The frequency of a common mutation of the methionine synthase gene in the Australian population and its relation to smoking and coronary artery disease

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Background Modest elevations in levels of circulating homocysteine are common in patients with vascular disease. Methionine synthase is a vitamin B_{12}-dependent enzyme catalysing the re-methylation of homocysteine to methionine; reduced methionine synthase activity results in elevated level of homocysteine.

Design A case–control study.

Methods We explored the frequency and distribution of a 2756A→G (D919G) mutation of the methionine synthase gene, detected by polymerase chain reaction genotyping, in 745 Australian Caucasian patients aged ≤65 years (550 men and 195 women) with and without angiographically documented coronary artery disease (CAD).

Results The frequency distributions of AA, AG and GG genotypes were 61.9%, 33.8% and 4.3%, respectively, and were in Hardy–Weinberg equilibrium. There was no correlation between the methionine synthase mutation and CAD from simple χ^2 comparison. However, the interactive term of life-time smoking dose with methionine synthase genotypes was predictive of both the number of significantly diseased vessels (≥50% luminal obstruction; $\chi^2=12.518$, $P=0.0019$), and the presence or absence of significant CAD ($\chi^2=7.045$, $P=0.027$). A stepwise logistic regression analysis showed that smokers who were also GG homozygotes had more severe CAD compared with smokers of other genotypes. The methionine synthase genotypes were not associated with any of the other established CAD risk factors assessed in our study.

Conclusions We conclude that the methionine synthase 2756A→G mutation is common, with homozygosity occurring in approximately 4% of white Australians, and that it has an interactive effect with life-time smoking dose to increase the severity of CAD. Smokers who are also GG homozygotes have additionally elevated CAD risk. *J Cardiovasc Risk* 5:289–295

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Introduction

Modest elevations of plasma homocysteine levels occur in approximately 20% of patients with coronary, cerebral, and peripheral vascular disease [1–10] and in those with venous thrombosis [11]. Levels are also generally increased in those with chronic renal failure [12–15] and in these patients there is greatly increased cardiovascular risk [14,15]. While the mechanisms mediating vascular changes are still unclear, a recent meta-analysis estimated the enhanced risk of coronary artery disease (CAD) associated with a 5 μmol/l elevation of total plasma homocysteine to be the same as that associated with a 0.5 mmol/l increase in total cholesterol [16].

Homocysteine is formed during the metabolism of the essential sulphur-containing amino acid methionine [17]. Most exogenous methionine (~90%) is taken up by cells to be incorporated into protein, while the remaining 10% is metabolised to homocysteine via S-adenosylmethione (AdoMet) and S-adenosylhomocysteine [18]. The homocysteine formed is either converted into cysteine via the trans-sulphuration pathway mediated by cystathionine beta synthase (CBS), a rate-limiting enzyme in the pathway, or it is re-methylated back to methionine by a folate- and vitamin B_{12}-dependent enzyme, methionine synthase. CBS is located on chromosome 21 [19] and methionine synthase on chromosome 1 [20–22]. Methionine synthase is one of the only two B_{12}-dependent mammalian enzymes, and it catalyses the re-methylation of homocysteine to methionine by concurrent demethylation of 5-methyltetrahydrofolate to tetrahydrofolate [20,23]. The production of 5-methyltetrahydrofolate is mediated by the enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR), the gene for which is located on chromosome 1 [24].

There has been considerable recent interest in CBS and MTHFR contributions to homocysteine metabolism, and to mild homocysteine elevation (hyperhomocysteinaemia).

Defective activity in these enzymes also results in rare inborn errors of metabolism associated with homocystinuria in which levels of plasma homocysteine are greatly increased [17,25–28]. In these inborn errors the phenotype features include precocious vascular disease [17,23]. In contrast to these uncommon mutations there is a common $C_{677}\rightarrow T$ substitution producing an alanine to a valine residue mutation at the MTHFR gene which results in thermolability of MTHFR and mild homocysteinaemia of the order found in patients with the usual forms of vascular disease [24,29,30]. However, in population studies the MTHFR mutation has not been shown to occur more frequently in patients with vascular disease than in those without [31–34]. Mutations at the CBS gene identified so far are too rare for effective population studies with sufficient power [17,27,28] to determine whether or not they are more frequent in patients with vascular disease. There are, as yet, no studies of methionine synthase gene variants that could produce mild hyperhomocysteinaemia.

