

Methylenetetrahydrofolate Reductase 677 C→T Mutation and Coronary Heart Disease Risk in UK Indian Asians

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Abstract—Plasma homocysteine concentrations are elevated in UK Indian Asians and may contribute to twice as many coronary heart disease (CHD) deaths in this group compared with European whites. The mechanisms underlying elevated homocysteine concentrations among Indian Asians are not well understood. In this study, we have investigated the extent to which the methylenetetrahydrofolate reductase (MTHFR) 677 C→T mutation accounts for elevated plasma homocysteine and increased CHD risk in Indian Asians compared with European whites. We investigated 454 male cases (with myocardial infarction or angiographically proven CHD: 224 Indian Asians, 230 European whites) and 805 healthy male controls (381 Indian Asians, 424 European whites). Fasting homocysteine concentrations, MTHFR 677 C→T genotype, and conventional CHD risk factors were measured. The prevalence of homozygous MTHFR 677T in Indian Asian controls was less than one third that in European white controls (3.1% versus 9.7%, $P < 0.001$). In Indian Asians, the TT MTHFR genotype was not associated with homocysteine concentrations and was not present in any of the Asian controls with hyperhomocysteinemia ($>15 \mu\text{mol/L}$). In contrast, among European whites, the TT MTHFR genotype was strongly related to elevated plasma homocysteine concentrations and was found in 27% of the European controls with hyperhomocysteinemia. Elevated homocysteine in Indian Asian compared with European white controls was accounted for by their reduced levels of B vitamins but not by the MTHFR 677T genotype. However, neither the TT MTHFR genotype nor B vitamin levels explained the elevated homocysteine concentrations in CHD cases compared with controls. TT MTHFR was not a risk factor for early-onset CHD in Indian Asians (odds ratio, 0.5; 95% confidence interval, 0.1 to 2.4; $P = 0.39$), unlike in European whites (odds ratio, 2.1; 95% confidence interval, 1.1 to 4.1; $P = 0.02$). We conclude that the MTHFR 677T mutation does not contribute to elevated plasma homocysteine concentrations or increased CHD risk in Indian Asians compared with European whites. Our results suggest that novel genetic defects and/or environmental factors influence homocysteine metabolism in Indian Asians residing in the United Kingdom. (*Arterioscler Thromb Vasc Biol.* 2000;20:2448-2452.)

Key Words: arteriosclerosis ■ genetics ■ nutrition

Premature coronary heart disease (CHD) mortality is almost 2-fold higher in UK Indian Asians compared with European whites.¹ Excess mortality in Indian Asians is not fully accounted for by the prevalence of conventional risk factors or that of diabetes and insulin resistance.²⁻⁴

Elevated plasma homocysteine is an independent risk factor for CHD.⁵⁻⁷ Previous studies suggest that the increase in CHD risk associated with a $5 \mu\text{mol/L}$ increase in total homocysteine is equivalent to a 0.5 mmol/L increase in total cholesterol.⁸ In North American and European white populations, an estimated 10% risk of coronary artery disease may be attributable to elevated homocysteine.⁸ Recent studies have shown that homocysteine concentrations are higher in UK Indian Asians compared with European whites and that elevated homocysteine may contribute to twice as many CHD

deaths among Indian Asians compared with Europeans.⁹ The precise mechanisms underlying elevated homocysteine concentrations among Indian Asians remain to be elucidated.

Plasma homocysteine concentrations are determined by genetic factors and by nutritional deficiencies of vitamins B₆, B₁₂, and folic acid.⁷ A mutation (677 C→T) in the enzyme methylenetetrahydrofolate reductase (MTHFR), which renders the enzyme thermolabile and functionally impaired, is common in North American and European populations.¹⁰⁻¹³ Homozygous MTHFR 677T is associated with raised plasma homocysteine concentrations,^{10,12,14-18} especially in the presence of low folate.^{11,14,17,19,20} Recent observations indicate that folate concentrations are 10% lower in Indian Asians than European whites,⁹ raising the possibility that the MTHFR 677T mutation may have a more important role in

