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Ryuichi Kawamoto · Katsuhiko Kohara
Yasuharu Tabara · Tetsuro Miki · Takaaki Doi
Hitoo Tokunaga · Ichirou Konishi

An association of 5,10-methylenetetrahydrofolate reductase (*MTHFR*) gene polymorphism and common carotid atherosclerosis

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Abstract Plasma homocysteine (Hcy) concentration has been shown to be influenced by a mutation in the gene coding methylenetetrahydrofolate reductase (MTHFR). Although plasma Hcy is related to atherosclerotic disorders, conflicting results have been reported about the association between *MTHFR* gene polymorphism and sclerotic lesions of the common carotid arteries. The effect of age–gene interaction on carotid arterial remodeling was investigated in elderly subjects with several risk factors for atherosclerosis. We evaluated sclerotic lesions of the common carotid arteries by ultrasonography in 326 patients (mean age ± standard deviation, 73 ± 12 years) and studied relations among the known risk factors for atherosclerosis, including *MTHFR* gene polymorphism and its interactions with age and sex. Of the 326 subjects studied, 136 had *MTHFR* genotype *CC*, 136 genotype *CT*, and 54 genotype *TT*. The three groups did not differ with respect to background factors such as age, history of cigarette smoking, blood pressure, lipids or uric acid, or in the incidence of atherosclerotic diseases. Spearman's rank correlation revealed a significant relationship between gender, age, Brinkman index, systolic blood pressure, triglycerides, HDL-cholesterol (HDL-C), uric acid, and *MTHFR* gene polymorphism. Multiple regression analysis using intima-media complex thickness (IMT) as a criterion variable and risk factors, including *MTHFR* gene polymorphism as explanatory variables showed that *MTHFR* gene polymorphism ($P = 0.039$) was a significant independent explanatory variable for IMT, along with gender (male) ($P < 0.001$), age ($P < 0.001$), systolic blood pressure (SBP) ($P = 0.047$), total cholesterol (T-C) ($P < 0.001$), and HDL-C ($P < 0.001$). Furthermore, a general linear model analysis revealed that interaction between age and *MTHFR* gene polymorphism was significantly associated with IMT, independently of age, SBP, T-C, and HDL-C in male subjects. However, age–gene interaction was not observed in female subjects. The findings of the present study confirm an association between *MTHFR* gene polymorphism and common carotid atherosclerosis in the Japanese population and further support the role of risk factor–gene interaction in common carotid atherosclerosis.

Key words Carotid atherosclerosis · Risk factor · *MTHFR* gene polymorphism · Age · Interaction

R. Kawamoto (✉) · T. Doi · H. Tokunaga · I. Konishi
Department of Internal Medicine, Nomura Municipal Hospital, 9-53 Nomura, Nomura-cho, Higashiuwa-gun, Ehime 797-1212, Japan
Tel. +81-894-72-0180; Fax +81-894-72-0938
e-mail: rykawamo@ehime.med.or.jp

K. Kohara · Y. Tabara · T. Miki
Department of Geriatric Medicine, Ehime University School of Medicine, Ehime, Japan

Introduction

Homocysteine (Hcy) is a sulfur-containing amino acid generated as an intermediate product in methionine metabolism. Hyperhomocysteinemia has been substantiated as a risk factor for occlusive vascular disease in 16.7%–46% of patients with coronary, cerebral, or peripheral arterial diseases (Nygard et al. 1995; Press et al. 1999; Selhub et al. 1995; Yoo et al. 1998). Recent studies have suggested that the risk of carotid artery stenosis is increased in subjects with even slightly elevated Hcy concentrations previously considered to be within the normal range (Selhub et al. 1995). Hcy can be transsulfurated to form cysteine or remethylated to form methionine. The latter reaction uses 5-methyltetrahydrofolate as a carbon donor: 5-methyltetrahydrofolate is synthesized from 5,10-methylenetetrahydrofolate through the action of methylenetetrahydrofolate reductase (MTHFR), which is found in endothelium or smooth muscle cells. Mutations in the gene coding for both of these enzymes leads to a group of disorders in which a marked elevation of circulating Hcy has been observed.

