The graded effect of hyperhomocysteinemia on the severity and extent of coronary atherosclerosis

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Abstract

It is not clear to what extent methylenetetrahydrofolate reductase (*MTHFR*) gene and hyperhomocysteinemia effect the severity and extent of coronary atherosclerosis in Asian populations. We examined the *MTHFR* genotypes and plasma homocysteine (HCY) concentrations in 192 Taiwanese and investigated their relationship with coronary artery disease (CAD), and the severity and extent of coronary atherosclerosis. The distribution of *MTHFR* genotypes was similar in 116 CAD patients and 76 non-CAD subjects. Homozygosity was noted in 8% of CAD patients and 13% of non-CAD subjects ($P = 0.33$; 95% CI, 0.2–1.6). The geometric mean of HCY values was higher in CAD patients ($11.10 \pm 1.51 \mu\text{mol/l}$) than in non-CAD subjects ($9.21 \pm 1.55 \mu\text{mol/l}$) ($P = 0.003$). HCY levels were higher in patients with multi-vessel disease ($P < 0.05$) or in patients with $\geq 90\%$ stenotic lesions ($P = 0.005$), compared with non-CAD subjects. The CAD risks in the top two HCY quartiles (≥ 14.0 and 10.1 – $13.9 \mu\text{mol/l}$) were 4.0 (95% CI, 1.7–9.2) and 3.2 (95% CI, 1.4–7.4) times higher than in the lowest quartile ($\leq 7.9 \mu\text{mol/l}$) ($P = 0.001$ and 0.007 , respectively). Linear regression analysis showed significant correlations between HCY concentrations and the severity and extent of atherosclerosis ($P = 0.0001$ for both). In conclusion, hyperhomocysteinemia appears to have a graded effect on the risk of CAD as well as the severity and extent of coronary atherosclerosis. Our findings do not support the homozygous genotype of *MTHFR* as a genetic risk factor for CAD in this Taiwanese population. Perhaps a further study including assessment of vitamin status is needed to better clarify the relationship between *MTHFR* genotypes and CAD. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Hyperhomocysteinemia; Methylenetetrahydrofolate reductase; Coronary artery disease

1. Introduction

As an important risk factor for coronary atherosclerosis, elevated plasma homocysteine (HCY) concentration, has recently received greater attention than have conventional risk factors such as smoking, hypercholesterolemia, hypertension and diabetes mellitus [1,2]. The exact mechanism for high HCY concentrations leading to the development of atherosclerosis remains unclear. Possible mechanisms include the effect on platelets, clotting factors, and the endothelium [3].

Hyperhomocysteinemia has been associated with genetic defects and/or nutrient deficiencies [4,5]. Methylenetetrahydrofolate reductase (*MTHFR*), the key enzyme in the remethylation of homocysteine to the methionine pathway, converting 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate, has been reported to contribute to hyperhomocysteinemia with its thermolabile variant [6]. The *MTHFR* gene has been cloned [7], and several mutations were found in it [8]. One of the nucleotide mutations ($677\text{C} \rightarrow \text{T}$; alanine to valine) commonly seen in the *MTHFR* gene in its homozygous state is responsible for the thermolabile phenotype and is associated with decreased *MTHFR* activity and an elevated fasting HCY concentration [9].

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Although the presence of a homozygous mutation has been reported as a risk factor for coronary artery disease (CAD) [9–11], some suggest that mutation alone may not be an independent risk factor for CAD [12–14].

Increasing severity of carotid disease with increasing HCY levels has been seen in asymptomatic adults [15], but reports on the relationship between the severity of CAD and HCY levels have been conflicting. Kang et al. [16] reported no correlation between HCY concentrations and severity of coronary stenosis. However, von Eckardstein et al. [17] did show a correlation between HCY levels and the number of occluded coronary arteries. In addition to significant stenosis, major clinical coronary events may be related to the extent of angiographically minor plaque [18]. The correlation between HCY concentrations and the extent of coronary atherosclerosis needs further investigation.

Because cardiovascular disease is now a leading cause of death in Taiwan, we undertook the present study to determine the prevalence of the *MTHFR* 677C→T mutation and HCY concentrations in our population and investigated their relationship with CAD. To our knowledge, this is the first study to examine the relationship between HCY levels and the severity and extent of coronary atherosclerosis in an Asian population. Our data indicate that hyperhomocysteinemia, but not *MTHFR* mutation, appears to be associated with CAD and has a graded effect on the risk of CAD as well as the severity and extent of coronary atherosclerosis.

