Polymorphisms of the methylenetetrahydrofolate reductase, vascular endothelial growth factor, endothelial nitric oxide synthase, monocyte chemoattractant protein-1 and apolipoprotein E genes are not associated with carotid intima-media thickness

Emin Alioglu MD¹, Ugur Turk MD¹, Sirri Cam MD², Abbasali Abbasaliyev MD³,
Istemihan Tengiz MD¹, Ertugrul Ercan MD¹

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BACKGROUND: Single nucleotide polymorphisms in the 5,10-methylenetetrahydrofolate reductase (*MTHFR*), vascular endothelial growth factor (*VEGF*), endothelial nitric oxide synthase (*eNOS*), monocyte chemoattractant protein-1 (*MCP-1*) and apolipoprotein E (*ApoE*) genes appear to be a genetic risk factor for atherosclerosis. Common carotid intima-media thickness (cIMT) provides information on the severity of atherosclerosis.

OBJECTIVE: To investigate the relationship between cIMT and gene polymorphisms associated with atherosclerosis in Turkish patients with coronary artery disease (CAD).

METHODS: Sixty-two patients with angiographically diagnosed stable CAD were divided into two groups according to their cIMT values (group 1: n=35, cIMT of 1 mm or greater; group 2: n=27, cIMT of less than 1 mm). *MTHFR* 677 C/T, *VEGF* -460 C/T, *eNOS* 894 G/T, *MCP-1* -2518 A/G and *ApoE* (E2, E3 and E4) gene polymorphisms (where A is adenine, C is cytosine, G is guanine and T is thymine) were analyzed by polymerase chain reaction and restriction fragment length polymorphism. Evaluations of cardiovascular risk factors and coronary atherosclerotic lesions were performed in all patients. Serum homocysteine and high-sensitivity C-reactive protein were measured and compared between the two groups.

RESULTS: Serum high-sensitivity C-reactive protein (P=0.04) and homocysteine (P=0.006) levels were higher in group 1 than in group 2. The ratio of multivessel CAD and previous myocardial infarction was significantly higher in group 1 than in group 2 (P=0.014). In the study population, no significant difference in cIMT was observed according to the polymorphisms studied. Only hyperhomocysteinemia (OR 1.17 [95% CI 1.01 to 1.35], P=0.033) and previous myocardial infarction (OR 3.76 [95% CI 1.10 to 12.81], P=0.034) maintained a significant correlation with cIMT on multiple logistic regression analysis.

CONCLUSION: cIMT is increased in patients with hyperhomocysteinemia, inflammation and extended CAD. *MTHFR* 677 C/T, *VEGF* -460 C/T, *eNOS* 894 G/T, *MCP-1* -2518 A/G and *ApoE* single nucleotide polymorphisms were not associated with increased cIMT.

Key Words: Atherosclerosis-related genes; Carotid intima-media thickness; Coronary artery disease; Homocysteine

Les polymorphismes géniques du méthylénététrahydrofolate réductase, du facteur de croissance endothéliale vasculaire, de l'oxyde nitrique synthase endothéliale, de la protéine-1 chimioattractante monocyttaire et de l'apolipoprotéine E ne sont pas associés à l'épaisseur intima-média de la carotide

HISTORIQUE : Les polymorphismes d'un nucléotide simple des gènes du 5,10-méthylénététrahydrofolate réductase (*MTHFR*), du facteur de croissance endothéliale vasculaire (*VEGF*), de l'oxyde nitrique synthase endothéliale (*eNOS*), de la protéine-1 chimioattractante monocyttaire (*MCP-1*) et de l'apolipoprotéine 1 (*ApoE*) semblent être un facteur de risque génétique d'athérosclérose. L'épaisseur intima-média de l'artère carotide commune (cIMT) est indicatrice de la gravité de l'athérosclérose. **OBJECTIF :** Explorer les relations entre la cIMT et les polymorphismes géniques associés à l'athérosclérose chez des patients turcs présentant une coronaropathie.

