Effect of the methylenetetrahydrofolate reductase 677C→T mutation on the relations among folate intake and plasma folate and homocysteine concentrations in a general population sample[1–3]

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ABSTRACT

Background: Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in folate and homocysteine metabolism. The common *MTHFR* 677C→T polymorphism decreases the enzyme's activity.

Objective: The objective of the study was to assess the effect of the polymorphism on the relations among folate intake, plasma folate concentration, and total plasma homocysteine (tHcy) concentration.

Design: The design was a cross-sectional analysis in a random sample (n = 2051) of a Dutch cohort (aged 20–65 y).

Results: At a low folate intake (166 μg/d), folate concentrations differed significantly among the genotypes (7.1, 6.2, and 5.4 nmol/L for the *CC*, *CT*, and *TT* genotypes, respectively; P for all comparisons < 0.05). At a high folate intake (250 μg/d), folate concentrations in *CT* and *CC* subjects did not differ significantly (8.3 and 8.6 nmol/L, respectively, but were significantly higher (P = 0.2) than those in *TT* subjects (7.3 nmol/L; P = 0.04). At a low folate concentration (4.6 nmol/L), *TT* subjects had a significantly higher (P = 0.0001) tHcy concentration than did *CC* and *CT* subjects (20.3 compared with 15.0 and 14.1 μmol/L, respectively), whereas at a high folate concentration (11.9 nmol/L), the tHcy concentration did not differ significantly between genotypes (P > 0.2; < 13.1 for all genotypes). The relation between folate intake and tHcy concentration had a pattern similar to that of the relation between plasma folate and tHcy concentrations.

Conclusions: At any folate intake level, *TT* subjects have lower plasma folate concentrations than do *CT* and *CC* subjects. Yet, at high plasma folate concentrations, tHcy concentrations in *TT* subjects are as low as those in *CT* and *CC* subjects. *Am J Clin Nutr* 2003;77:687–93.

KEY WORDS Methylenetetrahydrofolate reductase, MTHFR, folate intake, plasma folate concentration, plasma homocysteine concentration, total homocysteine, tHcy, metabolism, Dutch population

INTRODUCTION

The enzyme methylenetetrahydrofolate reductase (MTHFR; EC 1.5.1.20) plays a key role in folate and homocysteine metabolism (1, 2). The 677C→T mutation in the gene that encodes for *MTHFR* (3) decreases the enzyme's activity (3, 4). The prevalence of this variant is relatively high in the general population; eg, the prevalence of homozygosity (TT) is 5–15% in several white populations (5).

The 677C→T polymorphism is associated with a higher plasma total homocysteine (tHcy) concentration (3, 6), which is most pronounced in subjects with the *TT* genotype who have a marginal folate status (7). The polymorphism is also associated with a lower plasma folate concentration (8–16). These metabolic changes are postulated to modify the risk of chronic diseases, including cardiovascular disease (17, 18), cancer (19), and dementia (20, 21), and the risk of neural tube defects (8, 22). Therefore, it is important to have detailed information on the consequences of the 677C→T genotype for the relations between folate intake, plasma folate, and tHcy. Yet the influence of the 677C→T genotype on the relation between folate intake and plasma folate and on that between folate intake and plasma tHcy has only scarcely been investigated (8, 23).

With the data from a large cross-sectional, population-based study, we described the effect of the *MTHFR* 677C→T mutation on the relation between folate intake and plasma folate and on the subsequent relation between plasma folate and tHcy. Data on the effect of the genotype on the relation between folate intake and tHcy are presented as well, to interpreted studies that have data only on the relation between folate intake and tHcy.

SUBJECTS AND METHODS

Subjects

We drew an age- and sex-stratified random sample of 3025 subjects out of the population of the Monitoring Project on Risk

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Factors for Chronic Diseases in the Netherlands (MORGEN Project) examined from 1993 to 1996 (n = 19 066) (24). The MORGEN Project is a cross-sectional investigation of the prevalence of risk factors for chronic diseases and certain chronic conditions. The population of that study consisted of a random sample of subjects aged 20–65 y from 3 cities in the Netherlands (Amsterdam, Doetinchem, and Maastricht) (25). The external Medical-Ethical Committee of the Toegepast-Natuurwetenschappelijk onderzoek (TNO) Toxicology and Nutrition Institute, which follows the guidelines of the Helsinki Declaration, approved the MORGEN study.

Data collection

Respondents completed 2 self-administered questionnaires: a general questionnaire and a semiquantitative food-frequency questionnaire (FFQ). Subsequently, trained research assistants performed a physical examination at the Municipal Health Services of the three above-mentioned cities where subjects were located.

From the general questionnaire, we extracted information on sex, age, and smoking. The semi-quantitative FFQ provided information on dietary habits. This questionnaire's relative validity for food groups was tested against twelve 24-h recalls, and its reproducibility for food groups was tested by using 2 questionnaires administered 12 mo apart. Overall, the relative validity and the reproducibility of this questionnaire were sufficient and similar to those of other FFQs (26). We calculated the intake of folate and also of other nutrients (vitamins B-2, B-6, and B-12 and methionine) that are closely related to folate (27). Furthermore, we extracted data on the consumption of alcoholic beverages. The FFQ did not collect information on the doses and contents of vitamin supplements. Hence, to reduce the misclassification of B vitamin intake, we excluded from all analyses subjects who used supplements that might have contained B vitamins (217 men and 371 women).

Blood sampling and biochemical determinations

During the physical examination, nonfasting venous blood samples were obtained. The samples were collected in evacuated tubes containing 7.5% K3-EDTA (Safety-Monovette tubes; Sarstedt, Tilburg, Netherlands) and centrifuged within 1 h (10 min at 3000 × *g* at room temperature). After centrifugation, the plasma, red blood cells, and white blood cells were separated and stored at −20 °C or −80 °C. The blood samples were stored at room temperature before centrifugation, but we found that this led to only a small increase in the measured tHcy concentration (24).

