Original Paper

Medical Principles and Practice

Med Princ Pract 2014;23:346–350
DOI: 10.1159/000363451

Received: November 13, 2013
Accepted: May 7, 2014
Published online: June 14, 2014

Intercellular Adhesion Molecule-1 K469E and Angiotensinogen T207M Polymorphisms in Coronary Slow Flow

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Key Words

ICAM1 polymorphism · Angiotensinogen polymorphism · Coronary slow flow · Atherosclerosis

Abstract

Objective: To investigate intercellular adhesion molecule-1 *(ICAM1)* and angiotensinogen *(AGT)* gene polymorphisms, as related to atherosclerosis and endothelial dysfunction, in coronary slow flow (CSF). ***Subjects and Methods:*** The participants in this study were 48 patients with CSF and 67 patients with normal coronary flow as controls. The K469E polymorphism of *ICAM1* (rs5498) and the T207M polymorphism of *AGT* (rs4762) were determined using the polymerase chain reaction amplification method. ***Results:*** Baseline demographic parameters were similar in both groups. The mean thrombolysis in myocardial infarction frame count was significantly higher in patients with CSF (23.8 ± 5.1) compared to the controls (13.3 ± 2.6, p < 0.001). A significant association was found between the *ICAM1* K allele and CSF (OR: 1.96, 95% CI: 1.15–3.35, p = 0.013). There was no difference in the frequency of *AGT* T207M genotypes in the patients with CSF and the control subjects. ***Conclusion:*** This study showed that K469E polymorphisms of *ICAM1* that play a role in atherosclerotic pathogenesis are related to CSF.

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Introduction

Cardiovascular diseases (CVD) worldwide are the most common cause of mortality, and atherosclerosis is the most common cause of CVD [1, 2]. Endothelial dysfunction is an important change seen in the subclinical stages of atherosclerosis [3, 4]. There are many molecules and cytokines that play important roles in endothelial functions. One molecule that is well studied is the intercellular adhesion molecule-1 *(ICAM1)* which is a member of the immunoglobulin family. It is released from endothelial cells, small muscle cells, macrophages, and lymphocytes [5, 6]. The important role which ICAM1 plays in inflammation and atherosclerosis is due to the induction of leukocyte adhesion and transmigration to vascular basal membranes [5, 6]. *ICAM1* K469E polymorphism (rs5498) increases the serum levels and functions of the *ICAM1* molecule and is closely related to the formation and progression of atherosclerosis [7–9].

Angiotensinogen *(AGT)* is a key molecule in the renin-angiotensin-aldosterone system (RAS), and it plays an important role in the regulation of blood pressure [10]. *AGT* is changed to angiotensin I via renin, and angiotensin I is converted to angiotensin II (Ag-II). Ag-II plays a role in the etiopathogenesis of atherosclerosis resulting from the release of cytokines and adhesion molecules

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1011–7571/14/0234–0346$39.50/0

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from vascular endothelial cells [11]. Previous studies showed that *AGT* gene polymorphisms lead to increased AGT levels and are related to hypertension and coronary artery disease [2, 12–14].

Atherosclerosis is a major underlying pathophysiological mechanism in CVD [1, 2]. Coronary slow flow (CSF) is described as the delayed angiographic passage of a contrast agent in the absence of stenosis in epicardial coronary arteries [15]. Previous studies have shown that endothelial dysfunction and diffuse atherosclerosis may be the underlying mechanisms in the etiopathogenesis of CSF, although the etiopathogenesis is still unclear [16, 17]. However, it was shown that certain polymorphisms are associated with CSF [18, 19]. We hypothesized that CSF is a subtype of atherosclerotic disease; hence, we aimed in this study to investigate the relationship between CSF with *AGT* T207M and *ICAM1* K469E polymorphisms (http://www.genenames.org) that were previously reported to be associated with atherosclerosis and endothelial dysfunction.