There have been numerous genetic association studies of the *MTHFR* C677T variant, particularly in the homozygous state, which have shown both the presence (Arruda et al. 1997; Cattaneo et al. 1997; Christensen et al. 1997; Kluijtmans et al. 1997; Morita et al. 1997) and absence (Anderson et al. 1997; Legnani et al. 1997; Ma et al. 1996;

Press et al. 1999; Rees et al. 1997; Schwartz et al. 1997) of significant associations with such end points as coronary heart disease, myocardial infarction, thrombo-occlusive disease, and cerebrovascular disease. However, the relationship between elevated plasma Hcy concentration and the end points of vascular disease appears to be consistent, even in those studies that failed to show an association with the *MTHFR* C677T variant.

Recently, we observed that risk factor–gene interaction can influence carotid atherosclerosis (Tabara et al. 2001). The conflicting evidence regarding an association between the *MTHFR* gene and atherosclerosis may suggest an interaction between risk factor and gene. Aging is a major risk factor for the development of atherosclerosis. Furthermore, plasma Hcy has been shown to increase with age. These findings may suggest a possible interaction between aging and *MTHFR* polymorphism, since it is also well demonstrated that the *MTHFR* genotype is significantly related to plasma levels of Hcy.

In the present study, we investigated the association between *MTHFR* polymorphism and carotid arterial remodeling in elderly subjects with several risk factors for atherosclerosis, with special emphasis on age–gene interaction.

Subjects and methods

Subjects. The subjects were consecutively enrolled from inpatients in the medical department of Nomura Municipal Hospital between August 1999 and December 2000. Those with cardiorenal or nutritional disorders that would affect blood pressure and lipid and glucose metabolism were excluded. Informed consent for the procedure was obtained from each patient. All procedures were approved by the Ethics Committee of Ehime University School of Medicine.

Ultrasound image analysis. An ultrasonograph (Hitachi EUB-565, Tokyo, Japan) equipped with a 7.5-MHz linear-type B-mode probe was used by a specialist in ultrasonography to evaluate sclerotic lesions of the common carotid arteries within three days of the blood biochemistry analyses. Patients were asked to assume a supine position, and the bilateral carotid arteries were observed obliquely from the front and rear. We measured the thickness of the intima–media complex (IMT) on the far wall of the common carotid artery at a point about 10 mm proximal to the bifurcation of the carotid artery (where the image is clearer, compared with the near wall) and the wall thickness near the 10-mm point on the B-mode monitor, and used the mean values for the study.

Other risk factors. We measured blood pressure in the right upper arm of seated patients using a standard sphygmomanometer. Cigarette smoking was quantified on the basis of the Brinkman index, which is a measure of daily consumption and duration of smoking. Blood biochemistry analyses [total cholesterol (T-C), triglycerides (TG), HDL-cholesterol (HDL-C), uric acid, and blood sugar] were carried out after fasting within two days of admission. Diabetes mellitus states was defined on the basis of previous treatment for diabetes mellitus. As for the presence of background atherosclerosis, those diagnosed as with transient ischemic attack, cerebral infarction, angina pectoris, or myocardial infarct were placed in the disease group.

MTHFR genotype analysis. Genomic DNA was extracted from peripheral blood lymphocytes using standard procedures. The DNA sample was amplified by polymerase chain reaction (PCR), and the restriction enzyme *Hin*fI was used to identify those with the C677T mutation, as described by Frosst et al. (1995). The PCR reaction generated a 198-bp fragment containing codon 677. The point substitution of T for C at codon 677 creates a *Hin*fI recognition sequence with resulting 175- and 23-bp fragments. Alanine-coding alleles therefore produced a 198-bp fragment that was easily distinguished from the 175-bp fragment generated by valine-coding alleles. Electrophoresis in a 4% agarose gel followed by ethidium bromide staining and UV illumination allowed detection of mutated alleles.

Statistical analysis. Statistical analysis was performed using SPSS 10.0J (Statistical Package for Social Science, Chicago, IL, USA). The prevalence of each genotype was compared by the χ-squared test. The differences among groups were analyzed by analysis of variance or by the Mann-Whitney U-test. The relations between IMT and risk factors, including genotype, were examined by Spearman's rank correlation and multiple regression analyses. A general linear model was employed to evaluate the significant contribution of risk factor–gene interactions to IMT. $P < 0.05$ was considered significant.