We measured the tHcy concentration, including the protein-bound and nonprotein-bound fractions of homocysteine, by using HPLC with fluorescence detection as described by Fiskerstrand et al (28), with some modifications (29). The within- and between-run CVs were 3.2% and 8.6%, respectively.

Plasma folate concentration was determined with the use of a *Lactobacillus casei* microbiologic assay (30), and plasma vitamin B-12 concentration was assessed with a *Lactobacillus leichmannii* assay (31). Both the folate and the vitamin B-12 assays were adapted to a microtiter plate format and carried out by a robotic workstation (Microlab AT Plus; Hamilton, Bonaduz, Switzerland). The within- and between-run CVs were 4.3% and 10.4% for folate and 1.1% and 1.7% for vitamin B-12.

DNA was extracted from frozen peripheral blood lymphocytes by a salting-out procedure. The presence of the 677C→T mutation was assessed by polymerase chain reaction, which was followed by restriction enzyme analysis with *Hin*FI (3). For 182 men and 204 women, the DNA extraction or genotyping was unsuccessful. The genotype distribution was in Hardy-Weinberg equilibrium.

Statistical analysis

Because of the exclusion of B vitamin supplement users (n = 588) and of subjects with a missing *MTHFR* genotype (n = 386), all analyses are based on data for 2051 men and women. The distributions of the plasma concentrations of tHcy, folate, and vitamin B-12 and the intake of all nutrients were skewed with a long tail toward higher values, and thus natural logarithmic transformations were applied to normalize these distributions. Inverse transformations were performed to provide geometric means and 95% CIs. Geometric means are provided throughout this report. After logarithmic transformation, the intakes of the nutrients (B vitamins and methionine) were adjusted for energy according to the method of Willett et al (32) as described elsewhere (33).

Mean geometric values of B vitamin and methionine intakes and of plasma concentrations of folate, vitamin B-12, and tHcy were calculated by *MTHFR* genotype (*CC*, *CT*, and *TT*), and *P* values for trends were calculated with univariate linear regression analyses with genotype as the independent variable.

The role of the 677C→T polymorphism in the relations between 1) folate intake and plasma folate concentration, 2) plasma folate and tHcy concentration, and 3) folate intake and tHcy concentration was described for men and women together, because no modification of effect by sex was observed for these relations. Modification of effect by the polymorphism in the above relations was evaluated in linear regression models with the inclusion of the appropriate interaction terms, eg, folate intake × genotype. A P value < 0.05 for the interaction term was considered to indicate a significant interaction.

Genotype-stratified relations were adjusted for age and sex. In multiple regression models, we also adjusted for alcohol consumption (drinks/d) and smoking (no or yes). Furthermore, we adjusted for vitamin B-12 intake in the relation with folate intake as explanatory variable and for plasma B-12 in the relation with plasma folate as explanatory variable. Because we did not have plasma levels of vitamins B-2 and B-6 and methionine, we could only adjust the associations for intakes of these nutrients.

To test whether the β coefficients differed significantly among the 3 genotypes, we calculated by hand the test statistic $(\beta_1 - \beta_2)/SE_{\beta_1 - \beta_2}$, which follows a Student's *t* test distribution. The SE of $\beta_1 - \beta_2$ was calculated with the use of the following formula: $SE_{\beta_1 - \beta_2} = \sqrt{[(SE_{\beta_1})^2 + (SE_{\beta_2})^2]}$. The 2-sided P value of this statistic was derived from a table giving the percentile values of the *t* distribution (34). To account for multiple comparison, we multiplied this P value by 3.

The mean adjusted plasma folate or tHcy concentrations (and 95% CIs) for each genotype within tertiles (based on the total population, rather than being genotype-specific) of folate intake or plasma folate concentrations were calculated by analyses of covariance, as implemented in the general linear model procedure (SAS Institute Inc, Cary, NC). Differences in mean concentrations compared with a referent category were tested with the use of Bonferroni's adjustment for multiple comparisons.

Findings were considered significant if the 2-sided P value was < 0.05. Data were analyzed with SAS statistical software, version 8.1 (SAS Institute Inc).

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TABLE 1
Dietary and blood indices in men and women aged 20–65 y by methylenetetrahydrofolate reductase (*MTHFR*) genotype[1]

	Subjects				
	All (n = 2051)	*CC* (n = 983)	*CT* (n = 907)	*TT* (n = 206)	*P* for trend
Folate intake (μg/d)	204 (124–330)	205 (124–358)	203 (125–316)	201 (111–309)	0.09
Vitamin intake					
B-2 (mg/d)	1.53 (0.88–2.93)	1.53 (0.86–2.92)	1.52 (0.88–2.93)	1.53 (0.90–2.82)	0.8
B-6 (mg/d)	1.77 (1.14–2.58)	1.78 (1.14–2.60)	1.76 (1.11–2.48)	1.75 (1.19–2.59)	0.09
B-12 (μg/d)	4.53 (1.86–12.1)	4.58 (1.93–12.1)	4.47 (1.82–11.5)	4.58 (2.08–12.0)	0.4
Methionine intake (mg/d)	1.81 (1.14–2.63)	1.81 (1.13–2.64)	1.80 (1.16–2.57)	1.83 (1.20–2.53)	0.9
Plasma folate (nmol/L)	7.4 (2.4–22.4)	8.0 (3.4–22.2)	7.2 (2.5–20.9)	6.3 (2.6–26.6)	<0.001
Plasma vitamin B-12 (pmol/L)	284 (102–638)	288 (105–735)	283 (105–649)	274 (101–683)	0.1
Plasma tHcy (μmol/L)	13.6 (7.8–39.5)	12.9 (7.5–22.5)	13.6 (8.1–33.4)	17.1 (8.1–67.1)	<0.001

[1] Geometric $\bar{x}$; 1st–99th percentile range in parentheses. tHcy, total homocysteine. The minimal numbers of valid observations were 2037 for all subjects, 928 for *CC* subjects, 904 for *CT* subjects, and 205 for *TT* subjects.