MÉTHODOLOGIE : Les chercheurs ont divisé en deux groupes 62 patients présentant une coronaropathie stable diagnostiquée par angiographie selon leurs valeurs de cIMT (groupe 1 : n=35, cIMT d'au moins 1 mm ; groupe 2 : n=27, cIMT inférieure à 1 mm). Ils ont analysé les polymorphismes géniques du *MTHFR* 677 C/T, du *VEGF* -460 C/T, de l'*eNOS* 894 G/T, du *MCP-1* -2518 A/G et de l'*ApoE* (E2, E3 et E4) (où A désigne l'adénine, C, la cytosine, G la guanine et T la thymine) par réaction en chaîne de la polymérase et par polymorphisme de restriction. Ils ont évalué les facteurs de risque cardiovasculaire et les lésions athérosclérotiques coronaires auprès de tous les patients. Ils ont mesuré l'homocystéine sérique et la protéine C réactive à haute sensibilité et les ont comparées entre les deux groupes.

RÉSULTATS : Les taux de protéine C réactive à haute sensibilité (P=0,04) et d'homocystéine (P=0,006) étaient plus élevés dans le groupe 1 que dans le groupe 2. Le ratio de coronaropathie plurivasculaire et d'infarctus du myocarde antérieur était considérablement plus élevé dans le groupe 1 que dans le groupe 2 (P=0,014). Au sein de la population à l'étude, on n'a observé aucune différence significative de la cIMT d'après les polymorphismes étudiés. Seuls une hyperhomocystéinémie (RRR 1,17 [95 % IC 1,01 à 1,35], P=0,033) et un infarctus du myocarde antérieur (RRR 3,76 [95 % IC 1,10 à 12,81], P=0,034) permettaient de maintenir une corrélation significative avec la cIMT à l'analyse de régression logistique multiple.

CONCLUSION : Une cIMT accrue s'associe à une hyperhomocystéinémie, à une inflammation et à l'étendue de coronaropathie. Les polymorphismes d'un nucléotide simple des gènes du *MTHFR* 677 C/T, du *VEGF* -460 C/T, de l'*eNOS* 894 G/T, du *MCP-1* -2518 A/G et de l'*ApoE* ne s'associaient pas à une augmentation de la cIMT.

¹Central Hospital, Department of Cardiology, Izmir; ²Celal Bayar University, Faculty of Medicine, Department of Medical Biology and Genetics, Manisa, Turkey; ³Central Oil Workers Hospital, Department of Cardiology, Baku, Azerbaijan

Correspondence: Dr Emin Alioglu, Department of Cardiology, Central Hospital, Bayrakli, Izmir 35010, Turkey. Telephone 90-232-341-6767, fax 90-232-341-6868, e-mail dreminalioglu@yahoo.com

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High-resolution ultrasound is a reliable, noninvasive method for detecting early structural and functional atherosclerotic changes in the arterial wall (1,2). Increased carotid intima-media thickness (cIMT) is a structural marker of early atherosclerosis that correlates with vascular risk factors, relates to the severity and extent of coronary artery disease (CAD), and predicts the likelihood of cardiovascular events in population groups (3-7). Therefore, ultrasonographic measurements of the carotid artery may provide a useful intermediate phenotype for the identification of atherosclerosis-related genes.

Several complex mechanisms have been hypothesized to play a key role in the development of atherosclerosis, including metabolic and inflammatory processes. Single nucleotide polymorphisms in the 5,10-methylenetetrahydrofolate reductase (*MTHFR*), vascular endothelial growth factor (*VEGF*), endothelial nitric oxide synthase (*eNOS*), monocyte chemoattractant protein-1 (*MCP-1*) and apolipoprotein E (*ApoE*) genes appear as a genetic risk factor for atherosclerosis (8-10).

