significant association between methylenetetrahydrofolate reductase *677T* allele and hyperuricemia among adult Japanese subjects

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Abstract

It is well known that high serum uric acid (SUA) is the cause of gout and a risk factor of cardiovascular diseases. Although SUA is thought to have an association with folate metabolism through elevated production and/or damaged renal excretion, studies on functional polymorphisms of folate metabolizing are still limited, showing inconsistent findings. We hypothesized that hyperuricemia would be associated with methylenetetrahydrofolate reductase (*MTHFR*) C677T and thymidylate synthase (*TS*) 28-bp tandem repeat polymorphism. Subjects were 793 healthy health checkup examinees (272 male and 521 female Japanese) aged 39 years or older. There was no clear difference in SUA means among those with different genotypes of *MTHFR* and *TS*, but a significant association between hyperuricemia (SUA ≥7mg/dL) and *MTHFR 677T* allele carriers was observed. The odds ratio of harboring *677T* allele adjusted for sex, age, body mass index, serum creatinine, systolic blood pressure, currents habits of smoking and drinking, and *TS* genotype was 2.77 (95% confidence interval, 1.38-5.56). The *TS* genotype was not significantly associated with hyperuricemia; the corresponding adjusted odds ratio was 1.36 (95% confidence interval, 0.75-2.48) for non–*33* genotype relative to *33* genotype. Because *MTHFR 677CC* was rarer both in <4 mg/dL group and ≥7 mg/dL group, the comparisons of SUA means were not useful to elucidate the roles of the polymorphism. This new view may partly explain the inconsistent results on the association of the *MTHFR* polymorphism with SUA.

Keywords: Genetic polymorphism; Japanese; Methylenetetrahydrofolate reductase; Thymidylate synthase; Uric acid
Abbreviations: BMI, body mass index; CI, confidence interval; MTHFR, methylenetetrahydrofolate reductase; OR, odds ratio; PCR, polymerase chain reaction; SUA, serum uric acid; TRP, tandem repeat polymorphism; TS, thymidylate synthase; UA, uric acid.

1. Introduction

It is well known that high serum uric acid (SUA) is the cause of gout. Elevated SUA was commonly detected in subjects with abnormal purine metabolism, reflecting overproduction of uric acid (UA) and/or insufficient UA excretion from the kidney. On average, SUA increases with age and is higher in men than in women. It was also reported that hyperuricemia was associated with obesity, hypertension, hyperlipidemia, renal insufficiency, and insulin resistance [1-3]. Furthermore, elevated SUA predicted the risk of cardiovascular and cerebrovascular diseases. A recent meta-analysis showed that measurement of SUA could improve

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the prediction of coronary heart disease risk in general populations [4]. Serum uric acid is usually considered as a marker of renal dysfunction, as well as a risk factor of renal disease progression.

Methylenetetrahydrofolate reductase (MTHFR) is an enzyme in organs including liver, to convert 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate, which is a methyl donor for methylation of homocysteine into methionine. The enzyme activity of MTHFR is partly determined by *MTHFR* C677T polymorphism, which alters its affinity for the substrate or cofactor [5]. Several reports examined the association between the *MTHFR* polymorphism and SUA, but the results were inconsistent [6-10]. It is also known that the other genetic polymorphisms may influence the folate metabolism, including *thymidylate synthase* (*TS*) 28-bp tandem repeat polymorphism (TRP). *TS* gene encodes the enzyme to metabolize 5,10-methylenetetrahydrofolate, the substrate of MTHFR. Therefore, the enzyme activity of TS might affect the efficiency of MTHFR.

Although biologic mechanisms have not fully been clarified, a possible link between SUA and folate metabolism is through *S*-adenocyl-homocysteine originating adenosine. Another possibility is the link of folate metabolism to atherosclerosis, which damages renal excretion of UA. We hypothesized that hyperuricemia would be associated with the genotypes of *MTHFR* C677T and *TS* 28-bp TRP. This study aimed to test the hypothesis among Japanese health checkup examinees.