Results

Background of subjects with the three MTHFR genotypes. Of the 326 subjects studied (mean age ± standard deviation, 73 ± 12 years), 147 were men (72 ± 12 years) and 179 were women (74 ± 12 years). With respect to *MTHFR* gene polymorphism, 136 had genotype *CC*, 136 genotype *CT*, and 54 genotype *TT*. The distribution of *MTHFR* genotypes was consistent with published data for Japanese subjects (Morita et al. 1997) and was in agreement with the expected Hardy-Weinberg ratio ($P = 0.36$). Among the three *MTHFR* genotypes, there were no differences in background factors, including age, history of cigarette smoking, blood pressure, lipids, and uric acid, or in the incidence of atherosclerotic diseases such as hypertension, diabetes mellitus, ischemic heart disease, or cerebral infarction (Table 1).

Carotid intima–media thickness and the MTHFR gene. Spearman's rank correlation showed that IMT was significantly correlated with sex, age, Brinkman index, systolic blood pressure (SBP), TG, HDL-C, uric acid, and *MTHFR* gene polymorphism (Table 2). To further investi-

Table 1. Characteristics of the subjects by *MTHFR* C677T allele

Risk factor	*MTHFR* C677T allele			
	CC genotype $N = 136$	*CT* genotype $N = 136$	*TT* genotype $N = 54$	*P* value
Sex, male, N (%)	61 (44.9)	58 (42.6)	28 (51.9)	0.516
Age (years)	74 ± 13	74 ± 11	71 ± 14	0.655
BMI (kg/m^2)	22.4 ± 4.0	21.4 ± 3.8	22.0 ± 3.6	0.120
Brinkman index[a]	315 ± 514	266 ± 411	332 ± 534	0.803
Systolic BP (mmHg)	134 ± 19	132 ± 21	136 ± 20	0.299
Diastolic BP (mmHg)	74 ± 13	75 ± 12	75 ± 10	0.813
Antihypertensive drug use, *N* (%)	65 (47.8)	63 (46.3)	28 (51.9)	0.789
Total cholesterol (mg/dl)	184 ± 37	176 ± 39	180 ± 43	0.227
Triglyceride (mg/dl)	90 ± 45	88 ± 44	106 ± 72	0.169
HDL-cholesterol (mg/dl)	52 ± 19	50 ± 18	48 ± 19	0.209
Uric acid (mg/dl)	5.2 ± 1.9	5.4 ± 1.9	5.5 ± 2.2	0.634
Fasting blood sugar (mg/dl)	124 ± 45	120 ± 50	130 ± 55	0.344
Diabetes mellitus, *N* (%)	31 (22.8)	31 (22.8)	17 (31.5)	0.396
Atherosclerotic disease, *N* (%)[b]	56 (41.2)	56 (41.2)	24 (44.4)	0.918
Ischemic heart disease, *N* (%)	12 (9.6)	9 (7.4)	5 (9.6)	0.798
Ischemic stroke, *N* (%)	44 (32.4)	49 (36.0)	21 (38.9)	0.657

Values are mean ± standard deviaton
MTHFR, methylenetetrahydrofolate reductase; BMI, body mass index; BP, blood pressure
[a] Brinkman index: daily cigarette consumption × duration of smoking in years
[b] Four cases had both ischemic heart disease and ischemic stroke

Table 2. Spearman's rank correlations between conventional risk factors and common carotid arterial intima–medial thickness

Risk factor	Correlation coefficient	*P* value
Sex, male	0.197	<0.001
Age (years)	0.390	<0.001
BMI (kg/m^2)	−0.019	0.737
Brinkman index	0.171	0.002
Systolic BP (mmHg)	0.125	0.024
Diastolic BP (mmHg)	−0.056	0.313
Antihypertensive drug use	0.188	0.001
Total cholesterol (mg/dl)	0.070	0.209
Triglyceride (mg/dl)	0.141	0.011
HDL-cholesterol (mg/dl)	−0.221	<0.001
Uric acid (mg/dl)	0.173	0.002
Diabetes mellitus	0.051	0.361
MTHFR C677T allele[a]	0.113	0.042

[a] An additive model (*CC* = 1, *CT* = 2, *TT* = 3) was used for *MTHFR* genotype

Table 3. Multivariate linear regression analysis for common carotid intima–medial thickness with conventional risk factors and the *MTHFR* genotype

Risk factor	β	*P* value
Sex, male	0.264	<0.001
Age (years)	0.400	<0.001
Systolic BP (mmHg)	0.113	0.047
Diastolic BP (mmHg)	−0.094	0.094
Antihypertensive drug use	0.082	0.098
Total cholesterol (mg/dl)	0.220	<0.001
HDL-cholesterol (mg/dl)	−0.201	<0.001
MTHFR C677T allele[a]	0.097	0.039
R^2 =	0.314	<0.001

