Carrier-state of two or three polymorphic variants of *MTHFR, IL-6* and *ICAM1* genes increases the risk of coronary artery disease

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Abstract

Background: Progression of coronary artery disease (CAD) depends on multiple genetic and environmental factors. Polymorphic variants of genes encoding proteins involved in endothelium dysfunction or proinflammatory state may genetically differentiate the human population and determine a susceptibility to the disease.

Aim: To find the relationship between single polymorphisms of *MTHFR, IL-6* and *ICAM1* genes, double or triple combinations among their polymorphic variants and CAD in a patient population from the Upper Silesia region.

Methods: The study population consisted of 177 patients with angiographically documented CAD and 202 blood donors with no signs of CAD. All examined individuals were white Polish Caucasians aged 18-55 years. The analysis of genetic polymorphisms was performed using the PCR-RFLP method.

Results: We observed a weak association between single gene polymorphism and the disease only in the case of the *MTHFR* T allele. We also found that the frequency of some double or triple combinations among analysed genes, especially for *MTHFR+ICAM1* and *MTHFR+ICAM1+IL-6* patterns, differentiated the entire patient group from controls (p=0.047 OR=1.75 and p=0.016 OR=1.75, respectively). These two combinations were also significantly more frequent in patients who were age-matched with controls, especially in the female subgroups (for *MTHFR+ICAM1* p=0.007, OR=10.32 and for *MTHFR+ ICAM1+IL-6*, p=0.005, OR=17.95 in females).

Conclusion: The present study showed that simultaneous carrier-state of *MTHFR, IL-6 and ICAM1* genes increased the risk of CAD.

Key words: coronary artery disease, polymorphisms, MTHFR, IL-6, ICAM-1

Kardiol Pol 2008; 66: 1269-1277

Introduction

Coronary atherosclerosis is a leading cause of coronary artery disease (CAD). Atherosclerotic lesions are characterised by local endothelial dysfunction as a response to a number of genetic and non-genetic factors [1]. The genetic susceptibility to CAD may depend on polymorphic genes, e.g., coding the adhesion proteins, homocysteine (Hcys) metabolising enzymes and cytokines. Increased homocysteine levels lead to chronic inflammatory state. Methylenetetrahydrofolate reductase (MTHFR) catalyses homocysteine remethylation into methionine. This enzyme takes part in reduction of 5,10-methylenetetrahydrofolate into 5-methyletetrahydrofolate, the predominant form of folates and carbon donor for the remethylation process. The common *MTHFR* 677C→T polymorphism increases thermal liability of the enzyme leading to its decreased activation [2], which is associated with elevated plasma Hcys levels. A meta-analysis including outcomes of 13 studies on the relation of *MTHFR* genotypes with Hcys levels showed that TT subjects had 25% higher Hcys levels than the ones with the CC genotype [3].

Adhesion proteins include the intercellular adhesion molecule-1 (ICAM-1, CD54), which is expressed on the surfaces of endothelial cells, smooth muscle cells and macrophages. Circulating adhesion molecules show solubility, allowing evaluation of their concentrations. Increased soluble ICAM-1 levels (sICAM-1) have been observed in patients with confirmed coronary and cerebral atherosclerosis [4, 5]. The 1405A→G *ICAM1* gene polymorphism may influence ICAM-1 affinity to its ligands and plasma protein levels [6].

Interleukin-6 (IL-6), a proinflammatory cytokine, stimulates synthesis of all acute phase proteins involved

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Received: 22 July 2008. **Accepted:** 01 October 2008.
The study was supported by the grant from Medical University of Silesia in Katowice, project number: NN-2-014/05 and NN-1-060/03.

in the inflammatory reaction, including C-reactive protein, fibrinogen and serum amyloid A [7]. IL-6 may promote atherosclerosis by inducing endothelial dysfunction, monocyte/macrophage recruitment, and extracellular matrix degradation. The –174G→C *IL-6* gene polymorphism provides a cAMP binding site and affects the effectiveness of transcription and plasma IL-6 levels [8].

