Polymorphism of Methylenetetrahydrofolate Reductase Gene (C677T MTHFR) is Not a Confounding Factor of the Relationship Between Serum Uric Acid Level and the Prevalence of Hypertension in Japanese Men

Htay Lwin, MD; Tetsuji Yokoyama, MD*; Nobuo Yoshiike, MD; Kyoko Saito, PhD*; Akio Yamamoto, MD**; Chigusa Date, PhD†; Heizo Tanaka, MD††

Background The association between serum uric acid (UA) and the prevalence of hypertension, and the relationship between methylenetetrahydrofolate reductase (MTHFR) polymorphism and hypertension remains unclear. The aim of the present study was to investigate whether the C677T MTHFR mutation genotype (VV) is independently associated with the prevalence of hypertension or blood pressure (BP), and examined any interaction of MTHFR and UA with BP.

Methods and Results Participants were randomly selected from all residents (aged 40–69 years) in a rural county of Japan, and the data for the men (n=335) were analyzed. ‘Hypertension’ was defined as systolic BP ≥140 and/or diastolic BP ≥90 mmHg and/or being administered antihypertensive medication. Serum UA level was independently associated with the prevalence of hypertension (odds ratio (95% confidence interval) = 2.7 (1.2–5.9), p=0.047) for the highest tertile of serum UA (≥398.5 µmol/L (6.7 mg/dl)) vs that of the lowest tertile (<321.2 µmol/L (5.4 mg/dl)), but the MTHFR mutation was not independently associated with prevalence of hypertension or BP. No interaction of the MTHFR mutation and serum UA with BP was found.

Conclusions The mutation of C677T MTHFR was not independently associated with the prevalence of hypertension or BP levels although serum UA was. Furthermore, the relationship between serum UA and BP was not modulated by the MTHFR mutation in Japanese men. (Circ J 2006; 70: 83–87)

Key Words: Blood pressure; C677T MTHFR; Hypertension prevalence; Japanese; Uric acid

Many clinical and epidemiological studies have reported that serum uric acid (UA) is significantly associated with blood pressure (BP), and with hypertension (HT). However, the association between serum UA and HT remains controversial and both Okada et al and Ueshima et al have reported that BP levels and a prevalence of HT did not correlate with an increased level of serum UA when other confounding factors were taken into account. Some lifestyle-related factors (eg, alcohol consumption, body mass index (BMI) and obesity) may be confounding factors for the association between serum UA and HT.

A common polymorphism of the 5-10 methylenetetrahydrofolate reductase gene (C677T MTHFR) mutation can reduce the enzyme activity in the transsulfuration and remethylation pathways, leading to hyperhomocysteinemia. When the blood folate and vitamin B12 levels are lower and thus causing vascular complications, but other studies reported that hyperhomocysteinemia was not an independent risk factor for HT or BP. Wilcken et al reported that the MTHFR mutation is associated with HT in males, but the association was not strong. Some studies have reported that the C677T MTHFR mutation is associated with HT in men, but not in women and another Japanese study reported that the mutation was associated with HT in women, but not in men. Some studies, including a Japanese study, found no association between the MTHFR mutation or the level of homocysteine and a history of HT or vascular disease, although others have shown that hyperhomocysteinemia is an important risk factor for vascular complications. Therefore, the relationship of the MTHFR mutation with BP levels or prevalence of HT remains controversial.

Although the mechanism of the relationship between MTHFR polymorphism and serum UA is still unknown, some studies have presumed that the MTHFR mutation could affect 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. It has been speculated that the C677T MTHFR mutation may be a risk factor for hyperuricemia in elderly Japanese men, and hence a genetic confounding factor in the relationship between serum UA and HT.

However, no study has examined the genetic factors that may be linked to the UA–BP relationship or whether there...
is a genetic factor plus UA on BP levels or the prevalence of HT. We investigated the hypothesis that the C677T MTHFR mutant genotype (VV) is associated with BP levels, or with the prevalence of HT, independently of serum UA, serum creatinine, and lifestyle-related factors (BMI, smoking, alcohol consumption, physical activity, animal protein intake, serum folate, serum vitamin B₁₂, and age) in Japanese men. We also examined whether there is an interactive effect of the MTHFR genotype and the level of serum UA on systolic, diastolic, and mean BP (SBP, DBP, and MBP).