2. Methods

2.1. Subjects

With consent, 192 subjects underwent coronary angiography at the National Taiwan University Hospital in 1997. In addition to conventional lipid analysis, blood samples were obtained for *MTHFR* gene analysis and measurement of plasma HCY levels. The indications for cardiac catheterization were stable angina in 70 (36%) individuals, unstable angina in 15 (8%), prognostic stratification following myocardial infarction in 31 (16%), atypical chest pain in 42 (22%), and other reasons including valvular heart disease, cardiomyopathy, and congenital heart disease in 34 (18%).

The coronary angiography was performed in multiple projections to assure full assessment of the anatomy and was recorded on 35-mm cine films. The degrees of narrowing of the intraluminal diameters of the arteries were measured with the caliper method and by visual estimation by experienced angiographers

who were unaware of the subjects' HCY and *MTHFR* gene status. CAD was defined as at least 70% stenosis in one or more of the three coronary arteries or their primary branches [19]. The severity score of coronary stenosis was obtained by use of the Gensini scoring system [20,21]. The extent score to estimate the percentage of the coronary artery involved in atheroma was obtained by the method described by Sullivan et al. [22].

2.2. Genetic analysis

Heparinized blood was collected at the time of angiography after 12 h of fasting. Leukocytes were isolated immediately from plasma. DNA extracted from frozen buffy coat cells by Instagene kit (Bio-Rad, USA) was stored at -20°C until analysis, and plasma was stored at -80°C for HCY determination. The DNA sample underwent the polymerase chain reaction (PCR) as described by Frosst et al. [9]. The primers for analysis of the 677C→T mutation were 5'-TGAAGGAGAAGGTGTCTGCGGGA-3' and 5'-AGGCGGTGCGGTGAGAGTG-3'. The PCR mixture (50 μl) contained 50 ng of genomic DNA, 20 pmol of each primer and 1 U of Taq polymerase (Perkin-Elmer, USA). The cycle parameters were 3-min initial denaturation at 94°C , followed by 35 cycles of 40 s at 94°C , 45 s at 65°C , and 45 s at 72°C . Final extension was performed at 72°C for 7 min. The amplified DNA fragment (198 bp) was then subjected to *HinfI* digestion and subsequent electrophoresis in a 4% agarose gel. The wild type remained 198 bp after digestion and was designated as C, whereas the mutant allele which could be digested into 175 and 23 bp was designated as T.

2.3. Homocysteine determination

The plasma was frozen at -80°C until analysis. The measurement of HCY was based on the method described by Fortin and Genest [23]. Samples containing 180 μl of plasma, 45 μl of 2.5 mM acetylcysteine, 0.9% NaCl, 4 mM EDTA, and 22.5 μl of 10% Tri-*n*-butylphosphine in dimethylformamide were incubated at 4°C for 30 min, and 225 μl of 0.6 M perchloric acid in 1 mM EDTA was added. After centrifugation at 3000 rpm for 5 min, 100 μl of the supernatant was mixed with 20 μl of 1.55 M NaOH, 250 μl of 0.125 M potassium tetraborate, pH 9.5, containing 4 mM EDTA, and 100 μl of ammonium-7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate (1 g/l of potassium tetraborate), and incubated at 60°C for 60 min. After cooling on ice, a sample volume of 15 μl was subjected to high-performance liquid chromatography analysis and detected by a fluorescence detector (Waters 474, USA).

2.4. Statistical analysis

We used a chi-square test and Student's *t*-test to evaluate the categorical and continuous variables, respectively. Because of the right-skewed distribution of HCY values, a log transformation was used for normalizing the distribution. We calculated crude and adjusted odds ratios (ORs) plus 95% confidence intervals (CIs) to evaluate the association of CAD with other variables. We used univariate and multivariate logistic regressions to estimate the association of HCY values with other variables. Tukey's pairwise comparison procedure was used for the comparison of HCY values between groups with one-, two-, and three-vessel disease and non-CAD. To evaluate the risk of CAD, we used age- and sex-adjusted logistic regression to estimate ORs and 95% CIs for each HCY quartile [24]. We also used linear regression to compare the severity and extent of atherosclerosis between HCY quartiles. A significant difference was defined at $P < 0.05$. Data analysis was performed with SAS Statistical Software (SAS Institute, Cary, NC).