In addition, there may be a functional interrelation between these genetic polymorphisms and their products. It has been reported that elevated Hcys levels are significantly correlated with IL-6 levels. However, this correlation was most potent in patients with TT genotype of 677C→T *MTHFR* polymorphism [9]. The 677T allele may also be significantly associated with adhesion molecule levels, particularly sVCAM [10]. Previous studies performed in the Polish population showed a trend to higher sICAM-1 levels in subjects with CC genotype of –174G→C *IL-6* polymorphism as compared to GG subjects [11]. Other reports suggested a synergistic relationship of –174G→C *IL-6* polymorphism with 1405A→G *ICAM1* polymorphism in patients with a family history of ischaemic stroke [12].

The aim of this study was to determine whether there is any potential relationship between a carrier-state of single and multiple proatherosclerotic variants of *MTHFR, IL-6* and *ICAM1* genes and IHD in a patient population from Upper Silesia region.

Methods

The study involved 379 Caucasian subjects living in the Upper Silesia region who were assigned to two groups: the study group and the control group.

Study group

The study group (CAD group) comprised 177 patients (58 females and 119 males) aged 25-55 years (mean 43.8±6.1 years) with CAD confirmed in coronary angiography (>50% stenosis of at least one main coronary artery). The patients were enrolled from 2000 to 2004 in the 1st Clinic of cardiology in the Silesian Center of Cardiology in Katowice. The coronary angiography was performed using Judkin's technique. Myocardial infarction (MI) was diagnosed according to the guidelines of the European Society of Cardiology [13]. The exclusion criteria were as follows: cardiomyopathy, collagenoses disease, coagulopathy and acute intoxication (e.g., carbon monoxide or amphetamine). Patients were also excluded when they had any chronic inflammatory or autoimmune disease such as asthma. Based on the medical history, all patients were characterised regarding CAD risk factors such as: smoking, hypertension, overweight/obesity, diabetes mellitus type 1 or 2 and family history of cardiovascular disease. Current smokers were patients smoking more than five cigarettes daily. Arterial hypertension was diagnosed if two independent measurements showed systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg. Body mass index (BMI) was calculated using the formula: BMI = body weight (kg)/height (m^2). The presence of risk factors related to the body weight in an individual patient was determined based on increased BMI, according to the standards of the Polish Cardiac Society: BMI <25 – normal weight, BMI ≥25 – <30 – overweight, and BMI ≥30 – obesity. Subjects with fasting blood glucose >7.0 mmol/l or taking oral anti-diabetic drugs or insulin were included in the diabetic patient group. Hypercholesterolaemia was diagnosed if serum total cholesterol (TC) was ≥5 mmol/l or if the study subject was treated with lipid-lowering agents.

The control group

The control group consisted of 202 healthy blood donors, who were free of CAD symptoms (48 females and 154 males), aged from 18 to 55 years (mean 35.4±10.4 years). Blood donors were randomly recruited from the Regional Blood Donor and Therapy Station from 2000 to 2003. The exclusion criteria were family history of MI, coronary artery disease or ischaemic stroke as stated in the questionnaire. Subjects enrolled in the control group did not undergo coronary angiography.

The study included only patients who gave their informed consent to participate. The study design was approved by the Ethics Committee (NN-013-297/II/02/05 and NN-6501-297/IV/02/06).

Biochemical analyses

The blood was collected for analyses after a fasting period from the antecubital vein. The TC, HDL cholesterol and triglyceride levels were measured in the fresh serum using commercially available enzymatic assays (Analco; Warsaw, Poland). The LDL cholesterol levels were calculated using the Friedewald formula [14].

Polymorphism analysis

All analysed polymorphisms were detected using PCR-RFLP with our own modifications of reaction conditions. DNA was isolated from blood lymphocytes using MasterPure Genomic DNA Purification Kit (Epicentre Technologies; Madison, WI, USA) following the procedure provided.

The 677C→T *MTHFR* gene polymorphism was detected using the above-described method [2] with the following PCR conditions: preliminary denaturation at 96°C over 5 min, 35 cycles including: denaturation at 93°C over 50 sec, annealing of primers at 60°C over 50 sec, elongation at 72°C – 30 sec and final elongation at 72°C over 7 min. The PCR product of 198 base pairs (bp) was digested with *Hinf*I restriction enzymes (Promega; Madison, WI, USA) producing specific fragments: 198 bp for CC genotype; 198, 175 and 23 bp for CT genotype and 175, 23 bp for TT genotype.

