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SHORT COMMUNICATION

Ming Zuo · Hisahide Nishio · Myeong Jin Lee
Kenji Maejima · Sigenobu Mimura · Kimiaki Sumino

The C677T mutation in the methylene tetrahydrofolate reductase gene increases serum uric acid in elderly men

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Abstract A common mutation, C677T, in the methylene tetrahydrofolate reductase gene (*MTHFR*) reduces the activity of MTHFR and increases total homocysteine levels in plasma. Increased homocysteine levels are reportedly associated with high serum uric acid levels. The relationship between the *MTHFR* mutation and uric acid metabolism remains unclear, however. To investigate whether the C677T *MTHFR* mutation is a risk factor for hyperuricemia, we performed *MTHFR* genotyping and clinical laboratory determinations, including serum uric acid, in 271 elderly Japanese men (age range, 40–79 years; mean, 52.6 years). The mean uric acid levels for the C/C, C/T, and T/T genotypes were 5.67, 6.00, and 6.39 mg/dl, respectively (P = 0.012). The T/T genotype was more frequent in subjects with high uric acid levels than in those with low uric acid levels (P = 0.038). These findings suggest that the C677T *MTHFR* mutation contributed to higher uric acid levels in subjects enrolled in this study. In conclusion, the mutation of the *MTHFR* gene may be a risk factor for hyperuricemia in elderly men.

Key words MTHFR · Homocysteine · Uric acid

Introduction

Methylene tetrahydrofolate reductase (MTHFR) is a regulating enzyme in the remethylation phase of homocysteine metabolism. A common mutation in the *MTHFR* gene (a C-to-T transition at nucleotide position 677) has been reported as a genetic risk factor for cardiovascular disease in Western countries (Frosst et al. 1995; Christensen et al. 1997). The mutation has also been reported to be common in the Japanese population and to be related to the occurrence of cardiovascular diseases (Nishio et al. 1996; Morita et al. 1998; Ou et al. 1998). The C677T *MTHFR* mutation converts an alanine to a valine residue in the MTHFR protein; this amino acid substitution decreases the activity of the enzyme, leading to high plasma homocysteine levels (Frosst et al. 1995; van der Put et al. 1995, 1996; Christensen et al. 1997; Gudnason et al. 1998; van der Put et al. 1998). Several lines of evidence indicate that plasma homocysteine is an independent risk factor in cardiovascular disease (Scott and Weir 1996) and stroke (Coull et al. 1990).

Hyperhomocysteinemia can result from genetic or nutrient-related disturbances of homocysteine metabolism. A significant positive correlation between plasma homocysteine and serum uric acid has been reported in patients with atherosclerosis and in control subjects (Kang et al. 1986; Araki et al. 1989; Coull et al. 1990; Malinow et al. 1995; Evers et al. 1997; Motti et al. 1998). Motti et al. (1998) found a relationship between the C677T *MTHFR* mutation and serum uric acid. Their data, however, were for an inseparable mixture of young and old, and men and women, in spite of the higher uric acid levels found in men (Reed et al. 1972; Brand et al. 1985; Freedman et al. 1995).

In this study, to investigate whether the C677T *MTHFR* mutation is a risk factor for hyperuricemia, we performed *MTHFR* genotyping analysis and clinical laboratory determinations, including serum uric acid, in elderly Japanese men.

Methods

Study population

Two hundred and seventy-one unrelated men aged 40 years or more were recruited into the study after their informed consent had been obtained. They underwent clinical examinations at Hyogo Health Service Association (Kobe, Japan). None of the subjects were diagnosed as having gout or renal failure (≥1.5 mg/dl of serum creatinine).

M. Zuo · H. Nishio (✉) · M.J. Lee · K. Sumino
Department of Public Health, Kobe University School of Medicine
7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan
Tel. +81-78-382-5541; Fax +81-78-382-5559
e-mail: nishio@med.kobe-u.ac.jp

K. Maejima · S. Mimura
Hyogo Health Service Association, Kobe, Japan

Body mass index (BMI) was computed as weight (kg) divided by height (m^2). Blood pressure was determined by standard sphygmomanometric procedures, with the subjects in a relaxed state. Data on alcohol intake and smoking habits were obtained by interview. The subjects were asked to quantify their consumption of alcohol in terms of the kind of alcoholic beverage usually drunk and the minimum consumption per week. Alcohol intakes were then converted into grams of alcohol per day. The number of cigarettes smoked per day was also noted.

Blood sample collection and DNA extraction

A venous blood sample was drawn from each of the participants. DNA was extracted, using a SepaGene Kit (Sanko Junyaku, Tokyo, Japan), and stored at −20°C until analysis. Serum was used for clinical chemistry laboratory examinations at Hyogo Health Service Association.