3. Results

3.1. Subjects

There were 142 men (74%) and 50 women (26%) (aged 21–93 years; mean, 59 ± 9 years). A total of 86 individuals (45%) had hypertension and 35 (18%) had diabetes mellitus. There were 66 current smokers (34%), and 64 (33%) had hypercholesterolemia with 22 (11%) taking lipid-lowering drugs (Table 1). There were 23 (12%) with obesity and five (3%) with a family history of CAD, and three patients (2%) had a history of ischemic stroke. The CAD group consisted of 116 patients (aged 36–93 years; mean 61 ± 10 years), including 51 (27%) with three-, 20 (10%) with two-, and 45 (23%) with one-vessel disease. The remaining of 76 (40%) subjects were free of significant stenosis (aged 21–84 years; mean 56 ± 12 years). A total of 65 CAD patients (27 with three-, ten with two-, and 28 with one-vessel disease) were diagnosed in the 70 subjects with stable angina. All 15 individuals with unstable angina had CAD (nine with three-, three with two-, and

Table 1
Crude and adjusted odds ratios for coronary artery disease associated with selective risk factors^a

Risk factor	Total (<i>n</i> = 192) <i>n</i> (%)	CAD (+) (<i>n</i> = 116) <i>n</i> (%)	CAD (−) (<i>n</i> = 76) <i>n</i> (%)	Crude OR (95% CI)	Adjusted OR ^b (95% CI)
<i>Age, years</i>					
<60	99 (52)	51 (44)	48 (63)	1.0	1.0
≥60	93 (48)	65 (56)	28 (37)	2.2 (1.2–3.9)	2.7 (1.3–5.4)
<i>Sex</i>					
Female	50 (26)	18 (16)	32 (42)	1.0	1.0
Male	142 (74)	98 (84)	44 (58)	4.0 (2.0–7.7)	2.6 (1.2–5.9)
<i>Hypertension</i>					
(−)	106 (55)	59 (51)	47 (62)	1.0	1.0
(+)	86 (45)	57 (49)	29 (38)	1.6 (0.9–2.8)	1.5 (0.8–3.0)
<i>Diabetes mellitus</i>					
(−)	157 (82)	89 (77)	68 (89)	1.0	1.0
(+)	35 (18)	27 (23)	8 (11)	2.6 (1.1–5.9)	2.2 (0.8–5.7)
<i>Hypercholesterolemia</i>					
(−)	128 (67)	71 (63)	57 (75)	1.0	1.0
(+)	64 (33)	45 (37)	19 (25)	1.9 (1.0–3.6)	2.5 (1.2–5.1)
<i>Cigarette smoking</i>					
(−)	126 (66)	66 (57)	60 (79)	1.0	1.0
(+)	66 (34)	50 (43)	16 (21)	2.8 (1.5–5.5)	2.0 (0.9–4.5)
<i>HCY</i>					
<10.1 μmol/l	96 (50)	47 (41)	49 (64)	1.0	1.0
≥10.1 μmol/l	96 (50)	69 (59)	27 (36)	2.7 (1.5–4.8)	2.1 (1.1–4.4)
<i>MTHFR genotype</i>					
CC	101 (53)	60 (52)	41 (54)	1.0	1.0
CT	72 (37)	47 (40)	25 (33)	1.3 (0.7–2.4)	1.0 (0.5–2.2)
TT	19 (10)	9 (8)	10 (13)	0.6 (0.2–1.6)	0.4 (0.1–1.4)

^a CAD, coronary artery disease; CC, non-thermolabile homozygotes; CI, confidence interval; CT, thermolabile heterozygotes; HCY, homocysteine; MTHFR, methylenetetrahydrofolate reductase; OR, odds ratio; TT, thermolabile homozygotes.

^b Multiple variable logistic regression analysis.