2. Methods and materials

2.1. Subjects

The subjects were selected from 864 residents 39 years or older in a rural area of Hokkaido, Japan, who attended a health checkup program in 2003. Among them, 52 examinees did not agree the use of their residual blood for the polymorphism study, another 10 examinees reported medication against gout, and DNA was not successfully extracted from 9 examinees. The remaining 793 subjects (272 males and 521 females) were eligible for the present analysis. The participants worked in fishing, dairy farming, or commerce, with roughly equal numbers for each. This study was approved by the Ethics Committee of Nagoya University School of Medicine (approval number 398).

2.2. Data collection

The participants were requested to answer a questionnaire on health and daily lifestyle at the occasion of the health checkup. Written informed consent to provide lifestyle information and residual blood for genotyping was obtained. All participants underwent physical examinations and routine biochemical analyses of blood and urine after overnight fasting. Biochemical analysis of the sampled sera was performed using an autoanalyzer (JCA-RX20; Nihon Denshi Co. Ltd, Aichi, Japan), and serum SUA was measured by the uricase-POD method. Height and weight were measured at the health checkup. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared.

2.3. Genotyping

DNA was extracted from buffy coat conserved at –40°C using a BioRobot EZ1 (QIAGEN Group, Tokyo, Japan). *MTHFR* C677T polymorphism (rs1801133) was genotyped by a polymerase chain reaction (PCR) with confronting 2-pair primers [11]. Each 25-μL reaction tube contained 50 to 80 ng DNA, 0.12 mmol/L dNTP, 12.5 pmol of each primer, 0.5 U AmpliTaq Gold (Perkin-Elmer, Foster City, Calif) and 2.5 μL of 10× PCR buffer including 15 mmol/L $MgCl_2$. The PCR was conducted with initial denaturation at 95°C for 10 minutes, 30 cycles of denaturation at 95°C for 1 minute, annealing at 60°C for 1 minute, and extension at 72°C for 1 minute and a final extension at 72°C for 5 minutes. 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. The 28-bp TRP in the 5′-terminal of the regulatory region of the *TS* gene was genotyped by PCR as previously reported [12].

2.4. Statistical analysis

Hyperuricemia was defined as SUA level equal to and more than 7.0 mg/dL. Serum uric acid means were examined with a *t* test or analysis of variance. Hardy-Weinberg equilibrium for genotype frequency was examined with a χ^2 test. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using an unconditional logistic regression model. Factors with a continuous value were adjusted as a continuous variable by the logistic model. Two-sided *P* values less than .05 were considered to be statistically significant. All statistical analyses were performed using STATA Version 7 (STATA Corp, College Station, Tex).

3. Results

Table 1 shows the characteristics of 272 males and 521 females in this study. Although there were several participants with abnormal biochemical results, they were superficially healthy and attended by themselves without any physical assistance. Current smokers were 29.4% in males and 9.2% in females, and current drinkers were 55.9% and 13.4%, respectively. The SUA mean was higher in males than in females ($P < .0001$).

The genotype frequency of *MTHFR* C677T was 36.8% for *CC*, 48.9% for *CT*, and 14.2% for *TT*. The distribution was in Hardy-Weinberg equilibrium ($P = .381$). In *TS* 28-bp TRP, 4 alleles (*2rpt*, *3rpt*, *4rpt*, and *5rpt*) were found in the present subjects, giving the genotype frequency of 2.0% for

Table 1
Mean, SD, minimum (Min.), and maximum (Max.) of participations' characteristics

Characteristics	Males (n = 272)				Females (n = 521)			
	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.
Age (y)	63.0	10.6	39	88	60.3	10.0	39	86
Weight (kg)	64.3	9.4	42.6	91.2	55.3	8.6	35.8	87.4
BMI (kg/m^2)	24.1	3.1	16.9	34.9	24.0	3.4	15.9	38.8
Systolic blood pressure (mm Hg)	139	19.0	100	200	135	19.4	90	210
Diastolic blood pressure (mm Hg)	89	11.0	64	120	84	10.5	60	120
UA (mg/dL)	5.8	1.3	0.8	9.6	4.6	1.1	1.3	9.0
Creatinine (mg/dL)	0.82	0.16	0.5	1.7	0.61	0.11	0.2	1.1
BUN (mg/dL)	15.9	4.0	7.8	36.3	14.1	3.5	5.8	26.5
Triglyceride (mg/dL)	112	75	34	625	93	50	22	433
Total cholesterol (mg/dL)	207	31	112	325	218	33	105	366
HDL (mg/dL)	53.8	12.4	32	126	61.9	13.7	29	114
AST (U/L)	25.0	8.6	12	77	23.6	9.6	11	106
ALT (U/L)	26.6	15.0	7	125	22.4	13.8	7	138
GGT (U/L)	41.4	47.4	8	415	22.0	20.9	4	192
Hematocrit (%)	44.2	3.6	31.0	53.3	39.6	3.1	27.3	62.8
C reactive protein (mg/dL)	0.156	0.42	0.006	4.005	0.097	0.279	0.003	3.886