The risk factors: body mass index, Brinkman index, triglycerides, uric acid, and diabetes mellitus were not retained in the final model
β, standard regression coefficient; R^2, multiple coefficient of determination
[a] An additive model (*CC* = 1, *CT* = 2, *TT* = 3) was used for the *MTHFR* genotype

gate whether the *MTHFR* genotype is an independent determinant of carotid IMT, multivariate linear regression analysis for common carotid IMT was performed with the following risk factors: age, sex, body mass index, Brinkman index, SBP, diastolic blood pressure (DBP), T-C, HDL-C, triglycerides, uric acid, diabetes status, antihypertensive drug use, and *MTHFR* genotype. The results showed that *MTHFR* gene polymorphism was significantly and independently associated with IMT, as were sex, age, SBP, T-C, and HDL-C (Table 3).

Age–MTHFR gene interaction. To further investigate whether age and genotype interactions could influence IMT, a general linear model for IMT was analyzed with the following parameters: sex, age, SBP, DBP, antihypertensive drug use, T-C, HDL-C, and *MTHFR* gene polymorphism, including interactions with age and sex (Table 4). In male subjects, the interaction of age and *MTHFR* gene polymorphism was significantly associated with carotid IMT, in addition to age (alone), SBP, T-C, and HDL-C. This finding indicates that the association between age and carotid IMT was significantly different between subjects with the *MTHFR TT* genotype and those who were *MTHFR* C carriers (*CC* + *CT*). On the other hand, age–gene interaction was not observed in female subjects.

Discussion

This study examined the relation between *MTHFR* gene polymorphism and atherosclerosis evaluated ultrasonographically in patients with risk factors. The presence of a T

Table 4. General linear model for common carotid intima–medial thickness with conventional risk factors and the *MTHFR* genotype

	Male		Female	
Risk factor	F	*P* value	F	*P* value
Age (years)	54.89	<0.001[a]	30.87	<0.001[a]
Systolic BP (mmHg)	0.371	0.543	6.579	0.012[a]
Diasytolic BP (mmHg)	3.060	0.082	0.458	0.499
Antihypertensive drug use	1.410	0.237	1.809	0.180
Total cholesterol (mg/dl)	7.534	0.007[a]	2.477	0.117
HDL-cholesterol (mg/dl)	7.819	0.006[a]	12.46	0.001[a]
MTHFR C677T allele[b]	3.559	0.061	0.015	0.902
Age-*MTHFR* C677T allele[b]	3.977	0.048[a]	0.157	0.692

[a] Significant variables
[b] A dominance model ($CC + CT = 1$, $TT = 2$) was used for the *MTHFR* genotype

allele was a significant risk factor for IMT thickening in addition to conventional risk factors, including sex, age, SBP, T-C, and HDL-C. Furthermore, this study showed an effect of age–gene interaction on IMT.

MTHFR is present in vascular endothelium and smooth muscle cells, and the Hcy level rises by localized variation in MTHFR, which lowers this activity. Causes of atherosclerosis attributed to Hcy include endothelial cell injury, accelerated proliferation of smooth muscle cells (Harker et al. 1976), LDL oxidation promotion, thrombosis formation by accelerated prothrombin activity, and, recently reported, a mechanism acting via active oxygen (Welch and Loscalzo 1998). Accordingly, the relation between *MTHFR* gene polymorphism and atherosclerosis by IMT thickening has been suggested to be affected by Hcy (Cattaneo et al. 1997; Christensen et al. 1997; Kluijtmans et al. 1997; Morita et al. 1997). Despite these findings, it is difficult to clarify the relation between the *MTHFR* T allele and carotid artery stenosis (Girelli et al. 1998; Nakata et al. 1998). The relation is difficult to elucidate, presumably because of the presence of many risk factors that compound atherosclerosis. As for IMT, Bova et al. (1999) reported a relation between carotid artery stenosis (>75%) and *MTHFR* gene polymorphism, while Kostulas et al. (1998) denied that a relationship existed. However, the latter studied patients with symptomatic cerebrovascular disorders (irrespective of carotid artery stenosis) as well as those with carotid artery stenosis (>50%), which are clearly different groups.