Table 2
Univariate and multivariate determinants of plasma homocysteine levels^a

Factor	Total (<i>n</i> = 192)	Plasma HCY, μmol/l	Univariate <i>P</i>	Multivariate <i>P</i>
	<i>n</i> (%)	Means ± S.D.		
<i>MTHFR</i> genotype				
CC	101 (53)	9.68 ± 1.49		
CT	72 (37)	10.80 ± 1.46	0.09	0.1
TT	19 (10)	12.55 ± 1.90	0.0001	0.005
<i>CAD</i>				
(−)	76 (40)	9.21 ± 1.55		
(+)	116 (60)	11.10 ± 1.51	0.003	0.049
<i>Age, years</i>				
<60	99 (52)	9.39 ± 1.45		
≥60	93 (48)	11.47 ± 1.58	0.001	0.02
<i>Sex</i>				
Female	50 (26)	8.94 ± 1.58		
Male	142 (74)	10.91 ± 1.51	0.01	0.08
<i>Hypertension</i>				
(−)	106 (55)	9.87 ± 1.55		
(+)	86 (45)	10.91 ± 1.52	0.1	0.3
<i>Diabetes mellitus</i>				
(−)	157 (82)	10.49 ± 1.54		
(+)	35 (18)	9.87 ± 1.52	0.5	0.08
<i>Hypercholesterolemia</i>				
(−)	128 (67)	10.38 ± 1.57		
(+)	64 (33)	9.97 ± 1.49	0.4	0.7
<i>Cigarette smoking</i>				
(−)	126 (66)	9.97 ± 1.55		
(+)	66 (34)	11.02 ± 1.51	0.1	0.3

^a CAD, coronary artery disease; CC, non-thermolabile homozygotes; CT, thermolabile heterozygotes; HCY, homocysteine expressed as geometric means $\pm$ S.D.; TT, thermolabile homozygotes.

three with one-vessel disease). Among the 31 persons for prognostic stratification of myocardial infarction, there were 15 patients with three-, seven with two-, and nine with one-vessel disease. A total of four patients (one-vessel disease) were found in the group of 42 subjects with atypical chest pain. Only one patient (one-vessel disease) came from the group consisting of 34 persons with other heart diseases. The mean severity and extent scores were 38.9 ± 28.6 and 38.1 ± 19.5 , 35.7 ± 22.3 and 38.7 ± 14.1 , 41.2 ± 23.4 and 39.1 ± 15.0 , 3.7 ± 9.5 and 4.3 ± 7.4 , and 1.5 ± 3.2 and 4.2 ± 7.3 in the individuals with stable angina, unstable angina, prognostic stratification of myocardial infarction, atypical chest pain and other heart diseases, respectively.

3.2. Risk factors of CAD

Univariate analysis showed that CAD was associated with the older age group (≥ 60 years), male individuals, diabetic and hypercholesterolemic patients, smokers, and subjects with HCY $\geq 10.1 \mu\text{mol/l}$ (median value).

Multivariate analysis revealed an elevated risk of CAD for the elderly, males, and individuals with hypercholesterolemia and HCY $\geq 10.1 \mu\text{mol/l}$ (Table 1). There was no significant difference in the distribution of MTHFR genotypes between CAD and non-CAD groups. Homozygosity was noted in nine (8%) of 116 CAD patients and ten (13%) of 76 non-CAD subjects ($P = 0.33$; 95% CI, 0.2–1.6). The T-allele frequency in the entire population was 0.29 (0.28 for CAD and 0.30 for non-CAD, $P = 0.74$).

3.3. Determinants of HCY levels

Univariate logistic regression indicated that HCY levels were significantly higher in the TT genotype, CAD, elderly, and male groups. In multivariate logistic regression, higher HCY levels were independently associated with the TT genotype, CAD, and old age (Table 2). The HCY levels (geometric means) were 12.55 ± 1.60 , 12.18 ± 1.42 , 9.68 ± 1.43 , and $9.21 \pm 1.55 \mu\text{mol/l}$ in the groups of three-, two-, one-vessel disease and non-CAD, respectively (Table 3). There were signifi-

cantly higher HCY levels in three-vessel (vs. one-vessel and non-CAD) and two-vessel groups (vs. non-CAD) ($P < 0.05$ for both). The HCY levels were 11.70 ± 1.51 and 9.39 ± 1.46 $\mu\text{mol/l}$ in CAD patients with ($n = 89$) and without ($n = 27$) severe stenosis ($\geq 90\%$), respectively. The HCY level was significantly higher in patients with severe stenosis as compared to non-CAD subjects ($P = 0.005$). However, there was no significant difference in HCY levels between CAD patients with stenosis less than 90% and non-CAD subjects ($P = 0.44$).