The 174G→C *IL-6* gene polymorphism was detected using a method described by Elghannam et al. [15]. The following amplification parameters were used: preliminary denaturation at 94°C over 1 min, 35 cycles: denaturation at 94°C over 30 sec, annealing of primers at 64°C over 30 sec, elongation at 72°C – 30 sec and final elongation at 72°C over 5 min. The amplification product of 305 bp was digested with *Hsp*92II enzyme (Promega; Madison, WI, USA, *Nla*III isoschizomer), resulting in fragments of 230 and 75 bp for GG genotype; 230, 121, 109 and 75 bp for GC genotype; and 121, 109 and 75 bp for CC genotype.

The 1405A→G *ICAM1* gene polymorphism was detected using the above-described method [16] with the following reaction conditions: preliminary denaturation at 96°C over 1 min, then 30 cycles including: denaturation at 96°C over 20 sec, annealing of primers at 64°C over 50 sec, and elongation at 72°C over 1 min. The reaction product of 226 bp was digested with *Bsh*1236I enzyme (Fermentas; Burlington, ON, Canada, *Bst*UI isoschizomer) obtaining the following fragments: 223 bp for AA genotype; 223, 139 and 87 bp for AG genotype and 139 and 87 bp for GG genotype.

Statistical analysis

The data analysis was performed using STATISTICA 6.0 (STATSOFT; Statistica, Tulsa, OK, USA) and EpiInfo-6 (Centers for Disease Control and Prevention [CDC], Atlanta, GA, USA). The results are presented as mean ± SD or numbers and percentages. The Shapiro-Wilk *W*-test was used to determine normal distribution of the quantitative data. The means of normally distributed data were compared with Student's t-test, while Mann-Whitney U-test was applied for parameters with other than normal distribution. The allele frequency was determined based on the genotype rates. Consistency of genotype distribution with Hardy-Weinberg equilibrium was evaluated with χ^2 test in all study groups. The genotype and allele frequencies were compared between the study groups using χ^2 test with Fisher's modification if the data count was below 10. The differences were significant if p was <0.05. The strength of association of individual alleles and genotypes with the disease was determined based on odds ratio (OR) within 95% confidence interval (CI). The relationship between the polymorphic variants and CAD was established based on univariate and multivariate logistic regression models with respect to traditional CAD risk factors, such as smoking, elevated TC, LDL cholesterol and triglyceride levels as well as overweight/obesity.

Results

General, biochemical and clinical characteristics of the study groups

Table I presents general and biochemical characteristics of the study groups. Due to significant differences regarding subjects' age between the two groups, age-matched subgroups were selected (n=142 in the CAD group and n=121 in the control group). In addition, gender-matched subgroups were selected. The CAD group had significantly higher TC, LDL cholesterol and triglyceride levels compared to the controls. The patient group was also characterised by higher BMI and number of smokers. Similar differences were found in the age-matched subgroups and in females. No differences in LDL cholesterol were observed in the male subgroups.

Table I. General and biochemical characteristics of the study groups

	CAD n=177	Controls n=202
Gender n (%)	♀ 58 (32.8) ♂119 (67.2)	♀ 48 (22.8) ♂154 (76.2)
Age [mean ± SD]	43.8±6.1*	35.4±10.4
Total cholesterol [mmol/l ± SD]	5.8±1.4*	5.2±1.4
LDL cholesterol [mmol/l ± SD]	3.9±1.2*	3.4±1.2
HDL cholesterol [mmol/l ± SD]	1.1±0.3	1.1±0.4
Triglycerides [mmol/l ± SD]	1.9±1.0*	1.4±0.7
BMI [mean ± SD]	26.8±4.3*	24.8±3.6
Smoking n (%)	100* (56.5)	47 (23.3)

*CAD – coronary artery disease, BMI – body mass index, SD – standard deviation*p <0.05 compared to the control group*

Table II shows the contribution of traditional risk factors in the presence of CAD. Multivariate regression analysis revealed that the most potent risk factor of CAD in the study group was smoking.

In the CAD group, 83.1% of patients had previous MI, including reinfarction in 14.2% of them. In almost 63% of patients the infarct-related artery was occluded, in 61.2% of subjects multivessel coronary artery disease was found. Arterial hypertension was present in 57.3% of the study patients. Conditions associated with IHD such as diabetes, ischaemic stroke and peripheral artery atherosclerosis occurred in a small number of patients (6.2%, 1.1% and 10.1% respectively).