The aim of the present study was to investigate the relationship between cIMT and gene polymorphisms associated with atherosclerosis in Turkish patients with CAD.

METHODS

Patient population

The atherosclerosis-related gene polymorphisms were analyzed in 62 unrelated Turkish patients with angiographically diagnosed stable CAD who were admitted to the Department of Cardiology at Central Hospital. The mean ($\pm$ SD) age was 57.1 ± 10.5 years, 65% were men and all participants were Caucasian. The patients were divided into two groups according to their cIMT values (group 1: $n=35$, cIMT of 1 mm or greater; group 2: $n=27$, cIMT of less than 1 mm). Acute coronary syndrome, severe valvular heart disease, autoimmune disease, inflammatory arteritis, chronic or acute infectious disease, use of steroids or anti-inflammatory drugs within the past three months, renal failure and cancer were accepted as exclusion criteria. All participants gave informed consent.

Laboratory measurements

Venous blood samples were taken in the morning, after an overnight fast (10 h to 12 h). Total cholesterol and glucose were determined by conventional methods. Plasma homocysteine was measured using a chemiluminescent immunoassay (Immulite 2000; Diagnostic Products Corporation, United Kingdom). The high-sensitivity C-reactive protein (hs-CRP) samples were analyzed by latex particle-enhanced immunoturbidimetric assay. Sera were obtained before and after training by centrifugation of vacutainer clotted tubes at 3000 rpm for 10 min. All serum samples were stored at -70°C until analysis. The method is sensitive to 0.3 mg/L. The total median inter-assay and intra-assay coefficients of variation for the assays were less than 10% for hs-CRP.

Evaluation of risk factors

Hypercholesterolemia was defined as a serum total cholesterol level greater than 200 mg/dL (5.8 mmol/L) and/or treatment with a statin. Diabetes mellitus was defined as a glucose level greater than 110 mg/dL (6.05 mmol/L) and/or treatment with one or more antidiabetic agents. Systolic and diastolic brachial blood pressure were measured twice at 5 min intervals with the patient in the supine position during the carotid echocardiography examination. The mean value of the two measurements was obtained. Hypertension was defined as a systolic blood pressure greater than 140 mmHg and/or a diastolic blood pressure greater than 90 mmHg, or, alternatively, as treatment with one or more antihypertensive agents. Hyperhomocysteinemia was defined as a serum homocysteine level greater than 12 $\mu\text{mol/L}$.

Coronary angiography

Coronary angiography was performed according to standard angiographic techniques. Angiographic scoring was done by interventional cardiologists who were blinded to the study protocol. If the coronary luminal narrowing exceeded 50%, the stenosis was considered significant. Patients

with significant stenoses were classified according to the number of stenotic vessels (single- or multivessel disease).

cIMT measurements

Ultrasound examinations were performed using a 7.5 MHz linear transducer (Sonos 4500; Hewlett-Packard, USA). The proximal part of the carotid bulb was identified, and the segment of the right common carotid artery that was 1 cm to 2 cm proximal to the bulb was scanned. The image was focused on the posterior (far) wall, and the resolution box function was used to magnify the arterial far wall. IMT was defined as a low-level echogenic grey band that did not project into the arterial lumen, and was measured during end-diastole as the distance from the leading edge of the second echogenic line of the far wall of the distal segment of the right common carotid artery. Measurements (at least five) were taken 1 cm to 2 cm below the bifurcation and the average measurement was taken as the cIMT. One physician performed all measurements throughout the study. Increased cIMT was defined as cIMT of 1.0 mm or greater, because the epidemiological data currently available indicate that a value of cIMT at or above 1 mm at any age is associated with significantly increased risk of myocardial infarction (MI) and/or cerebrovascular disease (11).