3.4. Relationship of HCY levels to CAD risk and atherosclerosis

The HCY distribution in our sample was skewed toward higher values, with the 95th, 90th, 75th, 50th, and 25th percentiles at 20.6, 17.8, 13.9, 10.0, and 7.9 $\mu\text{mol/l}$, respectively, and a maximum and a minimum of 55.0 and 3.0 $\mu\text{mol/l}$. The value of the 95th percentile in non-CAD subjects was 16.2 $\mu\text{mol/l}$. Table 4 shows that subjects in the top two HCY quartiles (≥ 14.0 and $10.1\text{--}13.9$ $\mu\text{mol/l}$) were 4.0 (95% CI, 1.7–9.2) and 3.2 (95% CI, 1.4–7.4) times more likely to have CAD than those in the lowest HCY quartile (≥ 7.9 $\mu\text{mol/l}$) ($P = 0.001$ and 0.007 , respectively). However, there was no significant difference in CAD risk between the two lower HCY quartiles. The severity score and extent score had significant correlations with HCY quartiles by linear regression analysis ($P = 0.0001$ for both).

4. Discussion

In our Taiwanese subjects, the allele frequency of MTHFR 677C $\rightarrow$ T mutation (0.29) and the frequency of the three genotypes (CC 53%, CT 37%, and TT 10%) were similar to those reported by McAndrew et al. [25] and Ma et al. [26]. However, the frequency of homozygous mutation may vary between different populations. Morita et al. [10] reported a 16% homozygous frequency in Japanese CAD patients, 8% higher than in ours. The result of our study does not support the 677C $\rightarrow$ T mutation as a CAD risk factor. Several recent studies also showed no associations between

MTHFR polymorphism and CAD [12,14,26,27]. One possible reason for these inconsistencies may be associated with differences in the ethnicity of the study subjects. MTHFR mutation exerts its influence on atherosclerosis through the action of HCY. However, the plasma HCY concentration is not only influenced by the gene mutation but also by non-genetic factors, such as folate status. Though homozygous mutation is associated with increased HCY levels in those with low folate levels [13,28], the effect of this genetic defect may be largely compensated by adequate folate intake [13,26]. The nutritional compensation may make a substantial difference between HCY concentrations in those with homozygous mutation. This might be another reason for the inconsistency between MTHFR mutation and CAD. It has been shown that the TT genotype has higher plasma HCY concentrations in our study. It is well established that an elevated plasma HCY level is a risk factor for atherosclerosis [29,30]. Therefore, individuals with the TT genotype may be at an increased risk for CAD. As the genetic mutation accounts only for a fraction of the variation in plasma HCY concentrations, a much larger sample size might be necessary to demonstrate a genotype-related risk than to show the risk associated with plasma HCY concentrations. Perhaps a longer study with multivariate analysis to dissect out confounding variables (e.g. folate intake) would be needed to clarify the association between MTHFR mutation and CAD.

Our study showed a positive relationship between plasma HCY and CAD, revealing significantly higher HCY levels in patients with multi-vessel disease or severe stenosis ($\geq 90\%$). von Eckardstein et al. [17] reported a similar result for the relationship between HCY levels and the number of stenosed vessels. A positive association between the HCY level and severe coronary atherosclerosis ($\geq 90\%$) has also been reported by Verhoef et al. [31]. However, some previous studies [16,27] reported no significant correlation between CAD severity and HCY levels. These conflicting results might be associated with the different definitions of stenosis severity between studies ($\geq 70\%$ by Kang et al. [16] and $\geq 50\%$ by Wilken et al. [27]). Further scrutiny might be necessary to clarify the relationship between HCY concentrations and less severe stenosis

Table 3

Comparison of HCY geometric means $\pm$ S.D. between groups of three-, two-, and one-vessel disease and non-CAD^a

	Three-vessel ($n = 51$)	Two-vessel ($n = 20$)	One-vessel ($n = 45$)	Non-CAD ($n = 76$)
HCY, $\mu\text{mol/l}$	$12.55 \pm 1.60^*$	$12.18 \pm 1.42^{\dagger,\ddagger}$	$9.68 \pm 1.43^{*,\ddagger}$	$9.21 \pm 1.55^{*,\ddagger}$

^a CAD, coronary artery disease; HCY, homocysteine.

* Three-vessel versus one-vessel and non-CAD, $P < 0.05$.

[†] Two-vessel versus non-CAD, $P < 0.05$.

[‡] Two-vessel versus one-vessel, $P = \text{NS}$.