Analysis of association of *MTHFR*, *IL-6* and *ICAM1* gene polymorphisms with CAD

Distributions of the genotype polymorphisms *MTHFR* 677C→T, *IL-6* -174G→C and *ICAM-1* 1405A→G were consistent with Hardy-Weinberg equilibrium. The carriers were heterozygous and homozygous patients with respect to the specific allele. The distribution of genotypes and alleles of *MTHFR*, *IL-6* and *ICAM1* genes is presented in Table III.

Analysis of single polymorphisms

Significantly higher frequency of single allele was found only for T allele of *MTHFR* polymorphism in the entire study group compared to the controls (p=0.047, OR=1.41 95% CI 0.99-1.89). The patients also had two-

Table II. Contribution of traditional risk factors in CAD development in the whole study groups and age- and gender-matched subgroups

Traditional risk factors OR, 95% CI, p	Whole study groups OR, 95% CI, p	Age-matched subgroups OR, 95% CI, p	Female subgroups OR, 95% CI, p	Male subgroups OR, 95% CI, p
Elevated TC [mmol/l]	1.38 (1.18-1.61), p <0.0001* 1.36 (1.13-1.64), p=0.0013**	1.33 (1.10-1.60), p=0.0027* 1.38 (1.10-1.73), p=0.0055**	2.80 (1.72-4.55), p=0.00003* NS**	1.22 (1.03-1.45), p=0.020* 2.55 (1.34-4.84), p=0.004**
Elevated LDL cholesterol [mmol/l]	1.36 (1.14-1.63), p=0.0006* NS**	1.35 (1.09-1.68), p=0.0052* NS**	3.32 (1.80-6.12), p=0.0001* 3.90 (1.75-8.68), p=0.0007**	NS* NS**
Elevated triglyceride [mmol/l]	1.94 (1.45-2.59), p <0.0001* NS**	1.79 (1.28-2.51), p=0.0007* NS**	3.69 (1.72-7.91), p=0.0007* NS**	1.78 (1.30-2.43), p=0.0003* NS**
Overweight or obesity [kg/m^2]	1.14 (1.08-1.21), p <0.0001* 1.16 (1.08-1.24), p <0.0001**	1.09 (1.02-1.16), p=0.014* 1.12 (1.03-1.21), p=0.0093**	1.26 (1.11-1.43), p=0.0003* NS**	1.13 (1.05-1.22), p=0.0009* 1.16 (1.07-1.27), p=0.0007**
Smoking (yes/no)	6.70 (4.15-10.82), p <0.00001* 7.70 (4.46-13.29), p <0.0001**	7.12 (3.98-12.71), p <0.00001* 8.81 (4.59-16.91), p <0.0001**	7.11 (2.67-18.93), p=0.00007* 12.57 (3.06-51.68), p=0.0003**	6.68(3.82-11.67), p<0.0001* 8.30 (4.30-16.01), p <0.0001**

** univariate analysis, ** multivariate analysis, OR – odds ratio, CI – confidence interval*

Table III. Frequencies of genotypes and alleles of *MTHFR, IL-6* and *ICAM1* genes in the whole study groups and age- and gender-matched subgroups