The *TT* genotype is more closely related to the rise of Hcy in patients with a low folic acid level (Christensen et al. 1997; Girelli et al. 1998; Ma et al. 1996; Schwartz et al. 1997) and high Hcy is lowered by folic acid fortification (Jacques et al. 1999). This finding suggests that in a group with low folic acid intake, the effects of *MTHFR* gene polymorphism on atherosclerosis via Hcy are more likely to appear; therefore, the folic acid level and the effects of food should not be disregarded. Although we did not measure folic acid intake, the mean age of the subjects in the present study, 73 suggests that the folic acid intake was probably low. Since the effect of aging on carotid IMT was more precipitous in subjects with the *TT* genotype, age-related low intake of folic acid may underlie the age–genotype interaction observed in the present study. However, the age–gene interaction was observed only in male subjects. Although we could not specifically explain the gender-specific effect of the age–gene interaction, it has recently been shown that elderly male carriers of the T allele of the *MTHFR* gene have higher serum uric acid than those without T allele (Zuo et al. 2000). Since a significant positive association between plasma Hcy and serum uric acid has also been reported (Lussier-Cacan et al. 1996), these findings may explain the age-dependent effect of Hcy plasma levels on atherosclerosis in male subjects. The age–genotype interaction observed in the present study supports the hypothesis that other factors may contribute to the effect of genetic variation to give rise to carotid atherosclerosis (Tabara et al. 2001).

In summary, we found a significant association between *MTHFR* gene polymorphism and common carotid atherosclerosis in subjects with risk factors for atherosclerosis. Furthermore, an interaction between age and the *MTHFR* gene was observed. This finding further supports the idea that risk factor–gene interaction could allow us to determine specific predictive information about the development of atherosclerosis.

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References

Anderson JL, King GJ, Thomson MJ, Todd M, Bair TL, Muhlestein JB, Carlquist JF (1997) A mutation in the methylenetetrahydrofolate reductase gene is not associated with increased risk for coronary artery disease or myocardial infarction. J Am Coll Cardiol 30:1206–1211

Arruda VR, von Zuben PM, Chiaparini LC, Annichino-Bizzacchi JM, Costa FF (1997) The mutation Ala677Val in the methylene tetrahydrofolate reductase gene: a risk factor for arterial disease and venous thrombosis. Thromb Haemost 77:818–821

Bova I, Chapman J, Sylantiev C, Korczyn AD, Bornstein NM (1999) The A677V methylenetetrahydrofolate reductase gene polymorphism and carotid atherosclerosis. Stroke 30:2180–2182

Cattaneo M, Tsai MY, Bucciarelli P, Taioli E, Zighetti ML, Bignell M, Mannucci PM (1997) A common mutation in the methylenetetrahydrofolate reductase gene (C677T) increases the risk for deep-vein thrombosis in patients with mutant factor V (factor V:Q506). Arterioscler Thromb Vasc Biol 17:1662–1666

Christensen B, Frosst P, Lussier-Cacan S, Selhub J, Goyette P, Rosenblatt DS, Genest J Jr, Rozen R (1997) Correlation of a common mutation in the methylenetetrahydrofolate reductase gene with plasma homocysteine in patients with premature coronary artery disease. Arterioscler Thromb Vasc Biol 17:569–573

Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJH, den Heijer M, Kluijtmans LAJ, van den Hauvel LP, Rozen R (1995) A candidate genetic risk factor for vascular disease: a common mutation in methyleneterahydrofolate reductase. Nat Genet 10:111–113

Girelli D, Friso S, Trabetti E, Olivieri O, Russo C, Pessotto R, Faccini G, Pignatti PF, Mazzucco A, Corrocher R (1998) Methylenetetrahydrofolate reductase C677T mutation, plasma homocysteine, and folate in subjects from northern Italy with or without angiographically documented severe coronary atherosclerotic disease: evidence for an important genetic-environmental interaction. Blood 91:4158–4163

Harker LA, Ross R, Slichter SJ, Scott CR (1976) Homocysteine-induced arteriosclerosis. The role of endotherial cell injury and platelet response in its genesis. J Clin Invest 58:731–741

Jacques PF, Selhub J, Bostom AG, Wilson PW, Rosenberg IH (1999) The effect of folic acid fortification on plasma folate and total homocysteine concentrations. N Engl J Med 340:1449–1454