Genetic evaluation

Genomic DNA was extracted from 200 μL of EDTA-anticoagulated peripheral blood leukocytes using the Qiagen mini blood DNA purification kits (Qiagen, Canada). Polymerase chain reaction (PCR) amplification was performed in a programmable thermal cycler GeneAmp PCR System 9700 (PerkinElmer Inc, USA). The *eNOS* genotyping for the Glu298Asp mutation was performed as described by Hingorani et al (12). The primers used were 5'-CATGAGGCTCAGCCCCAGAAC-3' (forward) and 5'-AGTCAATCCCTTTGGTGCTCAC-3' (reverse), where A is adenine, C is cytosine, G is guanine and T is thymine. DNA was amplified for 30 cycles, each cycle comprising denaturation at 95°C for 1 min, annealing at 60°C for 1 min, extension at 70°C for 1 min with a final extension time of 5 min at 70°C . The initial denaturation stage was performed at 95°C for 5 min. PCR products were digested with the restriction enzyme MboI at 37°C for 16 h. In the presence of T at nucleotide 894, which corresponds to Asp 298, the 206 base pair (bp) PCR product was cleaved into two fragments of 119 bp and 87 bp. The PCR products were separated on 2.5% agarose gel and identified by ethidium bromide staining.

The *MCP-1* -2518 genotype was determined using a PCR-restriction fragment length polymorphism (RFLP) assay using a PvuII site affected by the G/A polymorphism (13). Amplification with the primers 5'-CCGAGATGTTCCAGACAG-3' (forward) and 5'-CTGCTTTGCTTGTCCTCTT-3' (reverse) generated a 930 bp product. Digestion with PvuII yields 708 bp and 222 bp fragments when G is at position -2518. PCR products from A/A homozygous individuals do not cut with PvuII. The products were separated in 3% agarose gel and stained with ethidium bromide.

VEGF -460 C/T genotyping was performed as described by Watson et al (14). The primers for *VEGF* were forward 5'-TGTGCGTGTGGGGTTGAGCG-3' and reverse 5'-TACGTGCGGACAGGCCCTGA-3'. PCR products (175 bp) were digested with the restriction enzyme BstUI at 37°C for 2 h. In the presence of C, the 175 bp PCR product was cleaved into two fragments of 155 bp and 20 bp. The PCR products were separated on 3% agarose gel and identified by ethidium-bromide staining.

The PCR-RFLP method previously described (15) was used for the analysis of the *MTHFR* 677 C/T polymorphism. Genotype analysis of *ApoE* gene polymorphisms was performed using the single-stage PCR-based method and digestion with the restriction enzyme Hha I, as previously described (16).

Statistical analyses

Cardiovascular risk factor frequencies were determined with descriptive statistics, by which genotype and allele distribution were evaluated using

TABLE 1
The clinical features and angiographic data of the groups

	Group 1* (n=35)	Group 2† (n=27)	P
Age, years	58.4±9.2	55.1±9	0.80
Male, n (%)	22 (63)	18 (67)	0.76
Diabetes mellitus, n (%)	16 (46)	15 (55)	0.44
Hypertension, n (%)	27 (77)	18 (67)	0.36
Hypercholesterolemia, n (%)	17 (48)	12 (44)	0.75
Heredity, n (%)	13 (37)	12 (44)	0.56
Cigarette smoking, n (%)	11 (31)	7 (25)	0.64
Homocysteine, µmol/L	11.7±4.5	8.5±4.2	0.006
hs-CRP, mg/L	19.0±25.0	9.0±8.0	0.04
Previous MI, n (%)	18 (51)	5 (18)	0.001
Single-vessel CAD, n (%)	10 (29)	17 (63)	0.01
Multivessel CAD, n (%)	24 (69)	10 (37)	0.01

Data presented as mean ± SD unless otherwise specified. *Patients had carotid intima-media thickness ≥1 mm; †Patients had carotid intima-media thickness <1 mm. CAD Coronary artery disease; hs-CRP High-sensitivity C-reactive protein; MI Myocardial infarction

χ^2 analysis. The relationships among the cIMT, genotypic variants and atherosclerotic risk factors were evaluated by the use of a multivariate logistic regression model. All analyses were performed with SPSS software version 11.0 (SPSS Inc, USA) and values were considered to be significant at $P<0.05$.