		Whole study groups		Age-matched subgroups		Female subgroups		Male subgroups	
Gene/genotypes and alleles		CAD n=177 (%)	Controls n=202 (%)	CAD n=142 (%)	Controls n=121 (%)	CAD n=58 (%)	Controls n=48 (%)	CAD n=119 (%)	Controls n=154 (%)
MTHFR	CC	75 (42.37)	103 (50.99)	58 (40.85)	59 (48.76)	23 (39.66)	25 (50.08)	52 (43.70)	78 (50.65)
	CT	85 (48.02)	89 (44.06)	69 (48.59)	56 (46.28)	29 (50.00)	21 (43.75)	56 (47.06)	68 (44.16)
	TT	17** (9.60)	10 (4.95)	15 (10.56)	6 (4.96)	6 (10.34)	2 (4.17)	11 (9.24)	8 (5.19)
	CT+TT	102 (57.63)	99 (49.01)	84 (59.15)	62 (51.24)	35 (60.34)	23 (47.92)	67 (56.30)	76 (49.35)
	C	235 (66.38)	295 (73.02)	185 (65.14)	174 (71.90)	75 (64.66)	71 (74.00)	160 (67.23)	224 (72.73)
	T	119* (33.62)	109 (26.98)	99 (34.86)	68 (28.10)	41 (35.34)	25 (26.00)	78 (32.77)	84 (27.27)
IL-6	GG	43 (24.29)	60 (29.70)	35 (24.65)	36 (29.75)	16 (27.59)	10 (20.83)	27 (22.69)	50 (32.47)
	GC	92 (51.98)	105 (51.98)	74 (52.11)	64 (52.89)	30 (51.72)	27 (56.25)	62 (52.10)	78 (50.65)
	CC	42 (23.73)	37 (18.32)	33 (23.24)	21 (17.36)	12 (20.69)	11 (22.92)	30**** (25.21)	26 (16.88)
	GC+CC	134 (75.71)	142 (70.30)	107 (75.35)	85 (70.25)	42 (72.41)	38 (79.17)	92 (77.31)	104 (67.53)
	G	178 (50.28)	225 (55.69)	144 (50.70)	136 (56.20)	62 (53.45)	47 (48.96)	116 (48.74)	178 (57.79)
	C	176 (49.72)	179 (44.31)	140 (49.30)	106 (43.80)	54 (46.55)	49 (51.04)	122* (51.26)	130 (42.21)
ICAM1	AA	53 (29.94)	72 (35.64)	43 (30.28)	45 (37.19)	20 (34.48)	19 (39.58)	33 (27.73)	53 (34.42)
	AG	113 (63.84)	122 (60.40)	92 (64.79)	70 (57.85)	33 (56.90)	29 (60.42)	80 (67.23)	93 (60.39)
	GG	11 (6.22)	8 (3.96)	7 (4.93)	6 (4.96)	5*** (8.62)	0 (0)	6 (5.04)	8 (5.19)
	AG+GG	124 (70.06)	130 (64.36)	99 (69.72)	76 (62.81)	38 (65.52)	29 (60.42)	86 (72.27)	101 (65.58)
	A	219 (61.86)	266 (65.84)	178 (62.68)	160 (66.12)	73 (62.93)	67 (69.79)	146 (61.34)	199 (64.61)
	G	135 (38.14)	138 (34.16)	106 (37.32)	82 (33.88)	43 (37.07)	29 (30.21)	92 (38.66)	109 (35.39)

significant difference compared to controls, ** significant difference (TT homozygotes vs. CC homozygotes), * significant difference (GG homozygotes vs. AA homozygotes), **** significant difference (CC homozygotes vs. GG homozygotes)*

Table IV. Carrier-state of several polymorphic variants differentiating CAD patients from controls

Genes (genotypes)	CAD n (%)	Controls n (%)	Univariate analysis OR (% CI), p	Multivariate analysis OR (% CI), p
		Whole study groups		
MTHFR+ICAM1 (CT+TT)+(AG+GG)	74 (41.81)	61 (30.20)	1.66 (1.06-2.59), 0.018*	1.75 (1.01-3.04), 0.047*
MTHFR+ICAM1+IL-6 (CT+TT)+(AG+GG)+(GC+CC)	58 (32.77)	44 (21.78)	1.75 (1.08-2.84), 0.016*	NS
		Age-matched subgroups		
MTHFR+ICAM1 (CT+TT)+(AG+GG)	61 (42.96)	36 (29.75)	1.78 (1.03-3.07), 0.027*	2.07 (1.06-4.05), 0.033*
MTHFR+ICAM1+IL-6 (CT+TT)+(AG+GG)+(GC+CC)	48 (33.80)	24 (19.83)	2.06 (1.13-3.78), 0.011*	2.12 (1.01-4.44), 0.045*

**p <0.05*

Table V. Carrier state of several polymorphic variants differentiating female subgroups

Genes (genotypes)	CAD n (%)	Controls n (%)	Univariate analysis OR (% CI), p	Multivariate analysis OR (% CI), p
MTHFR+IL-6 (CT+TT)+(GC+CC)	25 (43.10)	16 (33.33)	1.52 (0.64-3.62), 0.304	5.19 (1.02-26.38), 0.043*
MTHFR+ICAM1 (CT+TT)+(AG+GG)	25 (43.10)	12 (25.00)	2.27 (0.91-5.72), 0.052	10.32 (1.84-57.95), 0.007*
MTHFR+ICAM1+IL-6 (CT+TT)+(AG+GG)+(GC+CC)	19 (32.76)	8 (16.67)	2.44 (0.88-6.92), 0.058	17.95 (2.26-142.29), 0.005*

**p <0.05*

fold higher of TT homozygotes (in comparison to CC homozygotes) (p=0.043, OR=2.33 95% CI 0.95-5.85). No differences were found with respect to the distribution of genotypes and polymorphisms of *IL-6* and *ICAM1* alleles between the whole study groups. Moreover, there were no significant differences between the age-matched subgroups (Table III).