MTHFR genotyping

Polymerase chain reaction (PCR) was carried out with a PCR thermal cycler (TP2000; Takara Biomedicals, Kyoto, Japan). Thirty microliters of reaction mixture contained 200 ng of genomic DNA in 1 × Expand High Fidelity buffer (Boehringer Mannheim, Amsterdam, Netherlands) with 1.5 mM $MgCl_2$, 250 μM dNTPs, 0.5 μM primers, and 0.7 units of Expand High Fidelity PCR System Enzyme Mix (Boehringer Mannheim). The sequences of the primers were as follows: 5′-TGAAGGAGGTGTCTGCGGGA-3′ and 5′-AGGACGGTGCGGTGAGAGTG-3′(Frosst et al. 1995). The conditions for PCR included initial denaturation at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 2 min, extension at 72°C for 3 min, and a final extension at 72°C for 7 min. The amplified products were digested with *Hinf* I (New England Biolabs, Beverly, MA, USA) at 37°C for 4 h. The C-to-T transition creates a new *Hinf* I site. The digested products were electrophoresed in a 3% agarose gel.

Biochemical determinations

The following clinical chemistry variables were determined in serum by standard enzymatic methods: total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, glucose, uric acid, creatinine, urea, total protein, alanine aminotransferase, aspartate aminotransferase, γ-glutamyltranspeptidase, and alkaline phosphatase. For each subject, a whole blood cell count was taken.

Statistical analyses

In the determination of *MTHFR* genotypes, Hardy-Weinberg equilibrium was assessed by χ^2 analysis. For statistical purposes, all variables showing a non-Gaussian distribution were logarithmically transformed. Analysis of variance (ANOVA) and Scheffe's *F*-test were used to compare the means among subjects divided according to *MTHFR* genotype. The odds ratio and 95% confidence interval (95% CI) were calculated to estimate the relative risk of hyperuricemia associated with *MTHFR* genotype. Study variables were correlated with uric acid levels, using Pearson's correlation coefficient test or Spearman's correlation coefficient by rank test. Multiple regression analysis was performed, using uric acid as the dependent variable, and a group of significantly correlated parameters (by Pearson's or Spearman's analysis) as independent variables. In the analyses, *MTHFR* genotypes were designated as follows: C/C = 0, C/T = 1, T/T = 2 (Motti et al. 1998). These values reflected the copy number of T alleles in a subject. The analyses were performed using the StatView statistical package (Abacus Concepts, Berkeley, CA, USA) and Microsoft Excel (Microsoft, Redmond, WA, USA).

Results

Study population

Table 1 shows some baseline clinical and laboratory data for the subjects who participated in the study. The mean age of the subjects was 52.6 years (range, 40–79 years). The percentages of subjects with hypertension (systolic blood pressure, ≥160 mmHg or diastolic blood pressure, ≥95 mmHg), diabetes mellitus or impaired glucose tolerance (Hemoglobin Aic [HbAic], ≥6.5%), hypercholesterolemia (plasma total cholesterol, ≥220 mg/dl), and hyperglyceridemia (triglycerides, ≥150 mg/dl) were 12.5%, 5.9%, 27.7%, and 29.1%, respectively.

Distribution of *MTHFR* genotypes

The distribution of the MTHFR genotypes was: C/C, 41% (95% CI, 35%–47%); C/T, 45% (95% CI, 40%–51%); and

Table 1. Clinical data for the subjects enrolled in the study

Variable	Mean	SD	Range
Age (years)	52.6	8.8	40–79
BMI (kg/m^2)	23.3	2.73	16.1–30.6
Systolic blood pressure (mmHg)	121.4	18.6	86–182
Diastolic blood pressure (mmHg)	79.1	12.9	48–122
Total cholesterol (mg/dl)	200.6	33.1	96–301
HDL-cholesterol (mg/dl)	56.9	16.1	26–129
Log triglycerides (mg/dl)	2.1	0.24	1.5–2.9
Glucose (mg/dl)	104.7	24.7	77–299
Total protein (g/dl)	7.25	0.39	5.9–8.4
Creatinine (mg/dl)	0.94	0.14	0.6–1.5
Blood urea nitrogen (mg/dl)	15.0	3.1	7.9–27.6
Uric acid (mg/dl)	5.92	1.36	2.4–10.8
Hematocrit (%)	44.2	3.0	35.8–51.7
Alkaline phosphatase (IU/l)	142.1	39.6	70–332
HbAic (%)	5.05	0.91	3.7–10.3
Alcohol (g/day)	24.5	21.8	0–99.4
Smoking (cigarettes/day)	10.9	14.2	0–60