RESULTS

Dietary and blood indexes by *MTHFR* genotype

The study population consisted of 1094 men and 957 women with a mean age of 41 y (range 20–65 y). The dietary intakes of folate; vitamins B-2, B-6, and B-12; and methionine did not differ significantly between the genotypes (**Table 1**). The plasma folate concentration was inversely associated with the presence of a *T* allele. Compared with *CC* subjects, *TT* subjects had an average plasma folate concentration 1.7 nmol/L lower and *CT* subjects had an average plasma folate concentration 0.8 nmol/L lower. The prevalence of a low plasma folate concentration (defined as <4.5 nmol/L) increased with the presence of the mutation: 8% in *CC* subjects, 11% in *CT* subjects, and 23% in *TT* subjects. There was no association between the 677C→T polymorphism and the plasma vitamin B-12 concentration.

We observed a strong positive trend ($P < 0.0001$) between the presence of the *T* allele and the tHcy concentration. On average, the presence of 1 *T* allele or 2 *T* alleles resulted in tHcy concentrations, respectively, 0.7 μmol/L and 4.2 μmol/L higher than those found in subjects with no *T* alleles. The prevalence of hyperhomocysteinemia (defined as tHcy > 17.4 μmol/L) increased dramatically with the presence of the mutation: 8% in *CC* subjects, 12% in *CT* subjects, and 38% in *TT* subjects.

The effect of the *MTHFR* 677C→T mutation on the slopes of the relations among folate intake and plasma folate and tHcy concentrations

The *P* value of the interaction term folate intake × genotype in the regression model describing the continuous relation between folate intake and plasma folate concentration was 0.02; the interaction term plasma folate × genotype in the model describing the relation between plasma folate and tHcy was 0.0001, and the interaction term folate intake × genotype in the model describing the relation between folate intake and tHcy was 0.0001. Because of these interactions, we have stratified these relations to genotype.

The β coefficients of the linear regression models of the above described relations are shown in **Table 2**. These β coefficients express the change in, respectively, logarithmically transformed plasma folate (nmol/L) and logarithmically transformed tHcy (μmol/L) that is associated with a 1-unit change in logarithmically transformed folate intake (μg/d) or logarithmically transformed plasma folate concentration (nmol/L). Because of this logarithmic transformation of the *x* variable as well as the *y* variable, the interpretations of these coefficients is as follows: a 1% change in the *x* variable corresponds to a β% change in the *y* variable. For example, a 1% increase in folate intake is associated with a 0.47% increase in plasma folate concentration, according to the first coefficient in Table 2.

The *MTHFR* 677C→T mutation and the relation between folate intake and plasma folate concentration

The slope of the age- and sex-adjusted relation between folate intake and plasma folate concentration was positive for all genotypes (Table 2). The steepest increase in plasma folate concentration with an increasing folate intake was observed in *TT* subjects (β = 0.77), whereas the least steep slope was observed in *CC* subjects (β = 0.47). Multiple adjustment (age, sex, alcohol consumption, smoking, and intake of vitamins B-2, B-6, and B-12 and methionine) attenuated the slopes for *CC* and *TT* subjects, but augmented the steepness of the slope in *CT* subjects. The slope in *CT* subjects differed significantly from that in *CC* subjects ($P < 0.015$).

The mean adjusted (for age; sex; intake of vitamins B-2, B-6, and B-12 and methionine; alcohol consumption; and smoking) plasma folate concentration for each genotype per tertile of folate intake is shown in **Figure 1**. The mean folate intake in the lowest tertile was 166 μg/d (minimum, 102; maximum, 190); that in the medium tertile was 205 μg/d (191–220), and that in the highest tertile was 250 μg/d (221–761). This figure illustrates both the above described steepness of the relation between folate intake and plasma folate and the effect of the genotype on the plasma folate concentration at a given folate intake. At a low folate intake, the plasma folate concentration of all genotypes differed significantly: *CC* compared with *CT*, $P = 0.0005$; *CC* compared with *TT*, $P = 0.0001$; and *CT* compared with TT, $P = 0.03$. At a medium folate intake, the plasma folate concentration differed significantly between *CC* and *TT* subjects ($P = 0.0007$) and between *CC* and *CT* subjects ($P = 0.003$), but not between *CT* and *TT* subjects ($P = 0.2$). At a high folate intake, the plasma folate concentrations in *CC* and *CT* subjects did not differ significantly ($P = 0.9$), but *TT* subjects had a lower plasma folate concentration than did *CC* ($P = 0.006$) and *CT* ($P = 0.04$) subjects, despite equally high folate intakes.

The *MTHFR* 677C→T mutation and the relation between plasma folate and tHcy concentrations

The slope of the age- and sex-adjusted relation between plasma folate and tHcy was negative for all genotypes (Table 2). The

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TABLE 2

β coefficients of linear regression models for the concentration of plasma folate or tHcy associated with a 1-unit change in either folate intake or plasma folate, stratified by methylenetetrahydrofolate reductase genotype[1]

	CC subjects (n = 938)[2]	*CT* subjects (n = 907)[3]	*TT* subjects (n = 206)[4]
Model 1: plasma folate vs. folate intake			
Simply adjusted[5] β	0.47 (0.34, 0.60)	0.68 (0.54, 0.82)	0.77 (0.40, 1.14)
Multiply adjusted[6] β	0.41[a] (0.24, 0.58)	0.76[b] (0.58, 0.94)	0.63 (0.16, 1.10)
Model 2: plasma tHcy vs. plasma folate			
Simply adjusted[5] β	−0.17[a] (−0.20, −0.13)	−0.25[b] (−0.29, −0.22)	−0.48[c] (−0.57, −0.39)
Multiply adjusted[6] β	−0.15[a] (−0.18, −0.11)	−0.22[b] (−0.25, −0.18)	−0.38[c] (−0.47, −0.28)
Model 3: plasma tHcy vs. folate intake			
Simply adjusted[5] β	−0.24[a] (−0.31, −0.16)	−0.36[a] (−0.44, −0.28)	−0.78[b] (−1.08, −0.49)
Multiply adjusted[6] β	−0.21 (−0.31, −0.11)	−0.30 (−0.41, −0.19)	−0.51 (−0.88, −0.14)

[1] 95% CIs in parentheses. The β values express the change in log plasma folate or log total homocysteine (tHcy) associated with a 1-unit change in log folate intake or log plasma folate, respectively.

