Genetic, dietary, and other lifestyle determinants of plasma homocysteine concentrations in middle-aged and older Chinese men and women in Singapore[1–3]

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ABSTRACT

Background: Epidemiologic studies have identified the plasma homocysteine concentration as a risk factor for atherothrombotic vascular disease. There is little information on the distributions and determinants of homocysteine concentrations in Asian populations.

Objective: The present study was designed to examine the relations between genetic and lifestyle factors and plasma homocysteine concentrations among Chinese in Singapore.

Design: Plasma total homocysteine, folate, vitamin B-12, and vitamin B-6 concentrations and genetic variation at the methylenetetrahydrofolate reductase (*MTHFR*) locus were measured in 486 Chinese men and women aged 45–74 y in Singapore. Data on dietary and other lifestyle factors were collected in face-to-face interviews.

Results: Men had higher plasma concentrations of total homocysteine than women ($P = 0.0001$). Age was positively associated with plasma homocysteine in both sexes (P for trend = 0.0001). Plasma concentrations of folate, vitamin B-12, and vitamin B-6 were inversely associated with homocysteine concentrations. Among individuals with low plasma folate, those possessing 2 copies of *MTHFR* mutant alleles had significantly higher homocysteine concentrations than did those with ≥1 copy of the wild-type allele. Cigarette smoking, daily coffee consumption, and physical inactivity were positively related to plasma homocysteine concentrations in both sexes ($P < 0.05$). However, these associations disappeared after adjustment for plasma folate concentrations.

Conclusions: Age, sex, plasma folate, vitamin B-12 and B-6 concentrations, and *MTHFR* genotype are independent determinants of plasma homocysteine in middle-aged and older Chinese in Singapore. These factors combined could account for up to 40% of the total variation in homocysteine concentrations in this Asian population. *Am J Clin Nutr* 2001;73:232–9.

KEY WORDS Homocysteine, folate, vitamin B-12, vitamin B-6, *MTHFR* genotype, methylenetetrahydrofolate reductase, physical activity, coffee, tea, alcohol, cigarette smoking, cholesterol, triacylglycerol, Chinese, vascular disease, cardiovascular disease, coronary artery disease, hyperhomocysteinemia

INTRODUCTION

Numerous case-control and prospective studies have shown a relation between moderate hyperhomocysteinemia and atherothrombotic vascular disease in the coronary (1–5), cerebral (6–8), and peripheral arteries (9–12). Homocysteine is a sulfydryl-containing amino acid derived from the metabolic demethylation of dietary methionine. There are 2 pathways in homocysteine metabolism, the remethylation and transulfuration pathways. The remethylation pathway is catalyzed by *5*-methyltetrahydrofolate-homocysteine *S*-methyltransferase (methionine synthase) with N^5-methyltetrahydrofolate as the methyl donor. The formation of this methyl donor depends on the presence of N^5,N^{10}-methylenetetrahydrofolate (derived from dietary folate) and the enzyme N^5,N^{10}-methylenetetrahydrofolate reductase (MTHFR). Vitamin B-12 (cobalamin) is an essential cofactor for methionine synthase. In the transulfuration pathway, homocysteine is first converted to cystathionine and then to cysteine by cystathionine β-synthase. Vitamin B-6 (pyridoxine) is an essential cofactor in the transulfuration pathway (13).

Individuals with a nutritional deficiency that leads to low blood concentrations of folate, vitamin B-12, or vitamin B-6 are at risk of hyperhomocysteinemia. It was suggested that about two-thirds of hyperhomocysteinemia in an elderly population in the United States resulted from inadequate blood concentrations of one or more of these B vitamins (folate, vitamin B-12, and vitamin B-6) (14, 15). In addition, genetic defects were associated with moderate to severe hyperhomocysteinemia or homocystinuria (16). A common point mutation (C-to-T substitution at nucleotide 677) in the coding region of the gene for MTHFR results in conversion of an alanine to a valine residue, which is associated with a thermolabile MTHFR variant that has ≈50% of normal enzyme activity (17). Homozygotes for the valine variant of *MTHFR* have an ≈50% increase in circulating homocysteine concentrations when folate intake is suboptimal (18–20).

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Data on the determinants of homocysteine concentrations in Asian populations are scarce. The diets and other lifestyle characteristics and the genetic backgrounds of Asian populations are distinct from those of Occidental populations. In this report, we describe the distribution of plasma total homocysteine concentrations by age and sex among middle-aged and older Chinese men and women in Singapore. Furthermore, we examine the associations of homocysteine concentrations with *1*) plasma concentrations and dietary intakes of folate, vitamin B-12, and vitamin B-6; *2*) lifestyle factors including cigarette smoking, physical activity, and consumption of alcohol, coffee, and tea; *3*) plasma concentrations of triacylglycerol and total, HDL, and LDL cholesterol; and *4*) polymorphism of the *MTHFR* gene.