It is established that inhibition of methionine synthase results in reduced intracellular homocysteine re-methylation and increased export of homocysteine from cells to plasma [35–38]. It has been shown that there are large inter-individual differences in homocysteine clearances and it is postulated that this can be explained by variable adaptation to impaired methionine synthase function, leading to increased homocysteine flux through alternate metabolic pathways [36–38]. The cloning of the gene has facilitated an exploration of the effects of DNA mutations on methionine synthase activity and homocysteine metabolism [20–22,39]. Several mutations have been identified at the locus which lead to deficient methionine synthase enzyme activity and markedly elevated homocysteine levels (cobalamin G disease) [40]. Among recently discovered mutations is a 2756A→G change, resulting in a glycine for aspartic acid substitution (D919G). It appears to be common, but with as yet unclear functional significance, although a recent study of van der Put *et al.* [41] in young subjects did not find a relationship between the mutation and plasma homocysteine levels. The frequency for the mutant 'G' allele was estimated at 15% and 20% in two small (n = 45 and n = 54) undefined populations [20,22].

Given the possible importance of methionine synthase in relation to circulating homocysteine and atherogenesis, we undertook the present study to determine the frequency of the 2756A→G point mutation at the methionine synthase gene in our population and to determine whether it could be relevant to CAD risk in addition to other known risk factors. We have explored this in a population in which the presence or absence of CAD is defined by coronary angiography.

Materials and methods

The patient population

We studied 745 Caucasian patients aged 65 years or less, both men (n = 550) and women (n = 195), consecutively referred to the Eastern Heart Clinic at Prince Henry Hospital for coronary angiography. We excluded only patients shown to have significant left main disease (> 50% luminal obstruction) because it was difficult to categorise this small proportion of the total (4.5%) within the classification system we used (see below). This small group of patients would be classified as having single vessel disease with the current system whereas it has a much worse prognosis and is always managed differently from other single vessel disease. A written consent was obtained from every patient after a full explanation of the study, which was approved by the ethics committee of the University of New South Wales.

A 4 ml venous blood sample was drawn into an ethylenediamine tetraacetic acid (EDTA) sample tube before the angiogram, after the patient has fasted for at least 6 h. The blood sample was centrifuged within 2 h and plasma and cellular components stored separately at –70°C in aliquots until analysis.

Measurement of lipid variables

The hospital's clinical chemistry department measured lipoprotein, apolipoprotein, total cholesterol, high-density lipoprotein cholesterol and triglyceride levels using standard enzymatic methods. The low-density lipoprotein cholesterol levels were calculated using the Friedewald formula. Levels of apolipoprotein AI, apolipoprotein B and lipoprotein (a) were measured in our laboratory using in-house enzyme-linked immunosorbent assay methodology as described previously [42].

Detection of the 2756A→G (D919G) substitution at the methionine synthase locus

DNA was extracted from the frozen cellular blood component by a salting-out method [42]. The extracted DNA was stored at 4°C until analysis. The DNA samples were subjected to amplification by the polymerase chain reaction and the restriction enzyme *Hae*III was used to identify those with the mutation, as described by Leclerc *et al.* [22]. The primers used were 5′-GAA CTA GAA GAC AGA AAT TCT CTA for the upper stream and 5′-CAT GGA AGA ATA TGA AGA TAT TAG AC for the downstream. The expected fragment sizes after the *Hae*III digestion were 189 bp for 'A' allele and 159 and 30 bp for the 'G' allele.

Biometric measurements

The patient's height and weight were routinely measured before the coronary procedures by either a registered nurse or an attending doctor who also interviewed the patients to record the medical history. The waist and hip circumferences were measured and the body mass index obtained from the ratio of weight (kg) to height squared (m).