[2–4] Minimal number of observations: [2] 925, [3] 901, [4] 205.

[5] Adjusted for age and sex.

[6] Adjusted for age, sex, intake of vitamins B-2 and B-6 and methionine, smoking (no or yes), and alcohol consumption (drinks/d). Models 1 and 3 were also adjusted for vitamin B-12 intake and model 2 for plasma vitamin B-12.

steepest decrease in tHcy with an increasing plasma folate concentration was observed in *TT* subjects (β = −0.48), and the least steep slope was observed in *CC* subjects (β = −0.17). All slopes differed significantly between the genotypes ($P < 0.015$). Additional adjustments for the intakes of vitamins B-2 and B-6 and methionine, plasma vitamin B-12 concentration, alcohol consumption, and smoking did not significantly change our results.

The mean adjusted (intake of vitamins B-2 and B-6 and methionine, plasma vitamin B-12 concentration, alcohol consumption, and smoking) tHcy concentration per tertile of the plasma folate concentration is shown in **Figure 2**. The mean plasma folate concentration in the first tertile was 4.6 nmol/L (minimum: 1.2, maximum: 6.2); that in the medium tertile was 7.5 (6.3–8.9) nmol/L, and that in the highest tertile was 11.9 (9.0–56.3) nmol/L. The figure shows the multiple adjusted results presented in Table 2: compared with *CC* and *CT* subjects, *TT* subjects had a steeper inverse relation between folate intake and tHcy. At a low plasma folate concentration, *TT* subjects had a significantly higher ($P = 0.0001$) tHcy concentration than did *CC* and *CT* subjects. Although the mean tHcy concentration in *CT* subjects was much lower than that in *TT* subjects, it was significantly higher ($P = 0.03$) than that in *CC* subjects. At a medium plasma folate concentration, the mean tHcy concentration in *TT* subjects remained the highest ($P = 0.0001$ for the comparison with *CC*

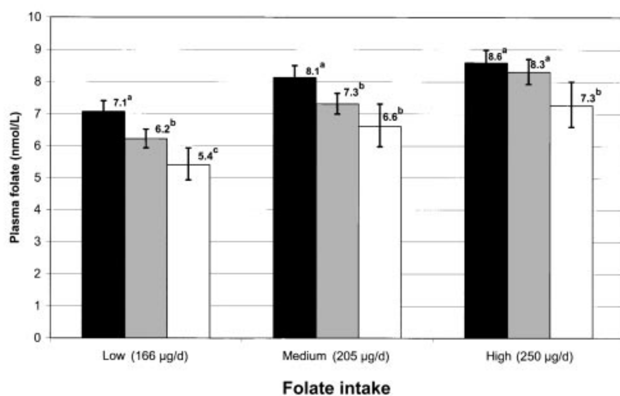

FIGURE 1. Geometric mean adjusted plasma folate concentrations according to the methylenetetrahydrofolate reductase genotypes stratified by tertiles of folate intake in Dutch men and women aged 20–65 y: *CC* subjects, ■; *CT* subjects, ■; *TT* subjects, □. Means were adjusted for age, sex, smoking (no or yes), alcohol consumption (drinks/d), and intakes of vitamins B-2, B-6, and B-12 and methionine. Values with different superscript letters are significantly different, $P < 0.05$. For a comparison of genotypes at the same folate intake, tertiles were based on the distribution of the entire population; thus, the numbers of *CC*, *CT*, and *TT* subjects with low, medium, and high folate intakes were 308, 297, and 78; 310, 313, and 60; and 319, 296, and 68, respectively.

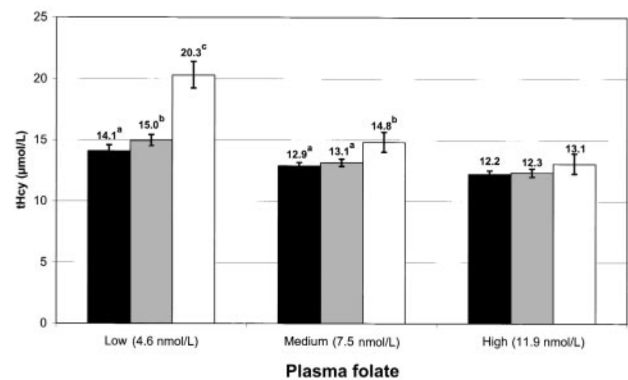

FIGURE 2. Geometric mean adjusted plasma total homocysteine (tHcy) concentrations according to the methylenetetrahydrofolate reductase genotypes stratified by tertiles of plasma folate concentration in Dutch men and women aged 20–65 y: *CC* subjects, ■; *CT* subjects, ■; *TT* subjects, □. Means were adjusted for age, sex, smoking (no or yes), alcohol consumption (drinks/d), intakes of vitamins B-2 and B-6 and methionine, and plasma vitamin B-12 concentrations. Values with different superscript letters are significantly different, $P < 0.05$. For a comparison of genotypes at the same folate intake, tertiles were based on the distribution of the entire population; thus, the numbers of *CC*, *CT*, and *TT* subjects with low, medium, and high folate intakes were 244, 328, and 105; 332, 306, and 51; and 352, 270, and 49, respectively.