Table 4

Age- and sex-adjusted association of HCY quartiles with CAD risk and atherosclerosis^a

	HCY quartile (range, $\mu\text{mol/l}$)			
	I (3.3–7.9) <i>n</i> = 48	II (8.0–10.0) <i>n</i> = 48	III (10.1–13.9) <i>n</i> = 46	IV (14.0–55.0) <i>n</i> = 50
CAD (+), <i>n</i> (%)	20 (41)	27 (56)	32 (70)	37 (74)
OR (95% CI)	1.0	1.8 (0.8–4.0)	3.2 (1.4–7.4)	4.0 (1.7–9.2)
<i>Severity score</i>				
Mean $\pm$ S.D.	15.0 $\pm$ 21.4	15.4 $\pm$ 20.0	30.1 $\pm$ 26.5	38.5 $\pm$ 29.0
Linear regression		Parameter	S.E.	<i>P</i> -value
Intercept		3.4	4.3	
HCY quartile		8.5	1.6	0.0001
<i>Extent score</i>				
Mean $\pm$ S.D.	16.4 $\pm$ 16.8	18.5 $\pm$ 18.6	26.0 $\pm$ 18.2	39.2 $\pm$ 22.8
Linear regression		Parameter	S.E.	<i>P</i> -value
Intercept		5.9	3.4	
HCY quartile		7.7	1.2	0.0001

^a CAD, coronary artery disease; CI, confidence interval; HCY, homocysteine; OR, odds ratio; S.D., standard deviation; S.E., standard error.

(70–90%). In addition, the inconsistency between the severity of coronary atherosclerosis and HCY concentrations may result from oversimplification of the atherosclerotic burden by estimating significant stenosis only. To better elucidate the correlation between the HCY concentrations and the severity of coronary stenosis, we employed the Gensini score to quantify the total atherosclerotic burden. The traditional classification of one-, two- and three-vessel disease used to categorize the severity of CAD is convenient, but may underestimate the amount of myocardium in jeopardy [32]. The Gensini score has been shown to provide a satisfactory functional stratification of coronary stenosis [20,21]. Recent studies have shown that coronary events are related to the progression of mild atherosclerosis and the rupture of minor plaques [18,33–35]. Pathologic findings have also revealed that more than 50% of the coronary tree is usually covered by raised atheroma before a clinical event occurs [35,36]. Sullivan et al. [22] and Nakagomi et al. [37] developed an extent score to reflect the load of minor plaques, which improved correlation with conventional CAD risk factors and was significantly related to the Gensini scoring severity of coronary disease. Therefore, evaluating the severity and extent of total atherosclerosis burden becomes important in conveying prognostic information. Our study showed that both the severity score and the extent score had good correlation with HCY quartiles. To our knowledge, we are the first to demonstrate the relationship between HCY levels and the severity and extent of coronary atherosclerosis, and we propose that hyperhomocysteinemia has a continuous influence on the initiation and progression of coronary atherosclerosis. Our study is limited to the use of coronary angiog-

raphy for determining both the severity and extent of atherosclerosis. This method may lack accuracy as compared to the use of intravascular ultrasound. However, it is not practical to use intravascular ultrasound to evaluate the entire coronary tree; coronary angiography is still the most readily available clinical method [37].

Our data showed that elevated HCY ($\geq 10.1 \mu\text{mol/l}$) has a graded effect on the risk of CAD. The subjects in the top two HCY quartiles (≥ 14.0 and 10.1 – $13.9 \mu\text{mol/l}$) were 4.0 and 3.2 times more likely to have CAD than those in the lowest quartile ($\leq 7.9 \mu\text{mol/l}$), respectively. This graded effect was similar to that in previous reports [30,31,38]. Glueck et al. [38] found that the relative risk for atherosclerotic events was 2.7 and 3.8 times higher in the top two HCY deciles (≥ 13.1 , $\geq 11.4 \mu\text{mol/l}$). Boushey et al. [30] reported that the OR for CAD with a $5\text{-}\mu\text{mol/l}$ HCY increment was 1.6 for men and 1.8 for women. Adequate dietary folate intake may be recommended for individuals (especially in elderly males) with HCY $\geq 10.1 \mu\text{mol/l}$.

The present study suggests that an elevated plasma HCY level, but not MTHFR mutation, is implicated as a risk factor for CAD. Hyperhomocysteinemia appears to have a graded effect on the risk of CAD as well as the severity and extent of atherosclerosis.

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