The analysis of gender-matched subgroups revealed that GG homozygous females with respect to *ICAM1* polymorphism as compared with females with 'wild' AA genotype were significantly more frequent in patients than in the control group (p=0.038, OR=1.95 95% CI 1.44-2.65). The C allele of *IL-6* polymorphism was significantly more often in the subgroup of healthy males than in blood donor males (p=0.035, OR=1.44 95% CI 1.01-2.05). The number of CC homozygous males compared to GG homozygotes was significantly higher in the patient group than in controls (p=0.033, OR=2.14 95% CI 1.00-4.60) (Table III).

Polygene analysis in the whole study group and age-matched subgroups

Due to lack or poor relationship of the single polymorphisms with CAD, we performed an analysis to determine whether there were any specific combinations of polymorphic variants of the analysed genes which allowed differentiation of patients with CAD from the controls. The frequencies of specific genetic patterns are shown in Table IV. Carriers of T allele of the *MTHFR* gene (subjects with CT+TT genotypes) and G allele of the *ICAM1* gene (subjects with AG+GG genotypes) were significantly more often in the patient group than in the controls (p=0.047, OR=1.75). It has also been documented that the rate of triple combination of *MTHFR+ICAM1+IL-6* variants differentiated the whole group of patients from the controls, although only in the univariate analysis (p=0.016, OR=1.75). The results for the whole group were confirmed in the age-matched subgroups. The simultaneous carrier state of *MTHFR* and *ICAM1* alleles as well as *MTHFR*, *ICAM1* and *IL-6* alleles was significantly more frequent in the age-matched patients (p=0.033, OR=2.07 and p=0.045, OR=2.12, respectively).

Polygene analysis in the gender-matched subgroups

Significant differences in the distribution of dual and triple combination of the carrier-state of the analysed polymorphisms were found only in females (Table V).

The *MTHFR+ICAM1* carrier females were more often in patients than in controls, similarly to *MTHFR+ICAM1+IL-6* female carriers. The multivariate logistic regression model

showed that these combinations may be considered independent risk factors of CAD in females (p=0.007, OR=10.32 and p=0.005, OR=17.95, respectively). The carrier-state of *MTHFR+ICAM1+IL-6* combination was even more strongly associated with CAD in females than smoking, higher TC level or BMI. Simultaneous carriers of T allele of *MTHFR* and C allele of *IL-6* are more frequent in female patients than in female blood donors (p=0.043, OR=5.19).

Discussion

Present study has indicated that T allele of 677C→T polymorphism of the *MTHFR* gene is more common in CAD patients than in controls, similarly to T allele carriers. Our previous studies carried out in a smaller group of patients (n=68) showed no correlation between T allele carriers and the presence of CAD [17]. In a group of 100 patients from the Szczecin area with a history of myocardial infarction (MI), also no correlation was found between 677C→T polymorphism and MI [18]. The number of TT homozygotes in that study was slightly lower (9%) compared to TT genotype rate in the control group (11%) [18]. In our study the percentage of TT homozygotes of the *MTHFR* gene in CAD patients (8.9%) was comparable to the rate reported by Goracy et al. [18] and the frequency observed in other studies [19]. A low number of TT genotype subjects may be supported by the hypothesis that TT genotype may be eliminated from the population due to increased infarct-related mortality [20]. A high percentage of TT homozygotes is present in Italian population with a relatively low prevalence of atherosclerosis as compared with other countries [21]. This may be explained by the Mediterranean diet, rich in folic acid, that compensates to some degree for the enzymatic defect of Hcys metabolism.