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TABLE 1. Clinical and Biochemical Characteristics of Cases and Controls, for European Whites and Indian Asians Separately

	European Whites		Indian Asians	
	Cases (n=230)	Controls (n=424)	Cases (n=224)	Controls (n=381)
Age, y	56±6‡	50±7	52±7‡	49±6
SBP, mm Hg	129±19	128±19	130±20	132±21§
Glucose, mmol/L	5.6±1.8	5.4±1.5	6.7±2.8‡	5.9±2.2§
Diabetes*	26 (11%)‡	17 (4%)	85 (38%)‡	59 (16%)§
Total cholesterol, mmol/L	5.3±1.1‡	5.6±1.1	5.2±1.1‡	5.7±1.0
HDL cholesterol, mmol/L	1.22±0.31‡	1.36±0.38	1.12±0.30‡	1.24±0.37§
Triglycerides, mmol/L	2.0±1.8‡	1.6±1.2	2.1±1.5	2.0±1.3§
Smoking, current or former†	193 (84%)‡	281 (67%)	59 (26%)‡	52 (14%)§
Vitamin B ₁₂ , pmol/L	379±165	397±185	314±221	318±166§
Serum folate, ng/mL	8.0±3.9	8.0±4.0	8.3±3.9‡	7.3±3.1§
Homocysteine, μmol/L	11.2±4.6‡	10.2±3.3	12.0±5.0‡	10.8±3.8§

SBP indicates systolic blood pressure. Values are mean±SD for continuous variables and numbers (and %) for categorical variables.

*Diabetes: physician diagnosis of diabetes or fasting plasma glucose >7.0 mmol/L.

†Former smoking status denotes cessation at least 6 months before the study.

‡ $P<0.01$ for cases compared to respective ethnic controls.

§ $P<0.01$ for Indian Asian controls compared to European white controls.

determining homocysteine concentrations in Indian Asians than in Europeans.

We tested the hypothesis that elevated homocysteine concentrations and increased CHD risk in Indian Asians are accounted for by an increased frequency of the MTHFR 677T allele compared with European whites.

Methods

Subjects

The study design has been described previously.⁹ In brief, we investigated 454 male patients with CHD (224 Indian Asians, 230 European whites) and 805 healthy male controls (381 Indian Asians, 424 European whites). Indian Asians were of North Indian descent and had been resident in the United Kingdom for a mean of 27±8 years. European whites were born in the United Kingdom. Consecutive CHD patients were identified from the cardiology outpatient, coronary care unit, and coronary angiography records of Hammer-smith and Ealing Hospitals. Patients were aged <60 years at diagnosis and were not studied within 3 months of myocardial infarction or coronary intervention. Indian Asian and European white male controls (aged 35 to 60 years) were identified at random from the age-sex registers of 56 general practitioners whose practices were within the area served by the 2 hospitals. We sent invitation letters by post, and the response rate was estimated at 57% in Indian Asians and 54% in European whites.

Criteria for CHD were as follows: (1) myocardial infarction (chest pain associated with ECG evidence of myocardial infarction and/or elevated cardiac enzymes) or (2) angiographically proven coronary artery disease (>50% stenosis in 1 or more major epicardial vessel). Exclusion criteria for both patients and controls included cardiomyopathy, serious organ disease, systemic illness, chronic alcohol abuse, serious psychiatric illness, and anticonvulsant therapy; additionally for controls, the presence of pathological Q waves on the ECG was an exclusion criterion. The study was approved by the local ethics committee, and all subjects gave written, informed consent.

Methods

Clinical history was recorded in all subjects. Blood pressure was calculated as the mean of 3 readings taken with a mercury sphygmomanometer, with the subject having been seated for 10 minutes;

a 12-lead ECG was recorded according to a standardized protocol. Samples for plasma homocysteine were taken in the fasting state (overnight), placed on ice, and centrifuged within 1 hour, and the separated plasma was stored at -70°C before assays. Additional fasting samples were collected for serum folate, vitamin B₁₂, glucose, total cholesterol, HDL cholesterol, and triglycerides. Total plasma homocysteine was measured by high-pressure liquid chromatography.²¹ Serum folate and vitamin B₁₂ were measured by radioassay (Simultrac, Becton-Dickinson), and lipid profiles were determined by using an Olympus AU800 multichannel analyzer. MTHFR genotype was determined from peripheral blood by polymerase chain reaction and *HinfI* digestion, with the use of primers as previously described.¹⁰ All assays and genotyping were performed blinded to racial group and case-control status.