Documentation of CAD and other medical conditions

Two cardiologists who were unaware that the patients were to be included in the study assessed the angiograms. The severity of coronary artery disease was determined by the

number of significantly stenosed coronary arteries. Each angiogram was classified as revealing either normal coronary arteries or having no coronary lesion with more than 50% luminal stenosis or as having one, two, or three major epicardial coronary arteries with more than 50% luminal obstructions. We obtained each patient's medical history using a questionnaire with standardised choices of answers to be ticked during the interview as described previously [42].

Statistical analysis

Hardy–Weinberg equilibrium was assessed using χ^2 analysis as described by Emergy [43]. The frequencies of the alleles and genotypes among different subgroups were compared using contingency table analysis. For the statistical analysis we coded the GG, GA and AA genotypes as 1, 2 and 3, respectively. Sex was also treated as a nominal variable and coded as 1 for male and 2 for female. We coded those with normal coronary arteries and those without significantly diseased vessels (< 50% luminal obstruction) as 0, and those with one or more significantly diseased vessels (≥ 50% luminal obstruction) as 1. In an expanded model to include the number of significantly diseased vessels, we assigned 0, 1, 2 and 3 as the ordinal variables for the absence of significant disease and significant disease of increasing severity, respectively. We used logistic regression analysis to evaluate associations between the A→G mutation at the methionine synthase gene and the presence of either significant CAD, or in the expanded model, the number of significantly diseased vessels. A forward-stepwise model was used to explore significant predictors and their interactive terms. The full model was compared with the reduced model to assess the effect of methionine synthase genotype by maximum likelihood χ^2 tests; only significant variables were retained in the model. Since sex and CAD may alter the risk profiles and their relationships, we analysed quantitative variables in sex–CAD strata. One-way analysis of variance (ANOVA) was used to assess the associations between the genotype distribution and quantitative variables. Two-tailed P values are reported.

Results

Methionine synthase genotypes and demographic characteristics of patients

The genotype distribution was in Hardy–Weinberg equilibrium in the total population and was not different between male and female patients ($\chi^2 = 2.50$, $P = 0.286$). The gene variant is common, in that 4.3% of our population was homozygous for the mutation and the G allele frequency was 21.2%. The distributions were not different in those with angiographically normal coronary arteries (GG homozygote: 4.8%, G allele: 23.0%) and those with angiographically demonstrable coronary arterial lesions (GG homozygote: 4.1%, G allele: 20.0%).

The quantitative variable values for different genotypes were compared using one-way ANOVA in a stratification by sex and with/without significant CAD (Table 1–4). Although there was no significant difference in any subgroup of patients with different genotypes for any of these listed variables, there were some trends. In the subgroup of male patients with significant CAD, GG homozygotes tended to have smoked more as indicated by their higher life-time smoking dose (Table 1). In contrast, the life-time smoking dose was much lower in GG homozygotes of male patients without significant CAD (Table 2). However the life-time smoking dose was higher in female GG homozygotes both with and without significant CAD (Tables 3,4). Lipoprotein(a) levels also tended to be higher in GG homozygote patients of both sexes. Although these differences were not statistically significant, they may exert cumulative effects on associations between methionine synthase A→G mutation and CAD.

The methionine synthase mutation and CAD

As shown in Table 5, there was no relationship between the presence or absence of significant CAD ($\chi^2 = 1.255$, df = 2, $P = 0.5318$ for men, and $\chi^2 = 0.912$, df = 2, $P = 0.6339$ for women). The prevalence of GG homozygotes in patients with CAD versus those without CAD were 3.0% versus 4.1% for men and 7.0% versus 5.3% for women. This lack of association was also found between the

Table 1 Demographic and lipid variables among male patients with significant CAD (n = 402) and different methionine synthase genotypes