BMI, Body mass index; HDL, high-density lipoprotein; HbAic, Hemoglobin Aic

T/T, 14% (95% CI, 10%–18%). The proportions of the *MTHFR* genotypes did not differ significantly from the values expected for the Hardy-Weinberg equilibrium (χ^2 = 0.23; degrees of freedom [df] = 1; P = 0.63). Allele frequencies were 0.63 (95% CI, 0.59–0.68) for the C allele and 0.37 (95% CI: 0.32–0.41) for the T allele. These findings were consistent with previous reports from Japan (Nishio et al. 1996; Morita et al. 1998; Ou et al. 1998).

Physical and biochemical variables in subjects grouped by *MTHFR* genotype

The distribution of physical and biochemical variables, in subjects grouped by MTHFR genotype, is shown in Table 2. For all variables, except for uric acid and creatinine, there were no significant differences between the groups of subjects with different MTHFR genotypes.

MTHFR genotype and uric acid level

The serum uric acid levels for each genotype are shown in Table 2. The serum uric acid levels in the subjects with different *MTHFR* genotypes differed significantly (F = 4.4575; P = 0.012). Homozygotes for the *MTHFR* mutation had a significantly higher mean uric acid level (6.39 ± 1.25 mg/dl; mean ± SD) than did those without the mutation (5.67 ± 1.34 mg/dl; Scheffe's F-test, P = 0.018), while heterozygotes had an intermediate value (6.00 ± 1.38 mg/dl). There was a trend towards increasing uric acid levels as the number of copies of the T allele increased.

As shown in Table 3, subjects were classified into two groups based on uric acid levels: a low uric acid group (<7.0 mg/dl) and a high uric acid group (≥7.0 mg/dl). The value of 7.0 mg/dl was the upper limit of the normal range of serum uric acid in males in our laboratory and was used as the cut-off value. The genotype frequencies differed significantly between the two groups (χ^2 = 8.549; df = 2; P = 0.014). The T/T genotype frequency in the high uric acid group was twice as high as that in low uric acid group (χ^2 = 4.31; df = 1; P = 0.038). The T allele frequency was also significantly greater in the high uric acid group than in the low uric acid group (χ^2 = 8.78; df = 1; P = 0.003).

Table 2. Clinical data for subjects grouped by methylene tetrahydrofolate reductase (*MTHFR*) genotype

	MTHFR genotypes			
Variable	C/C[a] (n = 111)	C/T[a] (n = 122)	T/T[a] (n = 38)	P value
Age (years)	53.9 ± 8.4	51.2 ± 7.9	52.4 ± 7.9	0.0745
BMI (kg/m^2)	23.3 ± 2.8	23.2 ± 2.4	23.6 ± 2.4	0.8022
Systolic blood pressure (mmHg)	121.2 ± 19.3	120.2 ± 18.8	125.6 ± 18.8	0.2917
Diastolic blood pressure (mmHg)	78.7 ± 12.9	78.4 ± 14.2	82.2 ± 14.2	0.2782
Total cholesterol (mg/dl)	198.8 ± 32.2	201.6 ± 35.6	203.5 ± 35.6	0.6878
HDL-cholesterol (mg/dl)	55.2 ± 16.2	58.2 ± 14.3	60.0 ± 14.3	0.3421
Log triglycerides (mg/dl)	2.1 ± 0.3	2.0 ± 0.2	2.0 ± 0.2	0.2050
Glucose (mg/dl)	102.8 ± 30.3	107.0 ± 18.6	104.3 ± 18.6	0.4349
Total protein (g/dl)	7.2 ± 0.4	7.3 ± 0.4	7.3 ± 0.4	0.9787
Creatinine (mg/dl)	0.92 ± 0.12	0.95 ± 0.13	1.00 ± 0.13	0.0055*
Blood urea nitrogen (mg/dl)	14.7 ± 2.9	15.1 ± 3.1	15.0 ± 3.1	0.5937
Uric acid (mg/dl)	5.67 ± 1.33	6.00 ± 1.25	6.39 ± 1.25	0.0123*
Hematocrit (%)	44.0 ± 2.9	44.6 ± 3.0	43.6 ± 2.6	0.1525
Alkaline phosphatase (IU/l)	139.3 ± 37.6	145.3 ± 35.3	140.3 ± 35.3	0.4906
HbAic (%)	5.1 ± 1.0	5.3 ± 0.8	5.0 ± 0.8	0.7146
Alcohol (g/day)	23.7 ± 22.1	26.0 ± 21.9	20.4 ± 18.0	0.3808
Smoking (cigarettes/day)	11.6 ± 15.9	11.0 ± 14.7	8.3 ± 14.7	0.4779