Statistical Methods

Data were analyzed by using the SPSS, version 8.0, statistical package. The independent-samples *t* test was used to compare continuous data between cases and controls and between the racial groups. ANOVA was used to compare homocysteine and folate concentrations between the MTHFR genotypes. Genotype distributions and other categorical variables were analyzed by the χ^2 test. The population variance in homocysteine attributable to MTHFR 677T was determined by linear regression. A logarithmic transformation of homocysteine concentrations was used for all analyses, and geometric means and approximate standard deviations are presented.

Results

Characteristics of Subjects

The clinical and biochemical characteristics of subjects are summarized in Table 1. Fasting homocysteine concentrations were higher in Indian Asian controls than in European white controls and were elevated in cases compared with controls in both racial groups.

Prevalence of the MTHFR 677T Allele in Control Subjects

The frequency of the MTHFR 677T allele was lower in Indian Asian controls compared with European white con-

TABLE 2. MTHFR Genotype and Homocysteine Concentrations in Cases and Controls, for Indian Asians and European Whites Separately

	MTHFR Genotype			P=
	CC	CT	TT	
Controls				
Indian Asians, n (%)	279 (73%)	90 (24%)	12 (3%)	
Fasting homocysteine, $\mu\text{mol/L}$	11.0 \pm 4.1	10.4 \pm 3.6	10.4 \pm 4.1	0.40
Serum folate, ng/mL	7.3 \pm 0.2	7.3 \pm 0.3	7.1 \pm 1.2	0.95
European whites, n (%)	188 (44%)	195 (46%)	41 (10%)	
Fasting homocysteine, $\mu\text{mol/L}$	10.1 \pm 2.9	10.1 \pm 3.0	11.6 \pm 5.5	0.007
Serum folate, ng/mL	8.0 \pm 0.2	8.1 \pm 0.3	7.0 \pm 0.5	0.28
CHD cases				
Indian Asians, n (%)	160 (71%)	61 (27%)	3 (1%)	
Fasting homocysteine, $\mu\text{mol/L}$	11.6 \pm 4.3	12.6 \pm 5.6	16.4 \pm 29.7*	0.22
Serum folate, ng/mL	8.4 \pm 4.1	7.9 \pm 3.6	7.3 \pm 4.4	0.62
European whites, n (%)	108 (47%)	91 (40%)	31 (14%)	
Fasting homocysteine, $\mu\text{mol/L}$	10.6 \pm 3.1	11.5 \pm 5.9	11.9 \pm 5.8	0.11
Serum folate, ng/mL	8.5 \pm 3.8	7.7 \pm 4.1	6.8 \pm 3.7	0.08

Values are geometric mean \pm SD.

*One outlier with homocysteine >50 $\mu\text{mol/L}$. Mean homocysteine for this group was 9.1 $\mu\text{mol/L}$ when this subject was excluded.

controls (15.0% versus 32.7%, $P<0.001$). The prevalence of homozygosity for MTHFR 677T in Indian Asian controls was less than one third that in European white controls (3.1% versus 9.7%, $P<0.001$).

Homocysteine Concentrations and MTHFR Genotype

In Indian Asian controls, there were no significant differences in homocysteine concentrations between subjects with TT compared with the CT or CC genotype (Table 2). In contrast, among European white controls, homocysteine concentrations were elevated in subjects with the TT genotype compared with the CT or CC genotype (Table 2). There were no significant differences in homocysteine concentrations between the MTHFR genotypes among cases in either racial group (Table 2).

After defining hyperhomocysteinemia according to the conventional criterion as a plasma homocysteine concentration $\geq 15 \mu\text{mol/L}$, we found that homozygous MTHFR 677T was not present among Indian Asian but was present in 27% of the European white controls with hyperhomocysteinemia (Table 3).