	GG	GA	AA	*P*
n	12 (3.0%)	140 (34.8%)	250 (62.2%)	
Age (years)	58.6 ± 1.6	56.9 ± 0.5	56.4 ± 0.4	0.3693
BMI (kg/m^2)	28.4 ± 0.9	28.6 ± 0.3	28.1 ± 0.2	0.5233
Waist : hip ratio	0.94 ± 0.02	0.97 ± 0.01	0.96 ± 0.01	0.3499
Total cholesterol (mmol/l)	5.3 ± 0.2	5.6 ± 0.08	5.4 ± 0.06	0.1593
Triglyceride (mmol/l)	2.20 ± 0.24	2.08 ± 0.08	2.02 ± 0.06	0.6407
HDL-C (mmol/l)	1.11 ± 0.07	1.03 ± 0.02	1.02 ± 0.02	0.4733
LDL-C (mmol/l)	3.23 ± 0.21	3.62 ± 0.07	3.49 ± 0.05	0.1236
Total cholesterol/HDL-C	5.00 ± 0.39	5.77 ± 0.14	5.70 ± 0.10	0.1749
Apo AI (g/l)	0.97 ± 0.07	0.92 ± 0.02	0.93 ± 0.02	0.7040
Apo B (g/l)	0.98 ± 0.06	0.92 ± 0.02	0.93 ± 0.02	0.5219
Lp(a) (mg/l)	341 ± 71	320 ± 25	311 ± 18	0.9001
Smoking dose (pack-years)	32.6 ± 7.0	28.8 ± 2.4	26.6 ± 1.7	0.5871
% Smokers	73.3%	77.5%	75.9%	

Data are expressed as mean ± SEM. *P* values were obtained using one-way ANOVA. CAD, coronary artery disease; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Apo, apolipoprotein; Lp(a), lipoprotein (a).

Table 2 Demographic and lipid variables among male patients without significant CAD (n = 148) and different methionine synthase genotypes

	GG	GA	AA	*P*
n	6 (4.1%)	53 (35.8%)	89 (60.1%)	
Age (years)	55.0 ± 3.1	54.9 ± 1.1	54.9 ± 0.8	0.9970
BMI (kg/m^2)	28.3 ± 1.4	28.0 ± 0.5	27.1 ± 0.3	0.2623
Waist : hip ratio	0.95 ± 0.02	0.98 ± 0.01	0.96 ± 0.01	0.3059
Total cholesterol (mmol/l)	5.1 ± 0.3	5.2 ± 0.1	5.1 ± 0.1	0.9763
Triglyceride (mmol/l)	1.41 ± 0.41	1.86 ± 0.15	1.83 ± 0.11	0.5877
HDL-C (mmol/l)	0.90 ± 0.13	1.01 ± 0.04	1.07 ± 0.03	0.2148
LDL-C (mmol/l)	3.22 ± 0.35	3.31 ± 0.11	3.25 ± 0.08	0.9018
Total cholesterol/HDL-C	5.38 ± 0.73	5.53 ± 0.23	5.15 ± 0.17	0.4132
Apo AI (g/l)	1.23 ± 0.19	1.08 ± 0.08	1.10 ± 0.05	0.7726
Apo B (g/l)	0.83 ± 0.09	0.91 ± 0.03	0.86 ± 0.02	0.3629
Lp(a) (mg/l)	389 ± 120	281 ± 42	245 ± 32	0.4548
Smoking dose (pack-years)	9.8 ± 9.5	28.5 ± 3.2	17.9 ± 2.5	0.0165
% Smokers	50.0%	67.9%	52.8%	

Data are expressed as mean ± SEM. *P* values were obtained using one-way ANOVA. CAD, coronary artery disease; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Apo, apolipoprotein; Lp(a), lipoprotein (a).

methionine synthase genotypes and the number of significantly diseased vessels ($\chi^2 = 5.73$, df = 8, $P = 0.677$), and for those with or without angiographically demonstrable coronary artery lesions ($\chi^2 = 1.47$, df = 2, $P = 0.478$). The prevalence of the GG homozygote in patients with 0, 1, 2, and 3 significantly diseased vessels were 4.6%, 1.7%, 4.5% and 3.8%, respectively, for men, and 5.1%, 6.5%, 6.1% and 7.4%, respectively, for women.