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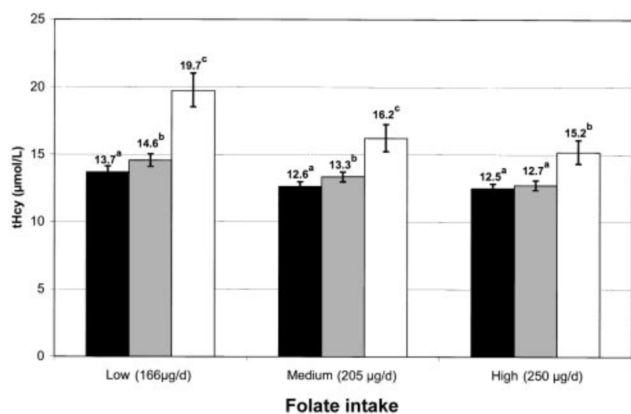

FIGURE 3. Geometric mean adjusted plasma total homocysteine (tHcy) concentrations according to the methylenetetrahydrofolate reductase genotypes stratified by tertiles of folate intake in Dutch men and women aged 20–65 y: *CC* subjects, ■; *CT* subjects, ■; *TT* subjects, □. Means were adjusted for age, sex, smoking (no or yes), alcohol consumption (drinks/d), and intakes of vitamins B-2, B-6, and B-12 and methionine. Values with different superscript letters are significantly different, $P < 0.05$. For a comparison of genotypes at the same folate intake, tertiles were based on the distribution of the entire population; thus, the numbers of *CC*, *CT*, and *TT* subjects with low, medium, and high folate intakes were 308, 297, and 78; 310, 313, and 60; and 319, 296, and 68, respectively.

subjects and $P = 0.0003$ for the comparison with *CT* subjects). The tHcy concentrations in *CT* and *CC* subjects no longer differed significantly ($P = 0.6$). At a high plasma folate concentration, none of the tHcy concentrations differed significantly (*CC* compared with *CT*, $P = 0.99$; *CC* compared with *TT*, $P = 0.2$; *CT* compared with *TT*, $P = 0.3$).

The *MTHFR* 677C→T mutation and the relation between folate intake and tHcy concentrations

Because folate intake is largely reflected in the plasma folate concentration, the patterns of steepness of the slopes of the relations between folate intake and tHcy and between plasma folate and tHcy were essentially the same (Table 2). The multiply adjusted (age, sex, alcohol consumption, smoking, and intake of vitamins B-2, B-6, and B-12 and methionine) relation between folate intake and tHcy attenuated the slopes for all genotypes and they were no longer significantly different.

The mean adjusted (for intakes of vitamins B-2, B-6, and B-12 and methionine; alcohol consumption; and smoking) tHcy concentration per tertile of folate intake is shown in **Figure 3**. As shown in Table 2, the steepest inverse slope between increasing folate intake and tHcy was observed in *TT* subjects. At a low folate intake, *TT* subjects had the highest tHcy concentration ($P = 0.0001$ when compared with *CC* and *CT* subjects), and the mean tHcy concentration in *CT* subjects was also significantly higher than that in *CC* subjects ($P = 0.02$). At a medium folate intake, tHcy concentrations also differed significantly by genotype (*CC* compared with *CT*, $P = 0.02$; *CC* compared with *TT*, $P = 0.0001$; *CT* compared with *TT*, $P = 0.0001$). At a high folate intake, the tHcy concentrations in *CC* and *CT* subjects no longer differed significantly ($P = 0.99$); however, despite the equally high folate intake, *TT* subjects still had a significantly higher mean tHcy concentration than did *CC* and *CT* subjects ($P = 0.0001$).

DISCUSSION

This population-based study identified 2 aspects of the *MTHFR* 677C→T polymorphism that have not been described in detail for a general population. First, at any folate intake level (including the highest level), *TT* subjects always had lower plasma folate concentrations than did *CC* or *CT* subjects. Second, *CT* subjects experienced an unfavorable effect of their *T* allele only when they had a low folate intake. Moreover, we confirmed a well-described gene × nutrient interaction; those with the *TT* genotype and a low plasma folate concentration or a low folate intake had higher tHcy concentrations than did *CT* and *CC* subjects. Conversely, at a high plasma folate concentration or a high folate intake, the difference in tHcy concentrations between the genotypes was small.

In the present study, 10% of the subjects were identified as homozygous for the 677C→T mutation; heterozygotes accounted for 44% of the subjects. These values are similar to those reported in other white populations (5). The fact that we had a complete data set for a subsample of the original random sample did not render our sample less representative: the distribution of age, lifestyle factors, biological cardiovascular disease risk factors (ie, blood pressure and total and HDL-cholesterol concentrations) was equal for subjects who were ($n = 2051$) and were not ($n = 974$) included.

The intake of folate and other relevant nutrients was assessed with a semiquantitative FFQ validated for the intake of food groups predominantly contributing to the intake of the studied nutrients (ie, vegetables, bread, milk and milk products, meat, and potatoes) (35). Furthermore, folate intake correlated with the plasma folate concentration in the same magnitude (Spearman correlation: 0.27) as in other Dutch studies (36, 37). Plasma folate concentrations were somewhat lower than those measured in other Dutch populations (8, 36, 38, 39). This difference may be attributable to the different methods used to measure the folate concentration (40, 41); in addition, we excluded all B vitamin supplement users, and they had higher plasma folate concentrations (data not shown).

The majority of the blood samples obtained for the tHcy measurement were from nonfasting subjects. Consumption of a normal breakfast is associated with a lower tHcy concentration (42), and a protein-rich meal results in a higher tHcy concentration (43). Nevertheless, the type of foods eaten by our subjects before the sampling of blood is most likely to have been independent of the genotype. Furthermore, our data showed no significant difference between the tHcy concentration in subjects who had fasted before the blood collection and that in subjects who had not (24). In addition, whole blood was stored at room temperature for a maximum period of 1 h before centrifugation. Because of a continuous synthesis and transport of homocysteine in blood cells at room temperature (44, 45), the tHcy concentrations in our samples might on average have been 0.4 µmol/L higher, as was indicated by a stability study (24). The small artificial increase in tHcy did not affect the ranking of our subjects according to their tHcy concentration (24). We conclude that the validity of our tHcy data was not seriously affected by the above mentioned factors.