Methods

Study Subjects
Participants were randomly selected from all residents (male and female, aged 40–69 years), of Shioso, Hyogo Prefecture, Japan in 1999 and 2000. Subjects gave written informed consent at enrollment. The cross-sectional studies were part of a longitudinal study to continuously monitor the changes in lifestyle and risk factors for cardiovascular disease during the past decade. In the present study, we analyzed men only (n=335) although both men and women were included in the study population. Shioso county, consisting of towns Y, I, S, H, and C, had a total population of 10,485 men aged 40–69 years (5,238, 2,489, 1,185, 1,044 and 829 in towns Y, I, S, H, and C, respectively). We conducted surveys in towns Y and H in 1999, and in towns I, S, and C in 2000. From all residents aged 40–69 years, we selected subjects by stratified random sampling based on town and decades of age (5 towns×3 age groups=15 strata). Based on an expected response rate of 75%, we selected a total of 520 men of whom 358 (68.8%) participated in this study. After excluding the missing data of 23 men, 335 men were finally enrolled for the current analysis. The study project was approved by the ethics review committee of the National Institute of Health and Nutrition, Japan, and the Medical Research Institute, Tokyo Medical and Dental University, Japan.

Assessment of Lifestyle Factors
Under the supervision of nurses or dietitians specifically trained for this study, each participant completed a standardized questionnaire that included items about dietary intake (including animal protein), personal and family history of disease, physical activity, smoking and alcohol consumption.

To evaluate dietary intake, we used the 24-h food recall method. Each subject kept a brief record of food intake during 1 day in advance of the examination, and the interviewer asked in detail about the foods that had been eaten during the previous day. The portion size was determined at this interview with the aid of food scales and real-size food photograph booklets. Energy intake was then calculated based on the Standard Tables of Food Composition in Japan, 4th edition.

Daily smoking and alcohol status were assessed by a questionnaire conducted during individual interviews. We classified daily smoking status into 3 groups: non-smokers (including ex-smokers), 1–19 cigarettes/day, and 20+ cigarettes/day. Alcohol intake was reported as the frequency of consumption and the usual amount and type of alcoholic beverage consumed. We classified these into 3 groups: non-drinkers (including ex-drinkers), 1–2 drinks/day, and 3+ drinks/day, where 1 drink is approximately 12 g of ethanol. Anthropometric measurements (height and weight) were measured in light clothing. BMI was defined as weight/height² (kg/m²).

Laboratory Methods
Venous blood samples were drawn into EDTA-tubes and serum plain tubes. Serum was then obtained by centrifugation at 3,000 rpm for 10 min at 4°C and subsequently used for biochemical assays. The blood cells were frozen and kept at −20°C until DNA extraction. Biochemical variables, including serum UA and serum creatinine, were measured by an enzymatic method on an autoanalyzer (Hitachi 7170). Plasma total homocysteine (tHcy) level was determined by high-performance liquid chromatography with fluorescence detection. Serum folate and serum vitamin B₁₂ were measured using a chemiluminescent analyzer ACS-180 (Chiron Corp, CA, USA). MTHFR genotyping was performed by the polymerase chain reaction method with slight modification, and using the sense primer (5′-CAA AGG CCA CCC CGA AGC-3′) and anti-sense primer (5′-AGG ACG GTG CGG TGA GAG TG-3′).)

BP Measurement
After at least 15 min of rest, BP was measured twice in the right arm while seated with a standard mercury sphygmomanometer by a trained staff physician, and the mean of the first and second values was used for the study. ‘Hypertension’ was defined as a SBP ≥140 mmHg and/or a DBP ≥90 mmHg and/or being administered antihypertensive medication.

Statistical Analyses
The allele frequency was determined by direct counting. The frequencies of the genotypes are shown as percentages. Deviation of the genotype distribution from the Hardy-Weinberg equilibrium was tested by the exact test. The adjusted odds ratio (OR) and 95% confidence interval (CI) were calculated to estimate the risk for HT among MTHFR genotypes, and tertiles of serum UA, and tertiles of serum folate, serum vitamin B₁₂, and serum UA were measured by an enzymatic method on an autoanalyzer (Hitachi 7170). Plasma total homocysteine (tHcy) level was determined by high-performance liquid chromatography with fluorescence detection. Serum folate and serum vitamin B₁₂ were measured using a chemiluminescent analyzer ACS-180 (Chiron Corp, CA, USA). MTHFR genotyping was performed by the polymerase chain reaction method with slight modification, and using the sense primer (5′-CAA AGG CCA CCC CGA AGC-3′) and anti-sense primer (5′-AGG ACG GTG CGG TGA GAG TG-3′).