BUN indicates blood urea nitrogen; HDL, high-density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ-glutamyltransferase.

2rpt2rpt, 26.9% for *2rpt3rpt*, 0.3% for *2rpt4rpt*, 0.6% for *2rpt5rpt*, 65.7% for *3rpt3rpt*, 1.3% for *3rpt4rpt*, and 3.3% for *3rpt5rpt*. The genotypes without *3rpt* were 2.9%, those with 1 *3rpt* 31.4%, and that with 2 *3rpt* alleles 65.7%, being in Hardy-Weinberg equilibrium ($P = .298$).

The genotype frequencies of the *MTHFR* and *TS* polymorphisms were shown according to SUA level and sex in Table 2. The *CC* genotype of *MTHFR* C677T was rarer in males with SUA less than 4 or 7 mg/dL or greater than males with SUA 4.0 mg/dL or greater and less than 7.0mg/dL. Among females, the percentage of the *CC* genotype decreased with SUA elevation, except those with SUA less than 4 mg/dL. The percentage of *TS 3rpt3rpt* genotype tended to decrease with SUA elevation in males, but not in females. There were no significant differences in SUA means among those genotypes, although a *P* value from analysis of variance was marginal in male *TS* ($P = .09$).

Table 3 shows the OR and 95% CI of hyperuricemia (≥7.0 mg/dL). The OR adjusted for sex and age was significant for *MTHFR* C677T (OR, 2.49; 95% CI; 1.29-4.80 for *NonCC* genotype relative to *CC* genotype), but not *TS* 28-bp TRP (model 1). Because the sex-age–adjusted ORs of BMI, creatinine, and systolic blood pressure were significant for hyperuricemia, these factors were added in the multivariate analysis, as well as potential confounding factors, current habits of smoking, and alcohol drinking (model 2). The estimates from model 2 were similar to those from model 1. When the genotypes of *MTHFR* and *TS* were

Table 2
Genotype frequencies (%) of *MTHFR* C677T and *TS* 28-bp TRP according to SUA levels

SUA	n	*MTHFR* C677T			*TS* 28-bp TRP		
		CC	*CT*	*TT*	*33*	*3 X*[a]	*XX*[a]
Males (mg/dL)		n = 102	n = 133	n = 37	n = 177	n = 87	n = 8
<4.0	19	26.3	52.6	21.1	84.2	15.8	0.0
4.0-4.9	46	45.7	45.7	8.7	65.2	32.6	2.2
5.0-5.9	79	44.3	39.2	16.5	68.4	27.8	3.8
6.0-6.9	78	37.2	50.0	12.8	64.1	33.3	2.6
≤7.0	50	24.0	64.0	12.0	54.0	42.0	4.0
Whole	272	37.5	48.9	13.6	65.1	32.0	2.9
Means ± SD	5.8 ± 1.3	5.7 ± 1.2	6.0 ± 1.4	5.6 ± 1.3	5.7 ± 1.3	6.0 ± 1.2	6.4 ± 1.2
Females (mg/dL)		n = 190	n = 255	n = 76	n = 344	n = 162	n = 15
<4.0	142	35.9	50.7	13.4	64.1	32.4	3.5
4.0-4.9	203	39.4	43.3	17.2	70.0	28.6	1.5
5.0-5.9	116	36.2	49.1	14.6	65.5	31.0	3.4
6.0-6.9	50	32.0	62.0	6.0	58.0	38.0	4.0
≤7.0	10	10.0	70.0	20.0	60.0	30.0	10.0
Whole	521	36.5	48.9	14.6	66.0	31.1	2.9
Means ± SD	4.6 ± 1.1	4.6 ± 1.0	4.6 ± 1.2	4.5 ± 1.1	4.5 ± 1.1	4.6 ± 1.1	4.7 ± 1.2

[a] "*X*" indicates an allele other than *3rpt*.