The product of MTHFR, 5-methyltetrahydrofolate, is the predominant circulating form of folate, whereas the enzyme substrate 5,10-methylenetetrahydrofolate is mainly found intracellularly (46,

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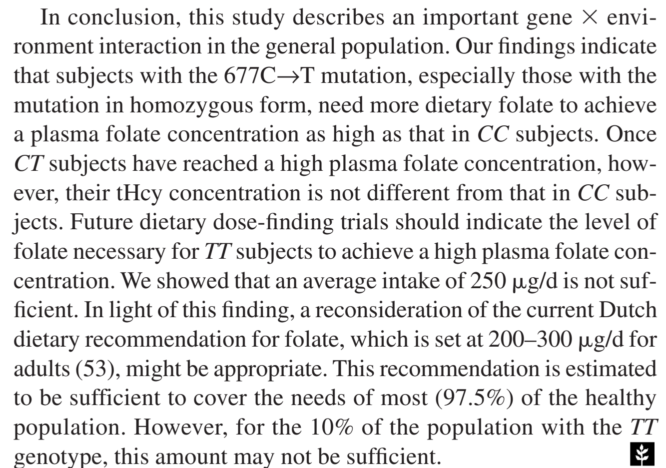
47). Because the 677C→T mutation results in a reduced specific enzymatic activity of MTHFR (≈34% residual activity in *TT* and ≈71% residual activity in *CT* relative to *CC*; 48), the result of the presence of a *T* allele may be a lower plasma folate concentration. Indeed, many investigators have reported a lower plasma folate concentration in *TT* subjects than in *CC* subjects (8–16). We were able to investigate whether differences in folate intake could (partly) explain this observation after adjustment for potential confounders. We found that this was not the case: for each folate intake tertile (where folate intake was equal for all genotypes), the adjusted plasma folate concentrations in *TT* subjects were always lower than those in *CC* or *CT* subjects (Figure 1). A comparable finding was briefly mentioned in another cross-sectional study, but the results were not shown (16). Very recently, the influence of the *MTHFR* 677C→T mutation on the response to dietary interventions was reported (23). The findings of those investigators, analogous to our results for low, medium, and high folate intakes (Figure 1), were that a gradient in plasma folate concentrations (*CC*→*CT*→*TT*) remained throughout the exclusion diet, the folate-rich diet, and the exclusion diet supplemented with 400 μg folic acid/d.

The observation that *CT* subjects have a lower plasma folate concentration than do CC subjects when folate intake is low has not been reported before. Yet, some researchers found that *CT* subjects in general had significantly lower mean plasma folate concentrations than did *CC* subjects (11, 13). In addition, in a finding analogous to the gene × nutrient interaction seen in our *TT* subjects, some researchers reported high tHcy concentrations in *CT* subjects with a low plasma or red blood cell folate concentration (5, 11). These findings imply that, to compensate for their *T* allele, *CT* subjects need more folate than do *CC* subjects. Once the folate intake of *CT* subjects is high, their plasma folate concentration is equal to that of *CC* subjects. This, however, is not the case in *TT* subjects.

Our findings confirm the suggestion that lower plasma folate concentrations in *TT* (or *CT*) subjects are not necessarily due to dietary insufficiencies but may reflect a direct effect of the *MTHFR* 677C→T polymorphism (11, 16, 49). Extra dietary folate will likely counteract the effect of the *TT* genotype (23), as was seen in *CT* subjects. The extra amount required could not be estimated from this cross-sectional study because of the limited range of folate intake in *TT* subjects; only dose-finding trials can answer this question.

Findings of Guenther et al (50) explain why extra folate may protect subjects with 1 or 2 *T* alleles from low plasma folate and high tHcy concentrations. In a system with bacteria, they showed that a mutation homologous to the human *MTHFR* 677C→T mutation was associated with an enhanced dissociation of flavin adenine dinucleotide (ie, the cofactor form of vitamin B-2). An optimal folate supply prevented the loss of flavin adenine dinucleotide and suppressed the inactivation of the enzyme (50).

The effect of the polymorphism on the relation between the plasma folate and tHcy concentration agrees with the findings in other studies (7, 11, 18, 51). So far, one other study has reported the modifying effect of the polymorphism on the relation between folate intake and the tHcy concentration (16). As in the present study, they found that, at a low folate intake, *TT* subjects had much higher tHcy concentrations than did *CT* and CC subjects. Alternatively, at high folate intakes, the tHcy concentration in *TT* subjects was similar to the concentrations in *CT* and *CC* subjects (16). These results agree with those in other trials, which show a steeper decrease in the tHcy concentration in *TT* subjects than in the concentrations in *CT* and *CC* subjects, in response to an increased folic acid intake (10, 23, 52).



In conclusion, this study describes an important gene × environment interaction in the general population. Our findings indicate that subjects with the 677C→T mutation, especially those with the mutation in homozygous form, need more dietary folate to achieve a plasma folate concentration as high as that in *CC* subjects. Once *CT* subjects have reached a high plasma folate concentration, however, their tHcy concentration is not different from that in *CC* subjects. Future dietary dose-finding trials should indicate the level of folate necessary for *TT* subjects to achieve a high plasma folate concentration. We showed that an average intake of 250 μg/d is not sufficient. In light of this finding, a reconsideration of the current Dutch dietary recommendation for folate, which is set at 200–300 μg/d for adults (53), might be appropriate. This recommendation is estimated to be sufficient to cover the needs of most (97.5%) of the healthy population. However, for the 10% of the population with the *TT* genotype, this amount may not be sufficient.