SUBJECTS AND METHODS

Study population

The subjects were participants in the Singapore Chinese Health Study, a population-based, prospective investigation of diet and cancer risk. From April 1993 through December 1998, 63 257 Chinese men and women aged 45–74 y enrolled in the study. The subjects were residents of government housing estates; 86% of the Singapore population lives in these facilities. At recruitment, a face-to-face interview was conducted in the subject's home by a trained interviewer who used a structured questionnaire. The questionnaire included a validated dietary component (including questions about coffee, tea, and alcoholic beverages) that assessed current intake patterns (21). Each subject was asked to estimate his or her usual intake frequencies and portion sizes for 165 food and beverage items during the past 12 mo. The questionnaire also requested information on demographics, lifetime use of tobacco (cigarettes, water pipe), current physical activity, reproductive history (women only), occupational exposures, medical history, and family history of cancer.

In April 1994, 1 y after the cohort study began, we started collecting blood and single-void urine specimens from a random 3% sample of study enrollees. A 20-mL blood sample was collected from each of these 486 subjects (216 men and 270 women). One 10-mL plain tube was used for the preparation of serum and one 10-mL heparin-containing tube was used for plasma. Immediately after blood collection, the tubes were stored on ice until further processing. All specimens were then kept at room temperature for 2 h before separation into their various components (plasma, serum, red blood cells, and buffy coat). All specimens were subsequently stored in a liquid nitrogen tank at −180°C until analyzed. Most blood samples were collected in the morning with no requirement that the subjects fast. However, we asked the subjects when they had eaten their last meal. The study was approved by the Institutional Review Boards of the University of Southern California and the National University of Singapore.

Laboratory measurements

Plasma total homocysteine concentrations were measured by using HPLC with electrochemical detection in the laboratory of the Department of Community, Occupational, and Family Medicine, National University of Singapore. The sample preparation procedure was as reported by Hughes and Ong (22).

Plasma folate and vitamin B-12 concentrations were analyzed with the Quantaphase II B-12/folate radioimmunoassay kit (Bio-Rad Laboratories Inc, Hercules, CA) (23). Plasma vitamin B-6 concentrations were determined with a radioenzymatic assay (Buhlmann Laboratories AG, Allschwil, Switzerland) on the basis of a method originally described by Shin et al (24).

Total and HDL-cholesterol concentrations were measured with the enzymatic, colorimetric method with sterol esterase, cholesterol oxidase, and 4-aminoantipyrine (25–27). HDL-cholesterol concentrations were measured in the supernate after centrifugation at $1500 \times g$ for 10 min at room temperature to precipitate the chylomicrons and LDL and VLDL cholesterol by using phosphotungstic acid and magnesium ions. Triacylglycerol concentrations were measured by using the enzymatic, colorimetric method with glycerol-3-phosphate oxidase and 4-aminoantipyrine (28). LDL-cholesterol concentrations were calculated with the Friedewald formula (29):

$$\text{LDL cholesterol} = \text{total cholesterol} - \text{HDL cholesterol} - \text{triacylglycerol}/5 \quad (1)$$

where all the variables are expressed in mg/dL. (To convert to mmol/L, multiply the product by 0.02586.)

Genomic DNA was isolated from blood leukocytes. The presence of a C-to-T substitution at nucleotide 677 of the *MTHFR* gene was assayed by using the methods of Frosst et al (17). The sense and antisense primers for the polymerase chain reaction are 5′-TGAAGGAGAAGGTGTCTGCGGGA-3′ and 5′-AGGACGGTGCGGTGAGAGTG-3′, respectively. Thirty-five cycles (95°C for 60 s, 62°C for 90 s, 72°C for 60 s) were used to amplify 198-base pair (bp) products. *Hinf*I (the restriction enzyme) recognition sequence digested the 198-bp fragment into 175-bp and 23-bp fragments and the analysis was conducted with polyacrylamide gel electrophoresis.

Data analysis

For each subject, we computed the usual daily intake of various nutrients by combining information obtained from the interview with nutrient values from the Singapore Food Composition Tables (21). Nutrient density expressed as weight per 4200 kJ (1000 kcal) was used in all data analyses to adjust for energy intake. The distributions of plasma homocysteine, folate, vitamin B-12, and vitamin B-6 concentrations and dietary intakes of various nutrients in the study population were markedly skewed toward high values, which were corrected to a large extent by transformation to logarithmic values. Therefore, the statistical analyses were performed on logarithmically transformed values. We present the geometric means and their 95% CIs, which were obtained by taking the antilog of the 95% CIs of logarithmically transformed values.