To take into account the many other factors contributing to CAD risk and CAD severity, we used a logistic regression model to assess the association between the methionine synthase genotypes and CAD in relation to age, waist : hip ratio, smoking dose, lipoprotein (a) and total cholesterol/high-density lipoprotein cholesterol. When the number of significantly diseased vessels was the ordinal response variable, the model containing the interactive term 'life-time smoking dose with methionine synthase genotypes' (–log likelihood: 777.07) was significantly better than the reduced model without the interactive term (–log likelihood: 784.87, $\chi^2 = 12.518$, P 0.0019). When the dichotomous variable 'with/without significant CAD' was the ordinal response variable, the above relationship was also significant ($\chi^2 = 7.045$, $P = 0.0295$). Patients with higher life-time smoking dose had added, although modest, risk if they were also GG homozygotes. The estimate for prediction of the CAD risk (regression coefficient: 0.0295) for the interactive term of 'life-time smoking dose' in pack-years and methionine synthase genotypes was about 22% higher compared with the smoking dose alone (0.0240) in a model which included other conventional independent predictors. However, the GG homozygosity posed no additional CAD risk unless the patients were also heavy smokers. For example, there were 8.0% more male GG homozygote patients having significant CAD than those of non-GG homozygotes (90% versus 82.0%) if they were also heavy smokers (≥ 40 pack-years). However, the difference was no longer present if they were non-smokers (50% versus 47.8%). The model used in the analysis fits the data well as assessed by goodness-of-fit tests ($P > 0.2354$).

Since men and women may have different risk profiles, we repeated the above analysis separately in the two sexes. The risk profiles were indeed different in men and women. In female patients only lipoprotein (a) and waist : hip ratio were significant predictors of the number of significantly diseased vessels. Including both these terms and the interactive term 'smoking dose with methionine synthase

Table 3 Demographic and lipid variables among female patients with significant CAD (n = 100) and different methionine synthase genotypes

	GG	GA	AA	*P*
n	7 (7.0%)	27 (27.0%)	66 (66.0%)	
Age (years)	58.4 ± 2.6	58.4 ± 1.3	57.9 ± 0.9	0.9455
BMI (kg/m^2)	30.6 ± 1.9	29.1 ± 0.9	28.1 ± 0.6	0.3947
Waist : hip ratio	0.88 ± 0.04	0.87 ± 0.02	0.87 ± 0.01	0.9779
Total cholesterol (mmol/l)	5.8 ± 0.4	6.0 ± 0.2	5.5 ± 0.1	0.1863
Triglyceride (mmol/l)	2.1 ± 0.6	1.8 ± 0.2	1.7 ± 0.1	0.5625
HDL-C (mmol/l)	1.17 ± 0.14	1.36 ± 0.07	1.25 ± 0.05	0.2793
LDL-C (mmol/l)	3.7 ± 0.4	3.8 ± 0.2	3.5 ± 0.1	0.4668
Total cholesterol/HDL-C	5.03 ± 0.58	4.67 ± 0.29	4.82 ± 0.20	0.8255
Apo AI (g/l)	1.06 ± 0.10	1.04 ± 0.06	1.07 ± 0.04	0.9330
Apo B (g/l)	1.08 ± 0.10	0.97 ± 0.05	0.95 ± 0.04	0.4599
Lp(a) (mg/l)	432 ± 135	373 ± 68	354 ± 46	0.8508
Smoking dose (pack-years)	27.3 ± 9.2	10.1 ± 4.3	16.6 ± 3.0	0.1932
% Smokers	57.0%	41.9%	50.0%	

Data are expressed as mean ± SEM. *P* values were obtained using one-way ANOVA. CAD, coronary artery disease; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Apo, apolipoprotein; Lp(a), lipoprotein (a).