RESULTS

There were no significant differences between the groups in relation to age, sex, smoking status, heredity, prevalence of diabetes mellitus, hypercholesterolemia and hypertension. Serum homocysteine (11.7±4.5 µmol/L versus 8.5±4.2 µmol/L; $P=0.006$) and hs-CRP (19.0±25.0 mg/L versus 9.0±8.0 mg/L; $P=0.04$) levels were significantly higher in group 1 than in group 2. In addition, the previous MI ratio was higher in group 1 than in group 2 (51% versus 18%; $P=0.001$). The ratio of multivessel CAD was significantly higher in group 1 than in group 2 (71% versus 37%; $P=0.01$). Table 1 shows the clinical features and angiographic data of the groups.

No significant difference in cIMT was observed according to the polymorphisms studied (Table 2). Only hyperhomocysteinemia (OR 1.17 [95% CI 1.01 to 1.35], $P=0.033$) and previous MI (OR 3.76 [95% CI 1.10 to 12.81], $P=0.034$) maintained a significant correlation with cIMT on multiple logistic regression analysis. However, there were no significant differences among genotypes in relation to age, sex, diabetes, hypercholesterolemia, hypertension, heredity, smoking, hs-CRP and cIMT.

DISCUSSION

The aim of the present study was to investigate the relationship between cIMT and gene polymorphisms associated with atherosclerosis in Turkish patients with CAD. In addition, the relationship among cIMT, cardiovascular risk factors and the extent of coronary atherosclerosis was evaluated. The principal finding from our study was that cIMT is increased in patients with hyperhomocysteinemia, inflammation and extended CAD. *MTHFR* 677 C/T, *VEGF* -460 C/T, *eNOS* 894 G/T, *MCP-1* -2518 A/G and *ApoE* single-nucleotide polymorphisms were not associated with increased cIMT.

Cardiovascular disease is the leading cause of mortality in Turkey and in the world. The most important recent epidemiological investigation on the Turkish population, the Turkish Adults Risk Factors (TEKHARF) study (17), has shown that the incidence of CAD in young individuals is common in this population. CAD has multifactorial etiologies resulting from the interaction of genetic predisposition and environmental risk factors such as smoking and hyperhomocysteinemia. Indeed, because a different intake of some nutritional factors (eg, vitamin B12, folate and vitamin B6) and methionine-rich foods (eg, eggs, packaged foods) may determine variations of the basal

TABLE 2
Distributions of the genotypes of 5,10-methylenetetrahydrofolate reductase (*MTHFR*), vascular endothelial growth factor (*VEGF*), endothelial nitric oxide synthase (*eNOS*), monocyte chemoattractant protein-1 (*MCP-1*) and apolipoprotein E (*ApoE*) genes

	Group 1* (n=35)	Group 2† (n=27)	P
<i>MTHFR</i> 677 C/T			
CC/CT/TT (%)	46/48/6	59/37/4	0.567
C/T	0.700/0.300	0.778/0.222	
<i>VEGF</i> -460 C/T			
CC/CT/TT (%)	11/37/52	7/63/30	0.130
C/T	0.300/0.700	0.371/0.629	
<i>eNOS</i> 894 G/T			
GG/GT/TT (%)	40/34/26	30/48/22	0.531
G/T	0.572/0.428	0.537/0.463	
<i>MCP-1</i> -2518 A/G			
AA/AG/GG (%)	34/54/12	37/50/13	0.975
A/G	0.614/0.386	0.630/0.370	
<i>ApoE</i>			
E2E3/E3E3/E3E4 (%)	12/74/14	19/70/11	0.712

*Patients had carotid intima-media thickness ≥1 mm; †Patients had carotid intima-media thickness <1 mm. A Adenine; C Cytosine; G Guanine; T Thymine

homocysteine levels (18,19), it is possible that the different dietary habits of the populations studied may also contribute to an explanation of the discrepant results.