Linear regression models were used to examine the relations between plasma homocysteine concentrations and dietary and other lifestyle variables. To assess the simultaneous effects of various predictors of plasma homocysteine and to provide effect estimates after adjustment for other factors, multiple linear regression models were used (30). We used analysis of covariance methods with age as a covariate to compare mean homocysteine concentrations for the different categories of predictors of homocysteine concentration. We used linear contrasts to test for trends across groups with ≥3 levels (30). A gene-folate product term was included in multiple linear regression models to assess the effect of the interaction between plasma folate and *MTHFR* genotype on plasma homocysteine concentration. Nine subjects were noninformative on *MTHFR* genotype; these individuals were excluded from all analyses involving the *MTHFR* variable.

TABLE 1
Distributions of selected subject characteristics in the Singapore Chinese Health Study

	Men (n = 216)	Women (n = 270)
Age (y)	58.2 ± 7.7[1]	57.1 ± 8.0
Weight (kg)[2]	62.5 ± 9.4	55.5 ± 8.8
Height (m)[2]	1.65 ± 0.06	1.56 ± 0.06
Body mass index (kg/m^2)	22.8 ± 3.2	22.8 ± 3.4
Level of education (%)[3]		
No formal education	12	38
Primary school	47	37
Secondary school	30	21
College or above	11	4
Cigarette smoking (%)[3]		
Never smoked	44	95
Former smoker	25	1
Current smoker	31	4
Alcohol consumption (%)[3]		
Nondrinker[4]	69	93
<1 drink/d	25	6
≥1 drink/d	6	1
Coffee consumption (%)		
None or occasional	32	30
1–3 cups/d	64	68
≥4 cups/d	4	2

[1] $\bar{x}$ ± SD.
[2,3] Significant sex difference: [2] $P < 0.001$ (two-tailed t test), [3] $P < 0.001$ (chi-square).
[4] Defined as <1 drink/mo.

Of the 486 subjects, 9% (n = 44) provided fasting blood samples (≥12 h postprandial) whereas 37% (n = 182) had eaten within the past 2 h. We examined plasma concentrations of homocysteine, vitamin B-12, vitamin B-6, and LDL, HDL, and total cholesterol according to the time elapsed since the last meal to determine whether higher concentrations occurred in subjects who were not fasting. No such increases were observed. This finding was not surprising because most of the meals eaten prior to blood sample collection were breakfasts (including foods such as bread, noodles, buns, and porridge). Therefore, all the subjects were included in these analyses.

The analyses were performed with the statistical program SAS, version 6.12 (SAS Institute Inc, Cary, NC). All the P values reported are two-tailed and significance was defined as $P < 0.05$.

RESULTS

The distributions of selected subject characteristics are shown in **Table 1**. The average body mass index (weight in kg divided by height in m^2) was 22.8 for both men and women. About two-thirds of the subjects had either no formal education or only a primary school education and women were less educated than men. Thirty-one percent of men and 4% of women were current cigarette smokers; 6% of men and 1% of women reported daily consumption of alcoholic beverages. About 70% of men and women drank coffee every day.

The distribution of plasma total homocysteine concentrations is shown for all subjects by sex and age in **Table 2**. Total homocysteine concentrations for all subjects ranged from 3.6 to 80.7 μmol/L. In both men and women, age was positively associated with homocysteine concentration (P for trend = 0.0001). There was an ≈1-μmol/L increase, on average, in homocysteine concentration per decade increase in age between 45 and 74 y. For any given age, the homocysteine concentration was ≈2 μmol/L higher in men than in women. This sex effect was highly significant (Table 2).

In Table 2 we also show the geometric mean plasma folate, vitamin B-12, and vitamin B-6 concentrations by age and sex. No age differences were found for folate, vitamin B-12, or vitamin B-6 in men or women. However, consistently higher concentrations of all 3 B vitamins were found in women than in men ($P < 0.001$).

The lower concentrations of folate and vitamins B-12 and B-6 in men than in women did not completely account for the higher homocysteine concentrations in men. After adjustment for folate and vitamins B-12 and B-6, the residual difference in plasma homocysteine concentrations between men and women remained significant ($P = 0.0001$).

We examined intercorrelations among plasma concentrations and dietary intakes of folate, vitamin B-12, and vitamin B-6. For plasma concentrations, the Spearman coefficients were 0.24 ($P < 0.001$) between folate and vitamin B-12, 0.31 ($P < 0.001$) between folate and vitamin B-6, and 0.24 ($P < 0.001$) between vitamins B-12 and B-6; the corresponding coefficients for dietary intakes of the vitamins were 0.12 ($P = 0.006$), 0.61 ($P < 0.001$), and 0.33 ($P < 0.001$), respectively. For correlations between plasma concentrations and dietary intakes of each vitamin, the correlation coefficients were 0.16 ($P < 0.001$) between plasma and dietary folate, 0.17 ($P < 0.001$) between plasma and dietary vitamin B-12, and 0.14 ($P = 0.002$) between plasma and dietary vitamin B-6.