Determinants of the Differences in Homocysteine Concentrations Between the Study Groups

Homocysteine levels were closely related to age, creatinine, and concentrations of vitamin B₁₂ and folate in both racial

TABLE 3. Prevalence of Homozygous MTHFR 677T in Subjects With and Without Hyperhomocysteinemia, for Indian Asian and European White Controls Separately

	Homocysteine Concentration		P=
	<15 $\mu\text{mol/L}$	$\geq 15 \mu\text{mol/L}$	
Indian Asians	12/334 (4%)	0/47 (0%)	0.19
European whites	32/390 (8%)	9/34 (27%)	0.001

groups (Pearson correlation coefficients of -0.12, 0.22, -0.34, and -0.34, respectively; all $P<0.001$). The main determinant of the difference in homocysteine between Indian Asian and European white controls was the reduced concentrations of folate and vitamin B₁₂ in Asians; adjustment for the prevalence of TT MTHFR had little additional effect (Table 4). In contrast, elevated homocysteine concentrations in CHD cases compared with respective controls were not accounted for by age, creatinine, folate, vitamin B₁₂, or MTHFR genotype in either racial group (Table 4).

MTHFR 677T as a Risk Factor for CHD

Among Indian Asians, the prevalence of the homozygous MTHFR 677T genotype was similar in cases and controls (Table 5). In Indian Asians, TT MTHFR genotype was not a risk factor for CHD, even in subjects with early-onset CHD (odds ratio, 0.5; 95% confidence interval [CI], 0.1 to 2.4; $P=0.39$). In contrast, among Europeans, the TT MTHFR genotype was more frequent in cases than controls (Table 5), and TT MTHFR was a significant risk factor for early-onset CHD (odds ratio, 2.1; 95% CI, 1.1 to 4.1; $P=0.02$).

Discussion

We tested the hypothesis that the MTHFR 677T allele is more frequent in Indian Asians and contributes to their increased plasma homocysteine concentrations and CHD risk compared with European whites. We found that the frequency of homozygosity for the MTHFR 677T mutation among Indian Asians was less than one third that in European whites. Unlike in European whites, the MTHFR 677T allele does not influence homocysteine concentrations in Indian Asians, despite their lower folate concentrations compared with those in Europeans. Our observations in Indian Asians contrast previous findings in American, Canadian, Dutch, Norwegian, Italian, Irish, and Japanese subjects in whom the homozygous MTHFR 677T genotype is more prevalent and is associated

TABLE 4. Difference in Homocysteine Concentrations Between Groups

Adjustment	Percentage Difference in Homocysteine Concentrations (95% CI)					
	IA vs EW Controls	P=	IA Cases vs Controls	P=	EW Cases vs Controls	P=
None	6.0 (1.9 to 10.4)	0.005	11.2 (5.4 to 17.2)	0.001	8.7 (3.5 to 14.0)	0.001
Age	6.2 (1.9 to 10.7)	0.004	8.1 (2.3 to 14.0)	0.005	8.7 (3.0 to 14.8)	0.002
Age+creatinine	6.7 (2.6 to 10.9)	0.002	6.0 (0.7 to 11.7)	0.03	7.1 (1.4 to 13.0)	0.013
Age, creatinine, folate, + vitamin B ₁₂	0.0 (-3.6 to 3.8)	0.99	7.0 (2.3 to 11.9)	0.003	6.2 (1.4 to 11.4)	0.013
Age, creatinine, folate, vitamin B ₁₂ , + MTHFR	0.5 (-2.9 to 4.2)	0.77	7.1 (2.3 to 12.2)	0.003	5.9 (0.9 to 11.2)	0.018

IA indicates Indian Asians; EW, European whites.

with elevated mean plasma homocysteine concentrations.^{11,13,14,18-20,22-25} In these populations, depending on the cutoff point for hyperhomocysteinemia, 21% to 73% of hyperhomocysteinemic subjects are homozygous for MTHFR 677T.¹² In the present study, homozygous MTHFR 677T was absent among Indian Asian but was found in 27% of the European white controls with hyperhomocysteinemia (>15 μmol/L). These observations demonstrate that the MTHFR 677 C→T mutation is not an important determinant of homocysteine concentrations in Indian Asians.