Subjects and Methods

Study Population

48 patients with CSF and 67 controls with normal coronary arteries participated in this study. Coronary angiography was performed in our Cardiology Clinic (Çanakkale, Turkey) between June 2010 and June 2013 on patients who had an indication for coronary angiography. All the subjects agreed to participate in the research and signed the informed consent form, and permission was obtained from the institution's Ethics Committee. The patients' complete history, results of the physical examination, risk factors for atherosclerotic heart disease, and medications were recorded. Patients who had been treated with antihypertensive drugs or those whose baseline blood pressure exceeded 140/90 mm Hg were diagnosed with hypertension. Diabetes mellitus was defined as fasting blood glucose higher than 126 mg/dl or the use of antidiabetic medications. Hyperlipidemia was defined as a total cholesterol level >200 mg/dl and/or a low-density cholesterol level >160 mg/dl. Exclusion criteria were patients with a known atherosclerotic disease, peripheral artery disease, visualized coronary plaques in coronary angiography, malignancy, chronic inflammatory disease, and renal and hepatic insufficiency. Peripheral blood samples from CSF patients and healthy controls were used to genotype point mutations of *ICAM1* and *AGT* genes.

Thrombolysis in Myocardial Infarction Frame Count and Definition of CSF

Angiographic equipment (GE Medical Systems, Innova 2100, USA) was used to perform coronary angiography with a femoral approach using Judkins catheters and iopramide as a contrast agent (Ultravist-370, Bayer Schering Pharma, Germany). The frame rate was 30 fps, and angiograms were recorded on a compact disc in DICOM format. Coronary blood flow was measured quantitatively using the thrombolysis in myocardial infarction (TIMI) frame count, which was determined for each major coronary artery in each participant according to the method first described by Gibson et al. [20]. The corrected TIMI frame counts for the left anterior descending coronary arteries (LAD) were calculated. The TIMI frame counts were divided by 1.7 because the LAD is usually longer than other major coronary arteries; thus, the TIMI frame count for this vessel is often higher. TIMI frame counts in the LAD and left circumflex (LCx) arteries were assessed at the right anterior oblique projection and in the right coronary artery (RCA) at the left anterior oblique projection. The mean TIMI frame count for each subject was calculated by adding the TIMI frame counts for LAD/1.7, LCx, and RCA, and divided by 3. The corrected cutoff values were 36.2 ± 2.6 frames for LAD, 22.2 ± 4.1 frames for LCx, and 20.4 ± 3 frames for RCA. Any values obtained above these thresholds were considered CSF [20].

Genotyping

Peripheral blood samples were collected from the patients and controls after a 12-hour overnight fasting period. All routine biochemical tests were carried out with the Cobas 6000 Integra (Roche) autoanalyzer device using the chemiluminescence method. Venous blood was collected in EDTA tubes for isolation of genomic DNA; the tubes were stored at –20°C. In all, 115 DNA samples from the patients with CSF and controls were genotyped using real-time PCR analysis. The total genomic DNA was extracted by either the MagnaPure Compact (Roche) technique or the Invitek kit extraction technique (Invitek®; Invisorb Spin Blood, Berlin, Germany). Target genes were amplified using real-time PCR methods (LightCycler 2.0, Roche) for the CSF cohort and the healthy controls. LightCycler FastStart DNA Master HybProbes, $MgCl_2$ (final concentration 3.0 mM), primer mix, PCR-grade water, and template DNA from the patients and controls were used for real-time amplification for each target gene.

The previously reported codon number of rs4762 is 207 (previously added as 174). T207M refers to a threonine-to-methionine exchange in codon 207. Real-time PCRs were performed using allele-specific sense and antisense SNP primers under the following conditions: initial denaturation at 95°C for 10 min, 45 quantification cycles at 95°C for 10 s, annealing at 60°C for 10 s, and extension at 72°C for 15 s. The melting cycle was 95°C for 30 s, 40°C for 2 min, and final cooling at 40°C for 30 s. The LightCycler 2.0 software program (Roche) was used to detect the mutated C allele (channel 640 at 58°C) and wild T genotype (channel 640 at 66°C) profiles for target analysis of *AGT* T207M.