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REFERENCES

1. Finkelstein JD. Methionine metabolism in mammals. J Nutr Biochem 1990;1:228–37.
2. Shane B. Folate metabolism. In: Picciano MF, Stokstad ER, Gregory JF, eds. Folic acid metabolism in health and disease. New York: Wiley-Liss Inc, 1990:65–78.
3. Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 1995;10:111–3 (letter).
4. Kang SS, Zhou J, Wong PW, Kowalisyn J, Strokosch G. Intermediate homocysteinemia: a thermolabile variant of methylenetetrahydrofolate reductase. Am J Hum Genet 1988;43:414–21.
5. Brattstrom L, Wilcken DE, Ohrvik J, Brudin L. Common methylenetetrahydrofolate reductase gene mutation leads to hyperhomocysteinemia but not to vascular disease: the result of a meta-analysis. Circulation 1998;98:2520–6.
6. Kluijtmans LA, Kastelein JJ, Lindemans J, et al. Thermolabile methylenetetrahydrofolate reductase in coronary artery disease. Circulation 1997;96:2573–7.
7. Jacques PF, Bostom AG, Williams RR, et al. Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. Circulation 1996;93:7–9.
8. van der Put NM, Steegers-Theunissen RP, Frosst P, et al. Mutated methylenetetrahydrofolate reductase as a risk factor for spina bifida. Lancet 1995;346:1070–1.
9. Ma J, Stampfer MJ, Hennekens CH, et al. Methylenetetrahydrofolate reductase polymorphism, plasma folate, homocysteine, and risk of myocardial infarction in US physicians. Circulation 1996;94:2410–6.
10. Nelen WL, Blom HJ, Thomas CM, Steegers EA, Boers GH, Eskes TK. Methylenetetrahydrofolate reductase polymorphism affects the change in homocysteine and folate concentrations resulting from low dose folic acid supplementation in women with unexplained recurrent miscarriages. J Nutr 1998;128:1336–41.
11. Harmon DL, Woodside JV, Yarnell JW, et al. The common 'thermolabile' variant of methylene tetrahydrofolate reductase is a major determinant of mild hyperhomocysteinaemia. QJM 1996;89:571–7.
12. Zittoun J, Tonetti C, Bories D, Pignon JM, Tulliez M. Plasma homocysteine levels related to interactions between folate status and methylenetetrahydrofolate reductase: a study in 52 healthy subjects. Metabolism 1998;47:1413–8.
13. Hustad S, Ueland PM, Vollset SE, Zhang Y, Bjorke-Monsen AL, Schneede J. Riboflavin as a determinant of plasma total homocys-

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teine: effect modification by the methylenetetrahydrofolate reductase C677T polymorphism. Clin Chem 2000;46:1065–71.