Our observations are consistent with those of several previous studies (20,21). Crouse et al (22) found a strong association between coronary artery status, verified by coronary angiography, and mean aggregate cIMT. Likewise, in our study, the occurrence of multivessel CAD was significantly higher in patients with increased cIMT.

Although CRP has been considered as a good biomarker of systemic inflammation, recent data suggest that CRP might also be the culprit in atherosclerosis (23). CRP induces the production of inflammatory cytokines from monocytes, and promotes monocyte chemotaxis. Therefore, it is conceivable that a high serum concentration of CRP levels enhance the progression of atherosclerosis and also reflect arterial wall inflammation. In the present study, hs-CRP levels were higher in patients with increased cIMT than in those without.

Other investigators performed population-based studies in which cIMT was evaluated for the number and incidence of cardiovascular risk factors, and acute coronary syndromes or stroke (4). A strong association between cIMT and risk of MI was established in the Rotterdam study (24). Our study group consisted of patients with angiographically proven coronary disease admitted with a diagnosis of chronic stable CAD. Patients with increased cIMT had a significantly greater history of MI than patients with normal cIMT.

Increased cIMT is related to various cardiovascular risk factors including hypertension, smoking, hypercholesterolemia and diabetes mellitus (25). There were no significant differences between the groups in relation to cardiovascular risk factors, except serum homocysteine levels, which were significantly higher in patients with increased cIMT.

Hyperhomocysteinemia is associated with a high incidence of CAD, stroke and peripheral vascular disease (26-28). Homocysteine appears to alter the anticoagulant properties of endothelial cells to a procoagulant phenotype. Mildly increased homocysteine causes dysfunction of the vascular endothelium (29). However, plasma homocysteine itself has been demonstrated to be an independent risk factor for increased carotid artery wall thickness (30,31). We also found that plasma homocysteine levels were significantly higher in patients with increased cIMT.

Hyperhomocysteinemia can result from genetic or nutrient-related disturbances of homocysteine metabolism. A significant proportion of individuals with mild hyperhomocysteinemia was shown

to have thermolabile 5,10-MTHFR, an enzyme that catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate and is, therefore, involved in the remethylation of homocysteine to methionine (32). The thermolability seems to be caused by a C/T substitution at nucleotide 677 of the MTHFR-encoding gene. The individuals homozygous for the 677 C/T mutated allele show a 70% decrease in MTHFR activity and a 10-fold increased risk of being in the upper fifth percentile of homocysteine distribution (33). However, the association between homozygosity for the mutation and increased plasma homocysteine levels is inconsistent (34), and some investigations have failed to show an increased risk of vascular disease among persons homozygous for the mutation (31,35,36). Serum homocysteine levels were not significantly different in the MTHFR 677 C/T genotypes in our study.

The role of the MTHFR gene polymorphism as a genetic determinant of the pathogenesis of vascular disease is less clear. Associations with carotid atherosclerosis have been inconsistent, with eight studies showing no association and seven studies showing higher cIMT or more frequent stenosis in T allele carriers or TT homozygotes (8). In our study, increased cIMT was not associated with MTHFR genotypes.