The 174G→C polymorphism of *IL-6* is associated with higher plasma interleukin-6 levels [8, 22, 23]. In the current study we have observed that C allele was slightly more often present in patients than in controls. The C allele frequency in our study (49.7%) remained similar to the previously reported [24]. We have found that CC homozygous males had higher risk of CAD compared to GG homozygous males. Our results confirmed the previously performed studies in a larger male group in which C allele carriers had higher risk of coronary artery disease compared to GG homozygotes. Smoking further increases this risk [24]. No relation between *IL-6* –174G→C polymorphism and CAD was observed in females. The lack of correlation most likely results from the influence of oestrogens on *IL-6* gene expression, which is peripherally controlled by 17-β-oestradiol in oestrogen-sensitive tissues. Therefore, negative feedback may occur in females, affecting the IL-6 levels [25]. There are also other studies available suggesting that the C allele of the *IL-6* gene is a risk factor of MI [26].

There were no significant differences in the distribution of genotypes and alleles of 1405A→G *ICAM1* polymorphism between patients and controls. We have only found a tendency to more common presence of G allele of the *ICAM1* gene in the entire group and subgroups of patients. The literature data indicate a relationship between 1405A→G polymorphism of *ICAM1* and the incidence of MI [27], stroke [28] and Graves' disease [29]. The GG genotype of *ICAM1* is also associated with a higher risk of peripheral artery atherosclerosis [30]. The A→G substitution determines change of lysine into glutamine acid at 469 protein site (K469E) in the fifth Ig-like domain. This domain is involved in binding the LFA-1 ligand of ICAM-1 which is expressed on leukocytes [31]. It has been shown that 1405A→G polymorphism of *ICAM1* correlated with protein levels in German children with asthma and may be considered as a predictor of asthma [6]. Recent reports also indicate a relationship of this polymorphism with plasma ICAM-1 levels in Chinese and European Americans [32].

Coronary atherosclerosis and CAD are multifactor diseases. The results of our analyses of single polymorphisms of *MTHFR*, *IL-6* and *ICAM1* genes with this condition as well as our previous studies [33-35] indicate that the input of a single genetic factor is rather weak. There is a high likelihood that a patient with atherosclerosis not linked to monogenetic familiar hypercholesterolaemia is a carrier of numerous alleles promoting atherosclerosis. Therefore simultaneous analyses of the relationship between carrier-state of various polymorphic variants and CAD may provide more reliable results. In this study we have noted that carriers of T allele of the *MTHFR* gene and G allele of the *ICAM1* gene were more often found in the patient group than among controls, as with carriers of triple combination of *MTHFR+IL-6+ICAM1*. The combination of *MTHFR+IL-6+ICAM1* was particularly strongly associated with disease in females, being more risky than smoking, elevated TC and overweight/obesity. However, due to the low number of participating females, the results require further confirmation on larger populations.

There are a few literature reports available on the coexistence of polymorphisms of several genes and atherosclerosis and CAD. The results of our previous studies carried out in smaller groups (n=146 in the CAD group and n=121 in the control group) showed that the carriers of three or four pro-atherosclerotic alleles of *ICAM1, APOE, PPARA* and *PAI-1* genes had an increased risk of CAD [33]. The other studies also documented that carrier state of four variants of *APOE, BChE, PPARγ2* and *ENOS* polymorphisms significantly increased the risk of early CAD as compared to the contribution of each gene alone [36].

Our study has some limitations. The number of participants in the study group was reletively low. The control group consisted of subjects free of family history

of CAD based on medical history. It was impossible to perform coronary angiography in the control group mainly due to ethical issues (lack of medical indications); therefore it cannot be excluded that some blood donors may suffer from CAD in the future. Regardless of these limitations the results of our studies allow us to state that simultaneous analysis of several genes may be useful in determining the genetic background of CAD in a specific population.

Conclusion

The current studies indicate that carrier state of several variants of *MTHFR*, *IL-6* and *ICAM1* genes increase the risk of coronary heart disease.