* P < 0.05
The values in different *MTHFR* genotypes (means ± SD) were compared by analysis of variance (ANOVA)
[a] See text for explanation of genotypes

Table 3. Odds ratios of hyperuricemia associated with *MTHFR* genotypes

	MTHFR genotype[a]			
Groups	C/C	C/T	T/T[b]	T allele frequency[c]
Low uric acid level (<7.0 mg/dl)	96 (45.1%)	92 (43.2%)	25 (11.7%)	0.33
High uric acid level (≧7.0 mg/dl)	15 (25.9%)	30 (51.7%)	13 (22.4%)	0.48
Odds ratio (95% CI)	1.0	2.09 (1.05–4.13)	3.33 (1.40–7.89)	

CI, Confidence interval
[a] χ^2 = 8.549; df = 2; P = 0.014; genotype frequencies differed significantly in the two groups
[b] χ^2 = 4.31; df = 1; P = 0.038; T/T genotype frequency was higher in the high uric acid group than in the low uric acid group
[c] χ^2 = 8.78; df = 1; P = 0.003; T allele frequency was higher in the high uric group than in the low uric acid group

Table 4. Correlation coefficient[a] between different variables and uric acid levels

	r	*P* value
MTHFR	0.177[b]	0.0036*
Age (years)	0.042	0.4938
BMI (kg/m^2)	0.293	<0.0001*
Systolic blood pressure (mmHg)	0.159	0.0089*
Diastolic blood pressure (mmHg)	0.290	<0.0001*
Total cholesterol (mg/dl)	0.026	0.6740
HDL-cholesterol (mg/dl)	−0.184	0.0023*
Log triglycerides (mg/dl)	0.247	<0.0001*
Glucose (mg/dl)	−0.105	0.0835
Total protein (g/dl)	0.076	0.2097
Creatinine (mg/dl)	0.328	<0.0001*
Blood urea nitrogen (mg/dl)	0.055	0.3685
Hematocrit (%)	0.128	0.0368*
Alkaline phosphatase (IU/l)	−0.125	0.0393*
HbAic (%)	−0.128	0.0368*
Alcohol (g/day)	0.044	0.4730
Smoking (cigarettes/day)	−0.125	0.0401*

* $P < 0.05$
[a] Pearson's coefficient, unless otherwise indicated
[b] Spearman's coefficient

Table 5. Multiple regression analysis using serum uric acid as dependent variable

	St. β	*P* value
Creatinine (mg/dl)	0.245	<0.0001*
Diastolic blood pressure (mmHg)	0.202	0.0168*
Log triglycerides (mg/dl)	0.156	0.0128*
BMI (kg/m^2)	0.137	0.0337*
MTHFR	0.109	0.0483*
HbAic (%)	−0.097	0.0829
Hematocrit (%)	0.000	0.9984
Alkaline phosphatase (IU/l)	−0.067	0.2331
HDL-cholesterol (mg/dl)	−0.076	0.2311
Systolic blood pressure (mmHg)	−0.023	0.7759
Smoking (cigarettes/day)	−0.097	0.1069
R	0.522	<0.0001

* $P < 0.05$
St. β, standardized β coefficient

The calculated odds ratios and 95% CI for the C/T genotype were 2.09 (1.05–4.13) and 3.33 (1.40–7.89) for the T/T genotype, compared with the C/C genotype (Table 3). Both of these effects were significant ($P = 0.03$ and $P = 0.005$, respectively), suggesting a codominant effect of the T allele on the risk of hyperuricemia.

Univariate regression analyses

On univariate analysis, serum uric acid showed a correlation with *MTHFR* genotype (number of copies of T allele per subject), BMI, systolic blood pressure (SBP), diastolic blood pressure (DBP), HDL-cholesterol, log-triglycerides, creatinine, hematocrit, alkaline phosphatase, HbAic, and smoking habits (Table 4). No relationship was found between uric acid level and other factors (age, total cholesterol, glucose, total protein, blood urea nitrogen, and alcohol consumption).

To exclude the possible biases caused by the presence of subjects with hypertension, diabetes mellitus or impaired glucose tolerance, hypercholesterolemia, and hyperglyceridemia, subjects with each of these conditions were left out of the list in turn. Leaving out subjects with any of these conditions did not change the results as to *MTHFR* genotype: the correlation coefficients between *MTHFR* genotype and uric acid level fluctuated between 0.167 and 0.220, and their *P* values remained below 0.001. The same could be said of creatinine, BMI, DBP, log-triglycerides, and HDL-cholesterol. Their significant correlation with uric acid level did not disappear even after we omitted the data that might be potential sources of bias.