Table 4 Demographic and lipid variables among female patients without significant CAD (n = 95) and different methionine synthase genotypes

	GG	GA	AA	*P*
n	5 (5.3%)	34 (35.8%)	56 (58.9%)	
Age (years)	54.0 ± 3.6	57.5 ± 1.4	57.1 ± 0.9	0.6708
BMI (kg/m^2)	28.8 ± 2.1	28.1 ± 0.8	27.2 ± 0.6	0.5566
Waist : hip ratio	0.79 ± 0.04	0.87 ± 0.01	0.84 ± 0.01	0.0759
Total cholesterol (mmol/l)	4.6 ± 0.5	5.4 ± 0.2	5.4 ± 0.1	0.3474
Triglyceride (mmol/l)	1.08 ± 0.49	1.69 ± 0.16	1.60 ± 0.12	0.4912
HDL-C (mmol/l)	1.17 ± 0.19	1.25 ± 0.06	1.35 ± 0.05	0.3713
LDL-C (mmol/l)	2.96 ± 0.50	3.34 ± 0.17	3.36 ± 0.12	0.7404
Total cholesterol/HDL-C	3.90 ± 0.73	4.50 ± 0.25	4.38 ± 0.18	0.7172
Apo AI (g/l)	1.36 ± 0.23	1.20 ± 0.08	1.19 ± 0.05	0.7717
Apo B (g/l)	0.82 ± 0.12	0.89 ± 0.04	0.85 ± 0.03	0.6868
Lp(a) (mg/l)	213 ± 143	308 ± 52	279 ± 38	0.7934
Smoking dose (pack-years)	27.3 ± 9.2	10.1 ± 4.3	16.6 ± 3.0	0.1932
% Smokers	40.0%	47.1%	33.0%	

Data are expressed as mean ± SEM. *P* values were obtained using one-way ANOVA. CAD, coronary artery disease; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Apo, apolipoprotein; Lp(a), lipoprotein (a).

Table 5 The distributions of the methionine synthase genotypes in patients with and without angiographically defined coronary artery disease (CAD)

Methionine synthase genotypes	Patients with significant CAD	Patients without significant CAD
Men		
GG	12 (3.0%)	6 (4.1%)
GA	140 (34.8%)	53 (35.8%)
AA	250 (62.2%)	89 (60.1%)
Women		
GG	7 (7.0%)	5 (5.3%)
GA	27 (27.0%)	34 (35.8%)
AA	66 (66.0%)	56 (59.8%)

The frequency distribution of the gentoypes among patients with CAD, expressed as number of patients in each group and the percentage frequency of each genotype within each subgroup provided in brackets, was not different from those without significant CAD ($\chi^2 = 1255$, df = 2, $P = 0.5328$ for men and $\chi^2 = 0.912$, df = 2, $P = 0.6339$ for women).

genotypes' in the model did not improve the model fit in female patients (–log likelihood: 185.26) compared with the reduced model (–log likelihood: 186.116). This was also true when the dichotomous variable 'with/without significant CAD' was used as the response variable ($P > 0.05$). However, in male patients the risk profiles for the number of significantly diseased vessels were similar to that of the total population. The model including the interactive term of smoking dose with methionine synthase genotype significantly improved the model fit (–log likelihood: 585.70) compared with the reduced model (–log likelihood: 591.325, $\chi^2 = 10.303$, $P = 0.0058$). The result was the same when we assessed the dichotomous variable 'with/without significant CAD' as the response variable in male patients ($\chi^2 = 6.827$, $P = 0.0329$). Goodness-of-fit tests also showed that the selected model was suitable for the data. We concluded that the interactive term is more predictive of CAD severity in male patients than the lifetime smoking dose alone.

Methionine synthase mutation and other medical conditions

Past history of myocardial infarction tended to be more prevalent among GG homozygotes than among AA homozygotes in both men ($P = 0.678$) and women ($P = 0.105$) as shown in Table 6. However, there was no consistent trend for any of the other medical conditions. None of the observations were statistically significant and this was confirmed using logistic regression analysis. In particular, we found no association between family history of premature CAD and methionine synthase genotypes in this patient population ($\chi^2 = 0.558$, df = 2, $P = 0.757$).