TABLE 2
Plasma concentrations of homocysteine and B vitamins by sex and age in the Singapore Chinese Health Study[1]

	Homocysteine[2]	Folate[2]	Vitamin B-12[3]	Vitamin B-6[4]
	μmol/L	*nmol/L*	*pmol/L*	*nmol/L*
Men[5]				
All (n = 216)	11.2 (10.8, 11.6)	12.2 (11.5, 12.9)	308 (292, 324)	29.4 (26.9, 32.0)
45–54 y (n = 77)	10.3 (9.7, 11.1)	12.5 (11.3, 13.9)	315 (292, 339)	32.1 (27.7, 37.1)
55–64 y (n = 86)	11.3 (10.6, 12.0)	12.4 (11.3, 13.7)	310 (289, 334)	28.0 (24.4, 32.1)
65–74 y (n = 53)	12.8 (11.8, 13.9)	11.3 (10.0, 12.8)	292 (266, 320)	27.8 (23.3, 33.1)
Women[5]				
All (n = 270)	9.1 (8.8, 9.4)	15.4 (14.6, 16.3)	346 (331, 363)	36.0 (33.3, 38.9)
45–54 y (n = 116)	8.2 (7.8, 8.6)	15.2 (14.0, 16.5)	365 (338, 393)	35.6 (31.6, 40.0)
55–64 y (n = 98)	9.4 (8.9, 9.9)	15.1 (13.8, 16.5)	326 (326, 300)	36.2 (31.9, 41.1)
65–74 y (n = 56)	10.3 (9.5, 11.0)	16.7 (14.8,18.7)	349 (313, 389)	36.8 (31.1, 43.6)

[1] Geometric mean; 95% CI in parentheses, adjusted for age. One-way ANOVA was used with log-transformed homocysteine values as the dependent variable and the age and sex grouping as the independent variable.
[2–4] Significant sex difference (two-tailed): [2] $P = 0.0001$, [3] $P = 0.0009$, [4] $P = 0.0006$.
[5] P for trend by age for homocysteine, $P = 0.0001$.

TABLE 3
Plasma homocysteine concentration by quartile of plasma B vitamins in the Singapore Chinese Health Study[1]

	Plasma homocysteine	
	Adjusted for age and sex[2]	Multivariate adjusted[3]
	μmol/L	
Folate (nmol/L)		
<10.4 (*n* = 122)	11.8 (11.3, 12.4)	11.4 (10.9, 12.0)
10.4–13.6 (*n* = 121)	10.3 (9.8, 10.8)	10.3 (9.8, 10.7)
13.7–18.5 (*n* = 122)	9.5 (9.1, 10.0)	9.6 (9.2, 10.1)
≥18.6 (*n* = 121)	8.8 (8.4, 9.2)	9.0 (8.6, 9.4)
β Coefficient[4]	−0.262[5]	−0.219[5]
Vitamin B-12 (pmol/L)		
<262 (*n* = 122)	11.4 (10.8, 12.0)	11.0 (10.5, 11.5)
262–328 (*n* = 121)	10.0 (9.6, 10.5)	9.9 (9.5, 10.4)
329–412 (*n* = 122)	9.5 (9.1, 10.0)	9.6 (9.2, 10.0)
≥413 (*n* = 121)	9.4 (9.0, 9.9)	9.7 (9.3, 10.2)
β Coefficient[4]	−0.194[5]	−0.120[5]
Vitamin B-6 (nmol/L)		
<21.9 (*n* = 121)	11.5 (10.9, 12.1)	10.8 (10.3, 11.4)
21.9–33.3 (*n* = 122)	9.8 (9.3, 10.3)	9.8 (9.4, 10.3)
33.4–50.4 (*n* = 122)	9.7 (9.3, 10.2)	9.9 (9.4, 10.3)
≥50.5 (*n* = 121)	9.4 (8.9, 9.9)	9.7 (9.2, 10.1)
β Coefficient[4]	−0.113[5]	−0.054[5]

[1] Geometric mean; 95% CI in parentheses. Data shown are for both sexes combined.
[2] One-way analysis of covariance with age and sex as the covariates and $\log_e$ concentration of homocysteine as the dependent variable.
[3] In addition to age and sex, the other 2 vitamins listed in the table were included in the multivariate regression models as covariates.
[4] Regression coefficients were based on $\log_e$ concentrations of homocysteine as the dependent variable and $\log_e$ concentrations of folate, vitamin B-12, and vitamin B-6 as the independent variables.
[5] $P < 0.01$ (two-tailed test).