In this study, homozygosity for MTHFR 677T was not a risk factor for CHD in Indian Asians. Combined with the low prevalence of MTHFR 677T in Indian Asians, our results exclude a role for this mutant allele as an important determinant of increased CHD mortality in Asians. Reasons underlying the low prevalence of the MTHFR 677T mutation in Indian Asians are not known. One possible explanation is that the MTHFR mutation may have been ‘bred out’ in this population, thereby conferring some protection against the adverse metabolic effects of reduced B vitamins. There has been some uncertainty as to the association of CHD with the MTHFR 677T allele among North Americans and European whites.^{14-19,22,26-29} Some investigators have found this mutation to be a significant risk factor for CHD,^{15,18,26} whereas others have found only a mild increase in the risk of CHD^{16,27}

or even no increase at all.^{14,17,19,22,28,29} However, critical analysis of these studies suggests that the inability of some studies to demonstrate an association of MTHFR 677T with CHD may have been due to selection of subjects from ethnically diverse populations³⁰ and the failure to take account of age at onset of CHD in the subjects studied. More recent data show a significantly higher frequency of homozygosity for the MTHFR 677T mutation in patients with early-onset CHD than in patients with later-onset CHD or in control subjects.³¹ The odds ratio for CHD in the young has been reported as 2.4 (95% CI, 1.2 to 4.7).³¹ In the present study, we found that the prevalence of homozygosity for MTHFR 677T was higher in European white cases with onset of CHD before the age of 50 years compared with controls. Our findings support the view that TT MTHFR is an important risk factor for premature CHD in Europeans.^{15,18,31}

We found that elevated plasma homocysteine concentrations in Indian Asian, compared with European, white controls, were explained by their low folate and B₁₂ concentrations. In contrast, homocysteine concentrations in Indian Asian and European white CHD patients, compared with controls, were only partially accounted for by differences in age, creatinine, vitamin status, and MTHFR genotype. In fact, folate concentrations were higher in Indian Asian cases compared with controls, probably reflecting dietary modifi-

TABLE 5. MTHFR Genotypes for Cases and Controls, Among Indian Asians and European Whites Separately

	MTHFR Genotype			OR for CHD	P=
	CC	CT	TT		
Indian Asians					
Controls	279 (73%)	90 (24%)	12 (3%)		
Patients					
CHD onset <50 years	83 (69%)	35 (29%)	2 (2%)	0.5 (0.1 to 2.4)	0.39
CHD onset ≥50 years	71 (72%)	26 (27%)	1 (1%)	0.3 (0.1 to 2.5)	0.25
All	160 (71%)	61 (27%)	3 (1%)	0.4 (0.1 to 1.5)	0.17
European whites					
Controls	188 (44%)	195 (46%)	41 (10%)		
Patients					
CHD onset <50 years	31 (38%)	35 (43%)	15 (19%)	2.1 (1.1 to 4.1)	0.02
CHD onset ≥50 years	74 (52%)	52 (37%)	16 (11%)	1.2 (0.6 to 2.2)	0.58
All	108 (47%)	91 (40%)	31 (14%)	1.5 (0.9 to 2.5)	0.14

Odds ratios (ORs) of CHD are for the homozygous MTHFR 677T genotype, in cases compared with respective ethnic controls, and are presented with 95% CI and P values. Age of onset of CHD could not be accurately determined in 13 (<3%) of the cases.

cations after the diagnosis of CHD. Because vitamin status and the MTHFR 677 C→T mutation do not account for elevated homocysteine in cases compared with controls, our results raise the possibility that other defects (genetic or environmental) in homocysteine metabolism may contribute to elevated homocysteine concentrations among CHD patients.

In summary, we have found that, unlike in European whites, the MTHFR 677T allele does not contribute to increased homocysteine concentrations or CHD risk in Indian Asians. Our findings raise the possibility that novel genetic defects and/or environmental factors may influence homocysteine metabolism in this ethnic group.

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