Kluijtmans LA, Kastelein JJ, Lindemans J, Boers GH, Heil SG, Bruschke AV, Jukema JW, van den Heuvel LP, Trijbels FJ, Boerma GJ, Verheugt FW, Willems F, Blom HJ (1997) Thermolabile methylenetetrahydrofolate reductase in coronary artery disease. Circulation 96:2573–2577

Kostulas K, Crisby M, Huang WX, Lannfelt L, Hagenfedt L, Eggertsen G, Kostulas V, Hillert J (1998) A methylenetetrahydrofolate reductase gene polymorphism in ischemic stroke and in carotid artery stenosis. Eur J Clin Invest 28:285–289

Legnani C, Palareti G, Grauso F, Sassi S, Grossi G, Piazzi S, Bernardi F, Marchetti G, Ferraresi P, Coccheri S (1997) Hyperhomocyst(e)inemia and a common methylenetetrahydrofolate reductase mutation (Ala223Val MTHFR) in patients with inherited thrombophilic coagulation defects. Arterioscler Thromb Vasc Biol 17:2924–2929

Lussier-Cacan S, Xhignesse M, Piolot A, Selhub J, Davignon J, Genest J Jr (1996) Plasma total homocysteine in healthy subjects: sex-specific relation with biological traits. Am J Clin Nutr 64:587–593

Ma J, Stampfer MJ, Hennekens CH, Frosst P, Selhub J, Horsford J, Malinow MR, Willett WC, Rozen R (1996) Methylenetetrahydrofolate reductase polymorphism, plasma folate, homocysteine, and risk of myocardial infarction in US physicians. Circulation 94:2410–2416

Morita H, Taguchi J, Kurihara H, Kitaoka M, Kaneda H, Kurihara Y, Maemura K, Minamino T, Ohno M, Yamaoki K, Ogasawara K, Aizawa T, Suzuki S, Yazaki Y (1997) Genetic polymorphism of 5,10-methylenetetrahydrofolate reductase (MTHFR) as a risk factor for coronary artery disease. Circulation 95:2032–2036

Nakata Y, Katsuya T, Takami S, Sato N, Fu Y, Ishikawa K, Takiuchi S, Rakugi H, Miki T, Higaki J, Ogihara T (1998) Methylenetetrahydrofolate reductase gene polymorphism: relation to blood pressure and cerebrovascular disease. Am J Hypertens 11:1019–1023

Nygard O, Vollset SE, Refsum H, Stensvold I, Tverdal A, Nordrehaug JE, Ueland M, Kvale G (1995) Total plasma homocysteine and cardiovascular risk profile. The Hordaland Homocysteine Study. JAMA 274:1526–1533

Press RD, Beamer N, Evans A, Deloughery TG, Coull BM (1999) Role of a common mutation in the homocysteine regulatory enzyme methylenetetrahydrofolate reductase in ischemic stroke. Diagn Mol Pathol 8:54–58

Rees DC, Liu YT, Cox MJ, Elliott P, Wainscoat JS (1997) Factor V Leiden and thermolabile methylenetetrahydrofolate reductase in extreme old age. Thromb Haemost 78:1357–1359

Schwartz SM, Siscovick DS, Malinow MR, Rosendaal FR, Beverly RK, Hess DL, Psaty BM, Longstreth WT Jr, Koepsell TD, Raghunathan TE, Reitsma PH (1997) Myocardial infarction in young women in relation to plasma total homocysteine, folate, and a common variant in the methylenetetrahydrofolate reductase gene. Circulation 96: 412–417

Selhub J, Jacques PF, Bostom AG, D'Agostino RB, Wilson PW, Belanger AJ, O'Leary DH, Wolf PA, Schaefer EJ, Rosenberg IH (1995) Association between plasma homocysteine concentrations and extracranial carotid artery stenosis. N Engl J Med 332:286–291

Tabara T, Kohara K, Nakura J, Miki T (2001) Risk factor-gene interaction in carotid atherosclerosis: effect of gene polymorphisms of renin-angiotensin system. J Hum Genet 46:278–284

Welch G, Loscalzo J (1998) Homocysteine and atherothrombosis. N Engl J Med 338:1042–1050

Yoo J-H, Chung CS, Kang SS (1998) Relation of plasma homocysteine to cerebral infarction and cerebral atherosclerosis. Stroke 29:2478–2483

Zuo M, Nishio H, Lee MJ, Maejima K, Mimura S, Sumino K (2000) The C677T mutation in the methylene tetrahydrofolate reductase gene increases serum uric acid in elderly men. J Hum Genet 45:257–262