14. DeLoughery TG, Evans A, Sadeghi A, et al. Common mutation in methylenetetrahydrofolate reductase. Correlation with homocysteine metabolism and late-onset vascular disease. Circulation 1996;94: 3074–8.
15. Schwartz SM, Siscovick DS, Malinow MR, et al. Myocardial infarction in young women in relation to plasma total homocysteine, folate, and a common variant in the methylenetetrahydrofolate reductase gene. Circulation 1997;96:412–7.
16. McQuillan BM, Beilby JP, Nidorf M, Thompson PL, Hung J. Hyperhomocysteinemia but not the C677T mutation of methylenetetrahydrofolate reductase is an independent risk determinant of carotid wall thickening—The Perth Carotid Ultrasound Disease Assessment Study (CUDAS). Circulation 1999;99:2383–8.
17. Brattstrom L, Wilcken DE. Homocysteine and cardiovascular disease: cause or effect? Am J Clin Nutr 2000;72:315–23.
18. Ueland PM, Refsum H, Beresford SA, Vollset SE. The controversy over homocysteine and cardiovascular risk. Am J Clin Nutr 2000;72:324–32.
19. Chen J, Giovannucci EL, Hunter DJ. MTHFR polymorphism, methyl-replete diets and the risk of colorectal carcinoma and adenoma among U.S. men and women: an example of gene- environment interactions in colorectal tumorigenesis. J Nutr 1999;129:560S-4S.
20. Yoo JH, Choi GD, Kang SS. Pathogenicity of thermolabile methylenetetrahydrofolate reductase for vascular dementia. Arterioscler Thromb Vasc Biol 2000;20:1921–5.
21. Nishiyama M, Kato Y, Hashimoto M, Yukawa S, Omori K. Apolipoprotein E, methylenetetrahydrofolate reductase (MTHFR) mutation and the risk of senile dementia—an epidemiological study using the polymerase chain reaction (PCR) method. J Epidemiol 2000;10:163–72.
22. van der Put NM, Eskes TK, Blom HJ. Is the common 677C→T mutation in the methylenetetrahydrofolate reductase gene a risk factor for neural tube defects? A meta-analysis. QJM 1997;90:111–5.
23. Ashfield-Watt PA, Pullin CH, Whiting JM, et al. Methylenetetrahydrofolate reductase 677C→T genotype modulates homocysteine responses to a folate-rich diet or a low-dose folic acid supplement: a randomized controlled trial. Am J Clin Nutr 2002;76:180–6.
24. de Bree A, Verschuren WMM, Blom HJ, De Graaf-Hess A, Trijbels FJM, Kromhout D. The homocysteine distribution: (mis)judging the burden. J Clin Epidemiol 2001;54:462–9.
25. Smit HA, Verschuren WMM, Bueno de Mesquita HB, Seidell JC. Monitoring van risicofactoren en gezondheid in Nederland (MORGEN-project): Doelstelling en werkwijze. [The monitoring project on risk factors for chronic diseases in the Netherlands (MORGEN-project): Aim and method.] Report number 263200001:1–93. Bilthoven: Rijksinstituut voor Volksgezondheid en Milieu, 1994 (in Dutch).
26. Ocke MC, Bueno de Mesquita HB, Pols MA, Smit HA, van Staveren WA, Kromhout D. The Dutch EPIC food frequency questionnaire. II. Relative validity and reproducibility for nutrients. Int J Epidemiol 1997;26:S49–58.
27. Sauberlich HE. Interactions of thiamin, riboflavin, and other B-vitamins. Ann N Y Acad Sci 1980;355:80–97.
28. Fiskerstrand T, Refsum H, Kvalheim G, Ueland PM. Homocysteine and other thiols in plasma and urine: automated determination and sample stability. Clin Chem 1993;39:263–71.
29. te Poele Pothoff MT, van den Berg M, Franken DG, et al. Three different methods for the determination of total homocysteine in plasma. Ann Clin Biochem 1995;32:218–20.
30. Molloy AM, Scott JM. Microbiological assay for serum, plasma, and red cell folate using cryopreserved, microtiter plate method. Methods Enzymol 1997;281:43–53.
31. Kelleher BP, Broin SD. Microbiological assay for vitamin B12 performed in 96-well microtitre plates. J Clin Pathol 1991;44:592–5.
32. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. Am J Clin Nutr 1997;65(suppl):S1220-8.
33. de Bree A, Verschuren WM, Blom HJ, Kromhout D. Association between B vitamin intake and plasma homocysteine concentration in the general Dutch population aged 20–65 y. Am J Clin Nutr 2001;73:1027–33.
34. Kleinbaum DG, Kupper LL, Muller F, Nizam A. Dummy variables in regression. Applied regression analysis and multivariate methods. Pacific Grove: Duxbury Press, 1998:317–60.
35. Ocke MC, Bueno de Mesquita HB, Goddijn HE, et al. The Dutch EPIC food frequency questionnaire. I. Description of the questionnaire, and relative validity and reproducibility for food groups. Int J Epidemiol 1997;26:S37–48.
36. Brussaard JH, Lowik MRH, vandenBerg H, Brants HAM, Goldbohm RA. Folate intake and status among adults in the Netherlands. Eur J Clin Nutr 1997;51:S46-50.
37. vanAsselt DZB, deGroot LCPGM, vanStaveren WA, et al. Role of cobalamin intake and atrophic gastritis in mild cobalamin deficiency in older Dutch subjects. Am J Clin Nutr 1998;68:328–34.
38. Nelen WL, Blom HJ, Steegers EA, den Heijer M, Thomas CM, Eskes TK. Homocysteine and folate levels as risk factors for recurrent early pregnancy loss. Obstet Gynecol 2000;95:519–24.
39. van den Berg M, de Jong SC, Deville W, et al. Variability of fasting and post-methionine plasma homocysteine levels in normo- and hyperhomocysteinaemic individuals. Neth J Med 1999;55:29–38.
40. Gunter EW, Bowman BA, Caudill SP, Twite DB, Adams MJ, Sampson EJ. Results of an international round robin for serum and whole-blood folate. Clin Chem 1996;42:1689–94.
41. Molloy AM, Mills JL, Kirke PN, Whitehead AS, Weir DG, Scott JM. Whole-blood folate values in subjects with different methylenetetrahydrofolate reductase genotypes: differences between the radioassay and microbiological assays. Clin Chem 1998;44:186–8.
42. Ubbink JB, Vermaak WJ, van der Merwe A, Becker PJ. The effect of blood sample aging and food consumption on plasma total homocysteine levels. Clin Chim Acta 1992;207:119–28.
43. Guttormsen AB, Schneede J, Fiskerstrand T, Ueland PM, Refsum HM. Plasma concentrations of homocysteine and other aminothiol compounds are related to food intake in healthy human subjects. J Nutr 1994;124:1934–41.
44. Andersson A, Isaksson A, Hultberg B. Homocysteine export from erythrocytes and its implication for plasma sampling. Clin Chem 1992;38:1311–5.
45. Malinow MR, Axthelm MK, Meredith MJ, MacDonald NA, Upson BM. Synthesis and transsulfuration of homocysteine in blood. J Lab Clin Med 1994;123:421–9.
46. Selhub J, Miller JW. The pathogenesis of homocysteinemia: interruption of the coordinate regulation by S-adenosylmethionine of the remethylation and transsulfuration of homocysteine. Am J Clin Nutr 1992;55:131–8.
47. Green JM, Ballou DP, Matthews RG. Examination of the role of methylenetetrahydrofolate reductase in incorporation of methyltetrahydrofolate into cellular metabolism. FASEB J 1988;2:42–7.
48. van der Put NM, van den Heuvel LP, Steegers-Theunissen RP, et al. Decreased methylene tetrahydrofolate reductase activity due to the 677C→T mutation in families with spina bifida offspring. J Mol Med 1996;74:691–4.
49. Lucock MD, Wild J, Schorah CJ, Levene MI, Hartley R. The methylfolate axis in neural tube defects: in vitro characterisation and clinical investigation. Biochem Med Metab Biol 1994;52:101–14.
50. Guenther BD, Sheppard CA, Tran P, Rozen R, Matthews RG, Ludwig ML. The structure and properties of methylenetetrahydrofolate reductase from *Escherichia coli* suggest how folate ameliorates human hyperhomocysteinemia. Nat Struct Biol 1999;6:359–65.
51. Bailey LB, Gregory JF III. Polymorphisms of methylenetetrahydrofolate reductase and other enzymes: metabolic significance, risks and impact on folate requirement. J Nutr 1999;129:919–22.
52. Malinow MR, Nieto FJ, Kruger WD, et al. The effects of folic acid supplementation on plasma total homocysteine are modulated by multivitamin use and methylenetetrahydrofolate reductase genotypes. Arteriol Thromb Vasc Biol 1997;17:1157–62.
53. Nederlandse Voedingsraad. Nederlandse voedingsnormen 1989 (Dutch Dietary Recommendations). Den Haag: Voorlichtingsbureau voor de voiding, 1992 (in Dutch).

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