Multiple regression analyses

To assess the independent contribution of the variables to serum uric acid levels, multiple regression analyses were done, including the variables that were significantly associated with serum uric acid on univariate analyses. As shown in Table 5, creatinine, DBP, log-triglycerides, BMI, and *MTHFR* genotype were found to be independently associated with serum uric acid levels. Systolic blood pressure, HDL-cholesterol, and alkaline phosphatase showed no consistent association with uric acid level.

Discussion

Several studies of twins, as well as epidemiological data about ethnic groups, have suggested that genetic factors intervene in serum uric acid level (Whitfield and Martin 1983; Emmerson et al. 1992; Tuomilehto et al. 1988). However, only two genetic disorders in which germline mutations cause hyperuricemia have been identified: hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficiency (Seegmiller et al. 1967) and mutant phosphoribosylpyrophosphate (PRPP) synthetase (Sperling et al. 1973; Akaoka et al. 1981).

We have identified one of the genetic factors, the C677T *MTHFR* mutation, that is associated with hyperuricemia in elderly men. The C677T *MTHFR* mutation is an independent risk factor for vascular diseases. Recent studies have suggested that elevated serum uric acid predicts ischemic heart disease and stroke (Persky et al. 1979; Okada et al. 1982; Brand et al. 1985; Freedman et al. 1995; Wannamethee et al. 1997; Lehto et al. 1998; Culleton et al. 1999), although uric acid was thought to be an inert end-product of purine metabolism without physiological significance, except in gouty diathesis. Therefore, the C677T *MTHFR* mutation may be a risk factor that is involved in multiple metabolic pathways related to vascular diseases.

In this study dealing with elderly men, a dose-dependent relationship was demonstrated between the number of copies of the T allele of the *MTHFR* gene and serum uric acid concentration (Table 2). Compared with the low uric acid group, the prevalence of the T/T genotype and the frequency of the T allele were higher in the group with

elevated uric acid levels. The calculated odds ratio and 95% CI of hyperuricemia showed an increased risk for the prevalence of the C/T and T/T genotype, compared with the C/C genotype (Table 3). Multivariate analysis also demonstrated that the C677T *MTHFR* mutation was an independent predictor of hyperuricemia (Table 5). However, there was no relationship between the *MTHFR* genotypes and serum uric acid levels in young men, young women, or elderly women in our preliminary study (data not shown).

It remains less clear how the mutated homozygosity (T/T genotype) increases the serum uric acid level. Motti et al. (1998) presented two possible mechanisms. First, in subjects with the T/T genotype, renovascular atherosclerosis or the complications of systemic vascular disease may decrease the renal clearance of uric acid, resulting in elevated serum uric acid levels. Second, adenosine originating from S-adenosyl-homocysteine, a precursor of homocysteine, could represent a link between the metabolic pathways of homocysteine and uric acid. These two mechanisms may work simultaneously in subjects with the T/T genotype.

The question then arises as to how much the C677T *MTHFR* mutation does contribute to hyperuricemia. In order to answer this question, we compared the C677T *MTHFR* mutation with other risk factors for hyperuricemia, using multiple regression analysis. Four other factors were also shown to be associated with hyperuricemia in this study: creatinine, DBP, triglycerides, and BMI. Compared with these four factors, the C677T MTHFR mutation, itself, may contribute less to hyperuricemia, because it showed a lower standard partial regression coefficient (standardized β coefficient) to serum uric acid, as shown in Table 5.

To reduce the risk of vascular disorders in those who carry the C677T *MTHFR* mutation, especially mutated homozygotes, supplementation with folate, vitamin B12, and vitamin B6 has been recommended, because these micronutrients help to maintain the activity of the enzymes that metabolize homocysteine (Malinow 1994; Scott and Weir 1996). For the mutated homozygotes, strategies for lowering serum uric acid level, such as weight control and reducing the intake of foods containing purines, should be adopted to prevent hyperuricemia or gout. According to Motti et al. (1998), of the three genotypes, the mutated homozygotes showed the strongest correlation and the steepest slope of the regression line between serum uric acid and the logarithmically transformed value of plasma homocysteine.

Thus, we have demonstrated that mutation of the *MTHFR* gene may be a risk factor for hyperuricemia in elderly Japanese men. We have also identified four other factors associated with hyperuricemia in this study, and proposed a medical management strategy for those who are homozygous for the C677T *MTHFR* mutation.

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