Plasma homocysteine concentrations were inversely associated with plasma concentrations of folate and vitamins B-12 and B-6 (**Table 3**). The results were similar for men and women; therefore, the data shown are for both sexes combined. After adjustment for age and sex, the geometric mean homocysteine concentration in subjects in the lowest quartile of plasma folate concentration was 34% higher than that of subjects in the highest quartile. Homocysteine concentrations decreased monotonically with increasing folate concentration (P for trend < 0.001). Similarly, inverse associations between plasma homocysteine and plasma vitamins B-12 and B-6 were seen in the study population. Compared with subjects in the highest quartile of vitamin B-12 or B-6, individuals in the lowest quartile had ≈20% higher homocysteine concentrations.

Folate, vitamin B-12, and vitamin B-6 exerted independent effects on homocysteine concentrations. Folate showed the strongest effect on homocysteine, followed by vitamin B-12; vitamin B-6 had the weakest effect (*see* regression coefficients in the multivariate adjusted column, Table 3). After adjustment for age, sex, and the other 2 vitamins, the geometric mean homocysteine concentration of subjects in the highest quartile of folate concentrations was 27% lower than that of subjects in the lowest quartile of folate concentrations. Similarly, the multivariate adjusted geometric means of plasma homocysteine for the highest and lowest quartiles of vitamin B-12 were 9.7 and 11.0 μmol/L, respectively (13% difference) and the corresponding means of homocysteine for the highest and lowest quartiles of vitamin B-6 were 9.7 and 10.8 μmol/L, respectively (11% difference).

We also examined the associations between plasma homocysteine concentrations and dietary intakes of folate, vitamin B-12, and vitamin B-6; these vitamin intakes were adjusted for total energy intake (**Table 4**). Again, the results were similar for men and women; therefore, the data shown are for both sexes combined. After adjustment for age and sex, the homocysteine concentration was inversely associated with each of the 3 B vitamins investigated. After further adjustment for the other 2 vitamins, dietary folate remained a significant predictor of plasma homocysteine concentration. However, dietary intakes of vitamins B-12 and B-6 were no longer associated with plasma homocysteine after adjustment for dietary folate (*see* regression coefficients in multivariate adjusted column).

The relations between plasma homocysteine and cigarette smoking, coffee consumption, and physical activity are shown in **Table 5**. Cigarette smokers had significantly higher homocysteine concentrations than nonsmokers, but among smokers there was no association between homocysteine concentration and number of cigarettes smoked per day. Subjects who drank coffee daily had higher homocysteine concentrations than those who drank coffee occasionally or not at all. Again, there was no

TABLE 4
Plasma homocysteine concentration by quartile of dietary intake of B vitamins in the Singapore Chinese Health Study[1]

	Plasma homocysteine	
	Adjusted for age and sex[2]	Multivariate adjusted[3]
	μmol/L	
Folate (μg)[4]		
<78.1 (*n* = 122)	10.7 (10.2, 11.3)	10.9 (10.3, 11.5)
78.1–96.1 (*n* = 121)	10.2 (9.7, 10.7)	10.2 (9.7, 10.7)
96.2–118.3 (*n* = 121)	9.7 (9.2, 10.2)	9.7 (9.2, 10.2)
≥118.4 (*n* = 122)	9.7 (9.3, 10.2)	9.6 (9.1, 10.2)
β Coefficient[5]	−0.15[6]	−0.14[6]
Vitamin B-12 (μg)[4]		
<1.18 (*n* = 121)	10.4 (9.9, 10.9)	10.4 (9.9, 10.9)
1.18–1.50 (*n* = 122)	10.5 (10.0, 11.0)	10.5 (10.0, 11.1)
1.51–1.82 (*n* = 121)	9.5 (9.1, 10.0)	9.5 (9.1, 10.0)
≥1.83 (*n* = 122)	9.9 (9.5, 10.5)	9.9 (9.4, 10.4)
β Coefficient[5]	−0.07[7]	−0.06
Vitamin B-6 (mg)[4]		
<0.63 (*n* = 122)	10.1 (9.6, 10.6)	9.6 (9.1, 10.2)
0.63–0.70 (*n* = 121)	10.4 (9.8, 10.9)	10.2 (9.7, 10.8)
0.71–0.80 (*n* = 122)	10.1 (9.6, 10.6)	10.3 (9.8, 10.9)
≥0.81 (*n* = 121)	9.8 (9.3, 10.3)	10.1 (9.6, 10.7)
β Coefficient[5]	−0.16[6]	0.01

[1] Geometric mean; 95% CI in parentheses. Data shown are for both sexes combined.
[2] One-way analysis of covariance with age and sex as the covariates and $\log_e$ concentration of homocysteine as the dependent variable.
[3] In addition to age and sex, the other 2 vitamins listed in the table were included in the multivariate regression models as covariates.
[4] Per 4200 kJ energy intake.
[5] Regression coefficients were based on $\log_e$ concentration of homocysteine as the dependent variable and $\log_e$ dietary intakes of folate, vitamin B-12, and vitamin B-6 as independent variables.
[6] $P < 0.01$ (two-tailed test).
[7] $0.01 < P < 0.05$ (two-tailed test).