The single nucleotide A to G polymorphism in the sixth exon of the *ICAM1* gene (K469E) resulted in an amino acid substitution from glutamic acid (E) to lysine (K) (rs5498). Genomic DNA (300 ng) was amplified by a real-time PCR in a final volume of 20 μl using the following conditions: initial denaturation at 95°C for 10 min, 45 quantification cycles at 95°C for 10 s, annealing at 60°C for 10 s, and extension at 72°C for 15 s. The melting cycle was 95°C for 30 s, 40°C for 2 min, and final cooling at 40°C for 30 s. The LightCycler 2.0 software program (Roche) was used to detect the mutated G allele (channel 640 at 66.5°C) and the wild A genotype (channel 640 at 55.8°C) profiles for target analysis of *ICAM1* K469E.

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Table 1. Baseline demographic and laboratory parameters of the study population

	CSF (n = 48)	Controls (n = 67)	p
Age, years	56.0±10.3	54.1±11.8	0.460
BMI	27.7±3.5	27.4±4.0	0.744
SBP, mm Hg	126.8±12.5	127.3±13.0	0.867
DBP, mm Hg	77.7±8.4	76.5±7.9	0.485
Heart rate, bpm	70±11	68±8	0.936
Males, n	26 (54.2)	27 (40.3)	0.141
Hypertension, n	22 (46.8)	31 (47.0)	0.986
Diabetes mellitus, n	13 (27.7)	13 (19.7)	0.322
Hyperlipidemia, n	21 (44.7)	16 (24.2)	0.022
Smoking, n	16 (34.0)	15 (22.7)	0.184
Family history, n	9 (13.6)	5 (10.6)	0.634
Total cholesterol, mg/dl	195.2±38.2	199.6±48.4	0.681
LDL, mg/dl	112.5±35.5	125.6±33.6	0.113
HDL, mg/dl	46.1±13.5	46.7±11.3	0.838
Triglycerides, mg/dl	150.1±115.6	132.7±75.4	0.824
TIMI frame counts			
LAD	37.7±9.3	19.8±5.4	<0.001
LCx	23.0±6.2	13.7±3.0	<0.001
RCA	26.4±7.4	14.7±4.3	<0.001
Mean	23.8±5.1	13.3±2.6	<0.001

Values in parentheses represent percentages. BMI = Body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; LDL = low-density lipoprotein; HDL = high-density lipoprotein.

Table 2. The polymorphic SNPs, genotype and allele frequencies of *ICAM1* and *AGT* genes in the patients with CSF and the control group

Gene/genotype frequency	Patients (n = 48)	Controls (n = 67)
ICAM1, n (%)		
EE	9 (18.8)	26 (38.8)
KE	27 (56.3)	33 (49.3)
KK	12 (25)	8 (11.9)
Allele frequency		
E	0.46	0.63
K[1]	0.54	0.37
AGT, n (%)		
TT	36 (75.0)	54 (80.6)
TM	11 (22.9)	12 (17.9)
MM	1 (2.1)	1 (1.5)
Allele frequency		
T	0.76	0.89
M[2]	0.24	0.1

[1] p value: 0.013, OR: 1.96 and CI (95%): 1.15–3.35.
[2] p value: 0.305, OR: 1.52 and CI (95%): 1.67–3.42.

Statistical Analysis

All statistical studies were carried out with the SPPS program (version 15.0, SPSS, Chicago, Ill., USA). All continuous variables were expressed with the mean ± standard deviation. All measurements were evaluated with the Kolmogorov-Smirnov test and the Shapiro-Wilk test, and a comparison of parametric and nonparametric values between the two groups was performed with the Mann-Whitney U test or Student's t test. Categorical variables (risk factors and polymorphisms) were analyzed using the χ^2 test. Risk estimations for the association of CSF with polymorphisms were calculated using ORs and 95% CIs by comparing genotypic combinations. $p < 0.05$ was accepted as statistically significant.