Table 3
Adjusted OR and 95% CI of SUA ≥7.0 mg/mL for *MTHFR* C677T and *TS* 28-bp TRP

Genotype		Model 1 [a]		Model 2 [b]		Model 3 [c]	
		OR	(95% CI)	OR	(95% CI)	OR	(95% CI)
All subjects (n = 793)							
MTHFR	*CC*	1	(Reference)	1	(Reference)	1	(Reference)
C677T	*NonCC*	2.49	(1.29-4.80)	2.82	(1.41-5.65)	2.77	(1.38-5.56)
TS	*33*	1	(Reference)	1	(Reference)	1	(Reference)
TRP	*Non 33*	1.68	(0.96-2.95)	1.43	(0.79-2.56)	1.36	(0.75-2.48)
Males (n = 272)							
MTHFR	*CC*	1	(Reference)	1	(Reference)	1	(Reference)
C677T	*NonCC*	2.20	(1.09-4.45)	2.46	(1.17-5.16)	2.40	(1.14-5.05)
TS	*33*	1	(Reference)	1	(Reference)	1	(Reference)
TRP	*Non 33*	1.79	(0.96-3.34)	1.58	(0.83-3.03)	1.51	(0.78-2.93)
Females (n = 521)							
MTHFR	*CC*	1	(Reference)	1	(Reference)	1	(Reference)
C677T	*NonCC*	5.40	(0.68-43.0)	5.41	(0.64-45.8)	5.58	(0.65-47.8)
TS	*33*	1	(Reference)	1	(Reference)	1	(Reference)
TRP	Non 33	1.32	(0.37-4.74)	1.21	(0.31-4.72)	1.35	(0.33-5.45)

[a] Adjusted for (sex) and age.
[b] Adjusted for (sex), age, BMI, creatinine, systolic blood pressure, current smoking, and current drinking.
[c] Adjusted for the factors of model 2 and genotypes of *MTHFR* and *TS* simultaneously.

put simultaneously in the model (model 3), substantial changes were not observed; OR, 2.77 (95% CI, 1.38-5.56), for *MTHFR non-CC* relative to *CC*.

4. Discussion

The present study demonstrated that *MTHFR 677T* allele carriers were associated with hyperuricemia but did not affected SUA means. The reason was that *MTHFR CC* genotype was relatively common among those with SUA levels between 4.0 and 6.9mg/dL, indicating that the *CC* genotype might contribute the optimal SUA levels. Accordingly, the simple comparison of SUA means among those with different genotypes could not evaluate the association with the *MTHFR* genotype appropriately. This new view may explain partly the inconsistency of the previous findings on the associations with the genotype. Although TS is an enzyme closely related with folate metabolism including methylation of homocysteine, the association with SUA was limited if any.

In 1998, Motti et al found an association between *MTHFR* C677T polymorphism and SUA among 155 middle-aged Italians [13]. Their data showed that increase in homocysteine was parallel to the increase in SUA levels, particularly in subjects with *677TT* genotype. Zuo et al [6] reported in 271 Japanese males that the polymorphism might be a risk factor of hyperuricemia in males 40 years or older. Their multiple regression analysis showed that creatinine, diastolic blood pressure, log(triglyceride), BMI, and *MTHFR* genotype were independently associated with SUA levels. In a study by Hong et al, similar results were found in 327 Korean males [7]. Golbahar et al reported that SUA was significantly higher in males and females with *TT* genotype than those with other genotypes among 518 Iranians [8]. On the other hand, there were 2 studies reporting no association between the *MTHFR* genotype and SUA mean in Japanese. They found that hyperuricemia had associations with hypertension and carotid atherosclerosis, but not with the *MTHFR* genotype among 335 males [9] and among 147 males and 179 females [10], respectively. Because they did not examine the association with hyperuricemia, their studies did not provide information about the effect of the *MTHFR* genotype on hyperuricemia. These studies were smaller in number than the present study.