Nitric oxide is a potent vasodilator produced by the action of nitric oxide synthase on L-arginine. Nitric oxide also inhibits platelet aggregation and adhesion, leukocyte chemotaxis and adhesion, adhesion molecule and chemokine expression, smooth muscle cell proliferation and migration, and low-density lipoprotein (LDL) oxidation (8). For these reasons, endothelial nitric oxide may have a prominent role in protection against atherosclerosis. Nitric oxide synthase exists in three forms – endothelial, neuronal and inducible. eNOS is presumed to be responsible for most of the endothelial and vascular effects of nitric oxide. Several polymorphisms of the eNOS gene have been identified, one of which, a G to T transversion at nucleotide 894 of exon 7, produces a glutamic acid to aspartic acid substitution at amino acid 298. This induces a conformational change that may reduce eNOS activity (37). This variant has been associated with enhanced vasoconstrictive response to phenylephrine, enhanced blood pressure response to endurance training, and hypertension, coronary disease and MI (38). No association of the 894 G/T variant with carotid atherosclerosis was found in four studies, although a fifth study demonstrated T homozygosity to be more frequent in subjects with plaque (8). In our study, increased cIMT was not associated with eNOS genotypes.

ApoE plays an important role in the development of atherosclerosis. It aids in cholesterol homeostasis and several hepatic receptors recognize ApoE. ApoE also directly modifies macrophage and T lymphocyte-mediated immune responses to inflammatory atherosclerosis. ApoE can also inhibit the proliferation of CD4 and CD8 T lymphocytes in lesions. ApoE has antioxidant activity demonstrated in a dose-dependent manner, with the E2 isoform having the greatest activity and E4 having the least. The ApoE gene encodes

three alleles – E2, E3 and E4. The E2 allele is associated with lower LDL cholesterol levels and the E4 allele is associated with higher LDL levels relative to the E3 allele (39). These observations led to the hypothesis that ApoE may play an important role in the development of CAD. Most studies of the ApoE E2/E3/E4 polymorphism have shown cIMT to be lower in carriers of the E2 allele and higher in carriers of the E4 allele, compared with E3/E3 homozygotes (8). These associations have not been totally consistent, however; de Andrade et al (40) and Hanon et al (41) showed higher cIMT in E2 carriers, Zannad et al (42) showed higher cIMT in E3/E3 homozygotes compared with E2 or E4 carriers, but on the right side only, and several studies (43-46) showed no association. The present study also demonstrates that ApoE genotypes were not associated with increased cIMT in patients with CAD.

VEGF is a potent angiogenic factor, and neovascularization has been shown to be important in atherosclerotic plaque development. There is some disagreement as to whether VEGF acts as a proatherosclerotic or antiatherosclerotic factor. Howell et al (9) reported that VEGF -2578 genotypes showed a significantly different distribution in patients without MI when stratified according to the number of diseased arteries – the AA genotype was a risk factor and the CC genotype was protective against atherosclerosis. There are no data in the literature regarding the effect of the VEGF gene polymorphism on carotid atherosclerosis. In our study, VEGF -460 C/T genotypes were not associated with increased cIMT.

MCP-1 plays an important role in atherosclerosis (47). MCP-1 shows potent chemotactic activity toward monocytes in response to immune, inflammatory and mechanical stimuli. Single nucleotide polymorphisms in the MCP-1 regulatory region (ie, -2518 G/A substitutions) have been identified and shown to affect transcription of the gene (13). It has also been reported that the GG genotype of the -2518 MCP-1 gene is associated with susceptibility to CAD (10). Tabara et al (48) reported that, although the plasma level of MCP-1 showed a significant association with cIMT, the -2518 A/G polymorphism did not directly correlate with cIMT, as was demonstrated in our study.

Limitations

There are several important limitations to the present study. First, the sample size was small; therefore, a larger study may be needed for further conclusion. Second, serum folate levels could have been measured, and folate's effect on cIMT and homocysteine levels evaluated.

CONCLUSION

Increased cIMT is associated with hyperhomocysteinemia, inflammation and more advanced CAD. MTHFR 677 C/T, VEGF -460 C/T, eNOS 894 G/T, MCP-1 -2518 A/G and ApoE single nucleotide polymorphisms were not associated with increased cIMT in Turkish patients with CAD.

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