Results

The clinical and laboratory findings for the subjects are given in table 1. Baseline demographic parameters were similar in both groups. The TIMI frame counts for each epicardial artery were higher in patients with CSF (23.8 ± 5.1) than in control subjects (13.3 ± 2.6), and the difference was statistically significant ($p < 0.001$).

Genotype properties and allele frequencies are given in table 2. The K allele of *ICAM1* was significantly related to the possibility of CSF (p = 0.013, OR: 1.96, 95% CI: 1.15–3.35). The AGT M allele and CSF were slightly related but did not reach statistical significance. In 38 patients with hyperlipidemia, the K allele was significantly related to an increased risk of CSF (p = 0.028, OR: 2.73, 95% CI: 1.11–6.75).

Discussion

This study showed that the K allele frequency of *ICAM1* was higher in patients with CSF than in controls and that K469E polymorphism was associated with CSF. There was no association between AGT T207M polymorphism and CSF. These findings support the aspect of endothelial dysfunction that may be the underlying mechanism of CSF.

Inflammation and inflammatory factors are important mechanisms in the formation and progression of atherosclerosis [21]. One of these inflammatory factors, *ICAM1*, is a cell surface glycoprotein released from endothelial cells, macrophages, and lymphocytes *and* plays an important role in the development of atherosclerotic plaques because it mediates activation of endothelial cells, triggers inflammation, and causes transmigration and adhesion of leukocytes to vascular basal membranes [22]. Epidemiologic studies have shown the relationship between *ICAM1* levels and coronary artery disease [5, 23]. Fur-

thermore, previous studies demonstrated that *ICAM1* gene mutations affect serum levels and activity in the ICAM1 molecule [24, 25]. In a meta-analysis study, *ICAM1* K469E polymorphism was found to be associated with CVD [8]. A study showed that plasma nitric oxide levels were reduced and endothelial functions were impaired in CSF patients, thereby indicating that endothelial dysfunction could cause CSF [25]. In our study the K allele of the *ICAM1* gene increased the risk of CSF almost 2-fold, thereby supporting the idea that atherosclerotic properties and endothelial dysfunction might be important in pathogenesis of CSF.

AGT, a peptide synthesized in the liver, is one of the basic molecules in RAS; it plays a role in blood pressure regulation, electrolyte balance, and vascular remodeling [26]. Ag-II, an important by-product of RAS, stimulates the release of cytokine from small muscle cells and the release of adhesion molecules from endothelial cells [11]. The *AGT* gene is located at lq42–43 and consists of five exons [27]. *AGT* T207M refers to a threonine-to-methionine exchange in codon 207 in exon 2. Borecki et al. [12] reported that *AGT* gene polymorphism is related to increases in Ag-II levels and hypertension. Findings of the association between *AGT* gene polymorphisms and coronary artery disease are different in the literature. Although some studies reported that *AGT* T207M polymorphism was associated with coronary artery disease and hypertension [14, 28], others reported that there was no relation [29, 30]. They concluded that ethnicity of the study population, other genetic polymorphisms and the number of participants may explain the different results. Our study, which included Caucasian patients, showed that the risk of disease was 1.5 times higher but it is not significant. These results may be due to the small size of the study population.

The limitations of this study include the following: (1) the small sample size, (2) it did not examine whether inflammatory markers, including ICAM1, AGT, Ag-II, and C-reactive protein, were affected by polymorphisms and other cytokines, and (3) it did not perform intravascular ultrasonography on patients to determine the extent of intimal thickening and calcifications.

Conclusion

The present study showed that the well-known relationship of the *ICAM1* K allele to endothelial dysfunction and atherosclerosis was associated with CSF. Our findings further support the hypothesis that endothelial dysfunction may be a responsible mechanism for CSF pathogenesis. We recommend that more studies should be done on the etiopathogenesis of CSF to clarify the current findings.

Acknowledgements

Our study on genetic polymorphisms in CSF was supported by the Research Fund of the Çanakkale Onsekiz Mart University (project No. TSA-2013-99).

We would like to thank Sengul Turunz and Ebru Sik for their help with the laboratory analysis.

Disclosure Statement

All authors declare that they have no competing interests.

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