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The correlation between the level of plasma tHcy and BP was tested by Pearson correlation analysis. ANOVA followed by Tukey’s multiple comparison test was used for comparison of the least square mean (LSM) of continuous variables between hypertensive and normotensive subjects, and between the MTHFR genotypes. Multiple linear regression analysis was used for assessment of interaction effect. Mantel-Haenszel chi-square test was used for group comparisons after adjustment for age. The multivariate-adjusted OR and CI were calculated by multiple logistic regression analysis. The multivariate-adjusted model included age, BMI, daily smoking, alcohol drinking, physical activity, animal protein intake, serum creatinine for the analysis for an association between serum UA and prevalence of HT, and the additional variables (serum folate, serum vitamin B₁₂, and serum UA) were included in the same multivariate model when an association of MTHFR genotype and prevalence of HT was analyzed. All statistical analyses were performed using SAS 8.2 software (Cary, NC, USA).
Results

Basic Characteristics and MTHFR Genotype Distribution

Table 1 shows the basic characteristics of all the study subjects and the distribution of the 3 MTHFR genotypes. The mean age was 53.1±8.9 years and the mean serum UA level was 562.8±83.3 μmol/L (6.1±1.4 mg/dl). The age-adjusted LSM of the serum UA level and of BP, body weight, height, BMI, and serum vitamin B12 among the 3 genotypes was not significantly different. The LSM of the plasma tHcy level in the VV genotype was significantly the lowest of the 3 genotypes. The frequencies of the C677T MTHFR mutant allele (V) was 43%. The frequency of mutant homozygotes of the C677T MTHFR genotype (VV) in the total, hypertensive, and normotensive men was 17.1%, 16%, and 17.4%, respectively (Table 2). The genotype distribution did not differ significantly from the Hardy-Weinberg equilibrium.

MTHFR Genotype and Prevalence of HT

Table 2 shows that there was no significant association between the follicle level in the VV genotype was significantly the lowest of the genotypes. The frequencies of the C677T MTHFR mutant allele (V) was 43%. The frequency of mutant homozygotes of the C677T MTHFR genotype (VV) in the total, hypertensive, and normotensive men was 17.1%, 16%, and 17.4%, respectively (Table 2). The genotype distribution did not differ significantly from the Hardy-Weinberg equilibrium.

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MTHFR Genotype and Prevalence of HT

Table 2 shows that there was no significant association between the
MTHFR genotype and the prevalence of HT after age adjustment, or after multivariate adjustment for age, BMI, daily smoking, alcohol consumption, physical activity, animal protein intake, serum UA, serum creatinine, serum folate, and serum vitamin B12. After age adjustment, the OR for the prevalence of HT in the highest tertile of serum UA level compared with the lowest tertile of serum UA was much higher than that of the medium tertile, showing an approximately linear dose–response relationship (p for trend=0.0027). Even after multivariate adjustment for age, BMI, daily smoking, alcohol consumption, physical activity, animal protein intake, and serum creatinine, the trend for the OR for the prevalence of HT according to tertile of serum UA was still significant (p for trend=0.047). Serum UA level was significantly higher in hypertensive subjects (LSM±standard error (SE)=380.7±7.7 μmol/L) than in normal subjects (LSM±SE=356.9±5.4 μmol/L, p=0.016 after adjustment of known confounding factors including selected lifestyle-related factors ie, age, BMI, daily smoking, alcohol consumption, physical activity, animal protein intake, serum creatinine).

Interaction of MTHFR Genotype and Serum UA or Other Lifestyle-Related Factors on Prevalence of HT or BP

There were no significant interaction effects of the MTHFR genotype and the serum UA level with MBP, SBP, and DBP (p=0.48 for overall test of interaction between MTHFR and serum UA with MBP, p=0.36 for that with SBP, and p=0.72 for that with DBP) even after multiple adjustment for age, BMI, daily smoking, alcohol consumption, physical activity, animal protein intake, serum creatinine, serum folate, and serum vitamin B12. There were also no significant interactions of the MTHFR genotype and BMI or alcohol consumption or daily smoking or physical activity with the prevalence of HT, or with MBP, SBP, and DBP (data not shown).