TABLE 5
Plasma homocysteine concentration by selected lifestyle variables in the Singapore Chinese Health Study[1]

	Plasma homocysteine	
	Adjusted for age and sex[2]	Multivariate adjusted[3]
	μmol/L	
Currently smokes cigarettes		
No (n = 408)	9.9 (9.6, 10.2)	9.9 (9.6, 10.1)
Yes (n = 78)	11.0 (10.3, 11.7)[4]	10.3 (9.7, 10.9)
Drinks coffee daily		
No (n = 149)	9.5 (9.1, 10.0)	9.7 (9.3, 10.1)
Yes (n = 337)	10.3 (10.0, 10.6)[5]	10.1 (9.8, 10.4)
Exercises weekly		
No (n = 313)	10.3 (10.0, 10.7)	10.1 (9.8, 10.4)
Yes (n = 173)[6]	9.7 (9.3, 10.1)[7]	9.8 (9.4, 11.1)
Time spent watching television (h/d)[8]		
<1 (n = 83)	9.6 (9.0, 10.2)	9.6 (9.1, 10.2)
1–4 (n = 370)	10.1 (9.8, 10.4)	10.0 (9.8, 10.3)
≥5 (n = 33)	10.8 (9.8, 11.9)	10.2 (9.3, 11.1)

[1] Geometric mean; 95% CI in parentheses.
[2] One-way analysis of covariance with age and sex as covariates.
[3] In addition to age and sex, plasma folate concentration was included in the multivariate regression models as an independent variable.
[4,5,7] Significantly different from "No" group (two-tailed test): [4] $P = 0.005$, [5] $P = 0.004$, [7] $P = 0.01$.
[6] Spend ≥0.5 h/wk in activities such as jogging, tennis, swimming laps, loading or unloading trucks, brisk walking, bowling, bicycling, tai chi, or chi kung.
[8] Trend was significant for unadjusted mean, $P = 0.04$ (two-tailed test).

dose-response relation between the number of cups of coffee consumed per day and homocysteine concentration. Two markers of physical activity (exercises weekly and time spent watching television) showed that physical inactivity was related to elevated homocysteine concentration. All 3 lifestyle factors (cigarette smoking, coffee consumption, and physical inactivity) were no longer associated with plasma homocysteine after adjustment for plasma folate concentration. Plasma homocysteine concentrations were not significantly associated with consumption of tea or alcoholic beverages after adjustment for age and sex.

The geometric mean concentrations of triacylglycerol and total, HDL, and LDL cholesterol in men were 1.90, 5.45, 1.24, and 3.28 mmol/L, respectively. The corresponding values in women were 1.63, 5.75, 1.43, and 3.47 mmol/L, respectively. Plasma homocysteine concentrations were not associated with plasma concentrations of triacylglycerol or total, HDL, or LDL cholesterol after adjustment for age and sex (data not shown).

Of the 477 subjects with informative *MTHFR* genotype, 57% (126 men and 147 women) were homozygous for the wild-type, or alanine, allele (*AA*), 35% (70 men and 95 women) were heterozygous (*AV*), and 8% (17 men and 22 women) were homozygous for the variant, or valine, allele (*VV*). Individuals with the *MTHFR-VV* genotype had the highest concentrations of homocysteine on average (geometric mean, 12.3 μmol/L), followed by persons with the *AV* genotype (10.0 μmol/L) and those with the *AA* genotype (9.7 μmol/L) after adjustment for age and sex. The difference in homocysteine concentrations between the *AV* and *AA* genotypes was not significant. Individuals with the *VV* genotype had significantly higher (by 25%) mean homocysteine concentrations than subjects with the *AA* and *AV* genotypes combined ($P < 0.0001$; **Table 6**). There was a significant interaction effect between *MTHFR* genotype and plasma folate concentration on homocysteine concentration in the study population ($P = 0.02$). When there was sufficient folate intake (plasma folate was above the median), *MTHFR* genotype had no effect on plasma homocysteine concentration. In contrast, when the folate intake was insufficient (plasma folate was below the median), the *VV* genotype was associated with an ≈30% increase in plasma homocysteine concentration relative to the *AA* and *AV* genotypes ($P < 0.0001$).