It has been reported that several host factors were associated with SUA levels. Serum uric acid levels increased with age [1,2] and were different between men and women possibly because of estrogens [14,15]. In the present dataset, BMI, serum creatinine, and systolic blood pressure had a significant association with hyperuricemia, so that these factors were adjusted in the analysis. The association of triglyceride with SUA has been reported in several previous studies [8,16], but the sex-age–adjusted OR of triglyceride for hyperuricemia was not significant in this study. When triglyceride was added in our multivariate analysis, there was no substantial change in the OR. A marked association between SUA and plasma homocysteine was reported [17], but the data of plasma homocysteine were not available in the present study.

A few germ line genetic mutations have been identified to cause hyperuricemia. It was found that UA was overproduced in those having defects of hypoxanthine-guanine phosphoribosyltransferase; the complete defects cause Lesch-Nyhan syndrome [18]. Accelerated purine synthesis as a consequence of phosphoribosyl pyrophosphate synthetase superactivity, as well as the deficiency in glucose-6-phosphatase activity, causes hyperuricemia [19]. However, the genetic factors of hyperuricemia in most cases are not fully understood. Genotypes reportedly associated with

hyperuricemia were *TGF-B* (transforming growth factor B) codon 25 *GG* genotype in renal transplant patients [20], *ecNOS* (endothelial constitutive nitric oxide synthase) variable number of TRP in males [21], alcohol dehydrogenase 2, and *NADH* dehydrogenase subunit-2 Leu237Met in male alcohol drinkers [22], G protein β-3 subunit G825T, and 5-hydroxytryptamine receptor 2A gene A1438G in Japanese workers [23]. *SLC22CA12* coding urate transporter (URAT1) was also reported to affect SUA [24]. Recent genomewide study in Taiwanese aborigines detected D4S2623 on chromosome 4q25 as a marker of gout [25]. The association between higher SUA and a δ-aminolevulinic acid dehydratase polymorphism was controversial [26,27].

The biologic role of MTHFR enzyme on SUA is not clearly established, but possible mechanism is as follows. First, adenosine originating from *S*-adenocyl-homocysteine could link the syntheses of homocysteine and UA [28], if preferentially incorporated into a precursor pool for UA under some lifestyle or physical conditions. Second, in those with *MTHFR 677TT* genotype, renovascular atherosclerosis or the complications of systemic vascular disease may reduce the renal clearance of UA, resulting in elevation of SUA [13]. Mazza et al [29] reported that homocysteine levels in normal subjects were associated with SUA and *MTHFR* genotype. Therefore, the enzyme activity of *MTHFR* could be involved indirectly in SUA level determination. The roles of MTHFR enzyme on low SUA and high SUA have to be examined separately.

There are several limitations of the present study. First, questions on disease history were included in our questionnaire, but not on drug usage. Gout was listed in the questionnaire, but not hyperuricemia. Accordingly, participants with gout were excluded, but those with nonsymptomatic hyperuricemia under medication could not be excluded. Second, those under medication of fenofibrate, losartan, and statin (drugs for hypertension and hyperlipidemia), which are known to reduce SUA [30], were not excluded, although the number seemed limited. Because there seemed no difference in the above drug usage among the different genotype of *MTHFR*, the influence of the drug usage on the ORs might be limited. Last, the statistical power was not large for females and for subgroup analysis among males. Among the female subjects, only 10 of 521 had SUA of 7.0 mg/dL or greater.

In summary, this study demonstrated the significant association of hyperuricemia (SUA ≥7.0 mg/dL) with *MTHFR* C677T, taking account of the potential confounding factors, sex, age, BMI, creatinine, systolic blood pressure, smoking, and drinking. Because *MTHFR 677CC* was rarer among male Japanese with SUA less than 4mg/dL or 7mg/dL or greater than those with SUA 4.0 to 6.9 mg/dL, the comparisons of SUA means among the different genotypes were found to be inappropriate to evaluate the role of the *MTHFR* genotype. This fact might partly explain the inconsistency in the studies previously reported by other investigators.

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