Discussion

In classical homocystinuria, an inborn error caused by deficient CBS activity, effective treatment to lower grossly elevated circulating homocysteine markedly reduces cardiovascular risk [44]. There is also no doubt that there is an association between mild homocysteine elevation and increased coronary risk [1–10]. However, as yet there is no conclusive evidence that genetically variant homocysteine-related enzymes known to result in mild homocysteine

Table 6 Relationship between methionine synthase genotypes and other medical conditions

	GG	GA	AA	*P*
Men				
Past history of myocardial infarction	47.6%	38.6%	41.0%	0.677
Angina	66.6%	70.1%	71.7%	0.967
Hypertension requiring treatment	60.6%	41.3%	40.1%	0.210
Type 2 diabetes	14.3%	10.4%	11.4%	0.839
Women				
Past history of myocardial infarction	58.3%	39.7%	30.7%	0.105
Angina	75.0%	71.5%	84.8%	0.065
Hypertension requiring treatment	50.0%	50.8%	56.0%	0.763
Type 2 diabetes	8.3%	15.9%	12.8%	0.729

The percentage in each cell represents the percentage of patients with that particular genotype who had the listed medical conditions. The *P* values were derived from χ^2 comparisons.

elevation are more common in patients with vascular disease [34]. We and others have shown that the MTHFR C_{677}→T mutation, which occurs in about 11% of white populations and is commonly associated with mild homocysteine elevation, is not associated with increased coronary risk [31–34]. There are also no data with regard to heterozygotes for CBS mutations and CAD risk, since all reported mutations are too rare for effective population studies. In the present study, we have demonstrated a relationship between the interactive term 'life-time smoking dose' with the 2756A–G mutation at the methionine synthase gene and the severity of CAD in 745 Australian Caucasian patients; the relationship, although modest, is nevertheless significant. Male patients who were smokers had significantly elevated risk if they were also GG homozygotes. The risk is additional to the risk already imposed by life-time smoking dose alone. The finding is consistent with the concept that the methionine synthase A→G mutation contributes modestly to an increase in risk of CAD and that this risk could be masked or modified by other risk factors, particularly cigarette smoking. That the interaction is significant in an analysis of all patients (in which sex was a significant independent predictor) in the study, and in male but not female patients when they are analysed separately, may be a reflection of the smaller numbers and lower smoking doses for the female patients or their different risk factor profiles.

In the absence of further data we can only speculate on the mechanism(s) mediating the smoking–methionine synthase mutation interaction. There is, however, a common factor in that both smoking [45] and disordered methionine metabolism [46] are associated with endothelial dysfunction as evidenced by reduced endothelial-dependent vasodilatation. Indeed, reduced endothelial-dependent vasodilatation may even result from the effects of passive smoking [45], and also during transient modest increases of circulating homocysteine after an oral methionine load [47]. Smoking-related effects may be associated with low circulating folate, perhaps diet-related, and low folate itself may diminish the re-methylation of homocysteine to methionine and increase circulating homocysteine. Thus, the effect of the mutation on methionine synthase activity and plasma homocysteine levels may only become manifest when there are additional factors involved, such as cigarette smoking. This would be consistent with the finding that there was no correlation between the mutation and homocysteine levels in a young Dutch population [41].

The present study also defines the frequency of the 2756A→G mutation of the methionine synthase gene in a large white population aged ≤ 65 years. We have established that it is common. The homozygote frequency could be determined for the whole population we studied (n - 745) since it was not different in those with and without CAD. The frequency was 4.1% (95% CI 2.7%–5.5%) for the GG homozygote and 20.0% (95% CI 17.1%–22.9%) for the 'G' allele. While the functional significance of the mutation has not as yet been identified, as mentioned, an interactive effect with folate or vitamin B_{12} metabolism, or both, is likely.

In conclusion, we have documented the frequency of the methionine synthase 2756A→G mutation and identified a significant interactive effect of life-time smoking dose with the methionine synthase genotype and the severity of CAD in Australian Caucasian patients documented angiographically. Male smokers had additionally elevated CAD risk if they were also GG homozygotes compared with the risk imposed by smoking alone, an interaction not identified in the smaller number of female patients studied. Lack of direct association between the methionine synthase mutation and CAD, and the difference between sexes suggest that its contribution to elevated risk of CAD is relatively small, but that it may be modified by other risk factors as evidenced by its enhancing effect on smoking-related risk.

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