DISCUSSION

To our knowledge, this is the first population-based study that describes the distribution of plasma total homocysteine in an Asian population. Furthermore, the present study identified age, sex, plasma folate and vitamins B-12 and B-6, and the *MTHFR* genotype as important predictors of homocysteine concentrations in middle-aged and older Singapore Chinese.

The mean homocysteine concentrations of Singapore Chinese are similar to those in populations in the United States (14, 31) and Norway (32). On the basis of a representative sample of the US population, Jacques et al (31) reported that the geometric mean serum total homocysteine concentrations in non-Hispanic white men aged 50–59 y, 60–69 y, and 70–79 y were 10.3, 10.5, and 11.7 μmol/L, respectively. The corresponding concentrations in non-Hispanic white women were 8.4, 9.6, and 10.6 μmol/L, respectively. There is no standard definition for hyperhomocysteinemia, but many previous studies have used the 95th percentile values in control samples as the cutoff point. Stampfer et al (1) reported a 95th percentile value of 15.8 μmol/L in US white men. In Norway, Nygard et al (33) reported 95th percentile values of 17.0 and 14.6 μmol/L in 40–42-y-old men and women, respectively. In the present study of Singapore Chinese aged 45–74 y, the 95th percentile values were 17.4 and 16.5 μmol/L in men and women, respectively.

Our study shows that the age- and sex-specific mean concentrations of plasma folate and vitamin B-12 in middle-aged and older Singapore Chinese are comparable with those in US whites (14, 15). However, the mean plasma vitamin B-6 concentration in Chinese is about one-half that of US whites (14). This could be a result of the lower intakes of vitamin B-6 by Chinese than

TABLE 6
Plasma homocysteine concentration by *MTHFR* genotype and plasma folate concentration in the Singapore Chinese Health Study[1]

	Plasma homocysteine	
	AA or *AV* genotype (n = 438)	*VV* genotype (n = 39)
	μmol/L	
All subjects	9.8 (9.6, 10.1)	12.3 (11.2, 13.4)[2]
By median folate concentration (nmol/L)		
<13.7	10.7 (10.3, 11.1)	13.8 (12.5, 15.3)[2]
≥13.7	9.1 (8.8, 9.4)	9.4 (8.1, 10.9)

[1] Geometric mean; 95% CI in parentheses, adjusted for age and sex. *AA*, homozygous for the wild-type (alanine) allele; *VV*, homozygous for the variant (valine) allele; *AV*, heterozygous.
[2] Significantly different from *AA* or *AV* genotype, $P < 0.0001$ (two-tailed test).

by US whites (14). In the present study of Singapore Chinese, significantly lower plasma concentrations of folate and vitamins B-12 and B-6 were found in men than in women. In an elderly population in the United States, a similar sex difference was observed for vitamin B-12, but folate and vitamin B-6 concentrations were comparable for men and women (14).

The present study shows an association between age and plasma total homocysteine that is similar to the associations found in previous studies conducted in whites. In general, there is a 1-μmol/L increase in plasma total homocysteine for each decade between 40 and 70 y of age (14, 15, 32, 34). High homocysteine concentrations in the elderly may be related to declining activity of cystathione β-synthase and possibly other enzymes involved in homocysteine metabolism (35). In addition, the age-related decline in renal function may contribute to the increase in plasma homocysteine concentrations with advancing age. Markers of glomerular filtration rate, such as creatinine and cystatin C concentrations (36, 37), were found to be significant, independent predictors of homocysteine concentrations (38, 39).

In the present study, the average plasma total homocysteine concentration was markedly higher in men than in women. This confirms the results of several previous studies (14, 32). In the Framingham Study (14), the sex difference was explained by intakes of vitamins B-12 and B-6. In the Hordaland Homocysteine Study (32), adjustment for vitamin intake did not completely explain the sex difference in homocysteine concentration. In the present study, adjustment for plasma concentrations of folate and vitamins B-12 and B-6 did not completely explain the difference in homocysteine concentrations between men and women. The residual sex difference was still significant; the values of men were 14% higher than those of women on average. Sex hormones may play a role in homocysteine metabolism. Elevated homocysteine concentrations were reported in women after menopause (40, 41), whereas reduced concentrations were measured in postmenopausal women on estrogen replacement therapy (42–45) and those treated with the partial estrogen agonist tamoxifen (46). Synthesis of creatine and creatinine, which is a function of muscle mass, is a major contributor to homocysteine formation (16) and this may explain the higher homocysteine concentrations in men than in women.