Plasma tHcy Level and HT

The age-adjusted geometric LSM, SE of the plasma levels of tHcy (13.9, 1.0 μmol/L), and serum vitamin B12 (437.3, 0.74 pmol/L) in hypertensive subjects did not differ significantly from those of normotensive subjects (13.9, 1.0 μmol/L and 425.6, 0.74 pmol/L, respectively), but the age-adjusted geometric LSM, SE of serum folate in hypertensive subjects (16.5, 2.3 nmol/L) was significantly higher than in normotensive subjects (14.0, 2.3 nmol/L, p=0.01). After additional adjustment for serum folate, serum vitamin B12, serum UA, serum creatinine, daily smoking, alcohol consumption, BMI, and physical activity, the geometric LSM of the plasma tHcy level of hypertensive subjects (13.6 μmol/L) was still not significantly different from that of normotensive subjects (13.5 μmol/L).

Discussion

Although some studies have shown a significant association between MTHFR mutation and SBP or HT in men and Inamoto et al have reported that MTHFR mutation was a risk factor for HT in women only; other studies have reported that the relationship between MTHFR polymorphism and BP or prevalence of HT remains unclear and inconsistent. No significant associations between MTHFR mutation and BP (MBP, SBP, and DBP) or prevalence of HT were found in the present study, which supports the studies of Nishio et al and Inamoto et al who found no association between MTHFR mutation and HT in men, although there was an association in women. MUTATION of MTHFR could reduce its enzymatic activity, and cause hyperhomocysteinemia; but the influence of the MTHFR mutation on plasma tHcy level also depends on lifestyle-related factors (serum folate, and serum vitamin B12 levels). In the present study, the plasma tHcy level was not significantly associated with SBP, DBP, or MBP (data not shown), and there was no significant difference between the level of plasma tHcy in hypertensive and normotensive subjects, even after adjustment for several confounding factors; however, in our previous report C677T MTHFR mutation was associated with increased plasma tHcy. Perry et al and Dalery et al also reported that there was no significant correlation between plasma tHcy level and the presence of HT or BP levels.

Many studies have reported that hyperuricemia is an independent predictor of HT in the general population, but its causative role is controversial. Several epidemiologic studies, including some Japanese studies, have failed to show UA as an independent risk factor for HT or BP or cardiovascular events. Although our study did not find an association between C677T MTHFR mutation and the prevalence of HT, we found that the serum UA level was significantly and independently associated with MBP, SBP, and DBP after age-adjustment and even adjustment for known confounding factors in the multivariate analysis in this Japanese population. We also found that serum UA levels were significantly higher in hypertensive subjects, and that the estimated risk of HT was also associated with the level of serum UA, even when the serum creatinine level and estimated creatinine clearance were considered as confounding factors for renal function. The higher serum UA concentration in the hypertensive subjects in our study may be a compensatory mechanism to counteract oxidative damage related to atherosclerosis and aging in humans. Recently, antioxidants such as vitamin E and fluvastatin have become important therapies in homocysteine-induced atherosclerosis caused by oxidative stress. Therefore, our results are compatible with many other clinical and epidemiologic studies. In addition, our study showed no significant interactive effect of MTHFR mutation and serum UA level with BP (MBP, SBP, or DBP), even after multiple adjustment for age, BMI, daily smoking, alcohol consumption, physical activity, animal protein intake, serum creatinine, serum folate, and serum vitamin B12. To the best of our knowledge, gene–UA interaction or gene–lifestyle interaction related to MTHFR polymorphisms in the general Japanese population has not been previously reported. Although we considered several confounding factors, including renal function, and lifestyle-related, nutritional and environmental factors, some limitations of this study are (i) the cross-sectional structure and (ii) the possibility that diuretic medication in the hypertensive subjects may have elevated the serum UA level and (iii) antioxidants supplements may also have affected the serum homocysteine concentration in the hypertensive subjects as well as the incidence of vascular complications. Unfortunately, we could not adjust for diuretic medication and antioxidant supplements when we analyzed for a relationship between serum UA and the prevalence of HT or BP because we did not have those data. Therefore, more longitudinal studies in the Japanese population should be conducted to take into account in several confounding factors.
In summary, our findings suggest that C677T MTHFR mutation is not independently associated with the prevalence of HT or BP level in Japanese men, but that the level of serum UA is, when selected lifestyle-related factors (BMI, daily smoking, alcohol consumption, physical activity, animal protein intake, serum folate, serum vitamin B12, and age), and serum creatinine are taken into account. The interaction of the MTHFR mutation and the serum UA level did not influence BP levels in Japanese men.

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