Plasma concentrations of folate, vitamin B-12, and vitamin B-6 are independent predictors of homocysteine concentrations in Singapore Chinese. Plasma folate showed the strongest association, followed by vitamin B-12 and then vitamin B-6. Similarly, dietary folate intake showed the strongest association with plasma homocysteine concentration and completely explained the associations between dietary vitamins B-12 and B-6 and plasma homocysteine. These findings are consistent with previous results (14, 15). In a meta-analysis of randomized intervention trials that assessed the effects of folate, vitamin B-12, and vitamin B-6 in lowering homocysteine concentrations, dietary supplementation with folate showed the strongest effect, reducing blood homocysteine concentration by 25%. Vitamin B-12 produced an additional 7% reduction in blood homocysteine concentrations, whereas vitamin B-6 did not produce a significant additional effect (47).

The prevalence of the *MTHFR-VV* genotype in the Singapore Chinese subjects was 8.0%. This is slightly lower than the prevalences of 13–16% in US whites (19, 48, 49) and 11–14% in Western Europeans (50–54) but is comparable with the prevalence of 10% in Japan (55). In the Singapore Chinese, a *MTHFR* genotype effect (higher homocysteine in *VV* homozygotes) was observed only among subjects with relatively low folate concentrations (below the population median). This folate-*MTHFR* gene interaction is consistent with results from studies conducted in Western populations (18, 20, 48, 56). MTHFR catalyzes the synthesis of 5-methyltetrahydrofolate, the main circulatory form of folate and the methyl donor in the remethylation of homocysteine to methionine (20). The *VV* genotype is associated with a thermolabile enzyme with 50% of normal activity (17). Our study results suggest that this lower efficiency in enzymatic activity can be sufficiently offset by a relatively abundant supply of the substrate (ie, folate) such that there is no resultant change in the remethylation process. However, this remethylation pathway becomes detectably inadequate, resulting in excess homocysteine, in the absence of sufficient substrate and the presence of suboptimal enzymatic activity.

In the present study, we observed that cigarette smoking, daily coffee consumption, and physical inactivity were positively associated with plasma homocysteine concentration. However, these associations disappeared after adjustment for plasma folate concentration. Several studies examined the associations between these lifestyle factors and plasma homocysteine concentration; the results were mixed. Osganian et al (57) found a positive association between cigarette smoking and serum homocysteine concentrations among 3524 schoolchildren in the United States. However, this positive association could be explained completely by serum folate, vitamin B-12, and vitamin B-6 concentrations. Stolzenberg-Solomon et al (58) noted a positive association between coffee consumption and plasma homocysteine concentration after adjustment for dietary folate intake. Nygard et al (32, 59) found that cigarette smoking, coffee consumption, and physical inactivity were each positively associated with plasma homocysteine; these associations remained significant after adjustment for dietary intakes of fruit and vegetables and use of vitamin supplements. In the present study, we noted that the inverse associations between plasma homocysteine concentration and plasma B vitamin concentrations were stronger than the inverse associations between plasma homocysteine and dietary B vitamin intakes. Therefore, residual confounding might explain the significant associations between the lifestyle factors (cigarette smoking, coffee consumption, and physical inactivity) and plasma homocysteine concentrations that were observed by Nygard et al (32, 59) and others (58) after adjusting for dietary B vitamin intakes.

In the present study, we did not find any significant relations between plasma homocysteine and plasma lipid concentrations or consumption of tea or alcoholic beverages. The results of previous studies were mixed. Nygard et al (32) reported positive associations between plasma homocysteine and both plasma total cholesterol and triacylglycerol. However, the triacylglycerol-homocysteine association disappeared after adjustment for intakes of fruit and vegetables and use of vitamin supplements. In the Physicians' Health Study, Stampfer et al (1) did not observe a significant association between plasma cholesterol and homocysteine. An inverse association between tea consumption and plasma homocysteine was noted by Nygard et al (59). However, the tea-homocysteine association was greatly attenuated after adjustment for use of vitamin supplements, intakes of fruit and vegetables, coffee drinking, and cigarette smoking. An inverse association between alcohol consumption and serum homocysteine concentrations was observed by Ubbink et al (60).

The authors noted that the effect of alcohol on homocysteine concentrations might be mediated by the folate content of the beer consumed. In the present study population, beer consumption was relatively low; only 7% of the alcohol consumed was beer, whereas 67% was hard liquor and 25% was wine. The low consumption of beer in our study population could be the reason for the lack of association between alcohol consumption and plasma homocysteine consumption.

In summary, the present study showed that age, sex, folate, vitamins B-12 and B-6, and *MTHFR* genotype are independent determinants of plasma homocysteine concentrations in middle-aged and older Chinese in Singapore. These 6 determinants combined could account for up to 40% of the total variation in plasma homocysteine concentrations in this Asian population.

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