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Nosicielstwo dwóch lub trzech polimorficznych wariantów genów *MTHFR*, *IL-6* i *ICAM1* zwiększa ryzyko choroby niedokrwiennej serca

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Streszczenie

Wstęp: Miażdżyca naczyń wieńcowych stanowi główne podłoże choroby niedokrwiennej serca (ChNS). Genetyczną podatność na ChNS mogą kształtować polimorficzne geny, kodujące m.in. białka adhezyjne, enzymy zaangażowane w metabolizm homocysteiny (Hcys) lub cytokiny, które są wskaźnikami dysfunkcji śródbłonka i stanu prozapalnego. Reduktaza metylenotetrahydrofolianowa (MTHFR) jest enzymem biorącym udział w procesie remetylacji Hcys do metioniny. Hcys wywołuje stres oksydacyjny stymulujący wytwarzanie prozapalnych cytokin. Polimorfizm 677C>T genu *MTHFR* obniża aktywność enzymu, co wiąże się z podwyższonym poziomem Hcys w osoczu. Wewnątrzkomórkowa cząsteczka adhezyjna 1 (ICAM-1) ulega ekspresji na powierzchni komórek śródbłonka naczyniowego oraz komórek mięśni gładkich. ICAM-1 bierze udział w adhezji i migracji leukocytów przez barierę komórek śródbłonka. Polimorfizm *ICAM1* 1405A>G może wpływać na powinowactwo ICAM-1 do swoich ligandów oraz na poziom białka w osoczu. Interleukina 6 (IL-6) to cytokina, która stymuluje syntezę wszystkich białek ostrej fazy zaangażowanych w odpowiedź zapalną. IL-6 aktywnie promuje proces miażdżycowy przez indukowanie dysfunkcji śródbłonka naczyniowego, rekrutację monocytów/makrofagów oraz degradację macierzy zewnątrzkomórkowej. Polimorfizm -174G>C genu *IL-6* wpływa na efektywność transkrypcji oraz poziom IL-6 w osoczu. Polimorfizmy wymienionych genów oraz produkty przez nie kodowane mogą być ze sobą funkcjonalnie powiązane. Podwyższony poziom Hcys koreluje znacząco z poziomem IL-6, zwłaszcza u osób z genotypem TT genu *MTHFR*. U osób z genotypem CC genu *IL-6* istnieje tendencja do wyższego poziomu sICAM-1 niż u homozygot GG.

Cel: Wyjaśnienie, czy istnieją związki między nosicielstwem pojedynczych oraz wielu „promiażdżycowych" wariantów genów *MTHFR, IL-6* i *ICAM1* a ChNS w populacji pacjentów z Górnego Śląska.

Metody: Badaniami objęto 379 osób rasy kaukaskiej (wiek 18–55 lat), w tym: 177 osób z potwierdzoną koronarograficznie ChNS (z >50-procentowym zwężeniem przynajmniej jednego głównego naczynia wieńcowego) oraz 202 krwiodawców bez objawów ChNS. Polimorfizmy wykrywano techniką RFLP-PCR. Dane analizowano przy użyciu programów: STATISTICA 6.0 oraz EpiInfo-6.

Wyniki: Związek między pojedynczym polimorfizmem a ChNS zaobserwowano jedynie w przypadku polimorfizmu 677C>T genu *MTHFR*. Stwierdzono wyższą częstość występowania allela T genu *MTHFR* w całej grupie chorych w porównaniu z grupą kontrolną (33,6 *vs* 27,0%, p=0,047, OR=1,41). Dwukrotnie wyższa była również częstość homozygot TT (w porównaniu z homozygotami CC) wśród osób z ChNS niż w grupie kontrolnej (9,6 *vs* 4,9%, p=0,043, OR=2,33). Nie stwierdzono różnic w rozkładzie genotypów polimorfizmów genów *IL-6* i *ICAM1* między całymi badanymi grupami. Wykazano, że częstości niektórych podwójnych i potrójnej kombinacji analizowanych genów, szczególnie w przypadku wzorów genotypowych dla *MTHFR+ICAM1* i *MTHFR+ICAM1+IL-6*, różnicują całą grupę chorych od całej grupy kontrolnej (p=0,047, OR=1,75 i p=0,016, OR=1,75, odpowiednio). Wymienione kombinacje genotypowe są również znacząco częstsze w podgrupie pacjentów z ChNS zgodnych wiekowo z kontrolą. Kobiet będących nosicielkami kombinacji *MTHFR+ICAM1* było znamiennie więcej wśród chorych niż w kontroli (43 *vs* 25%), podobnie jak i nosicielek wzoru genotypowego *MTHFR+ICAM1+IL-6* (33 *vs* 17%). Wieloczynnikowy model regresji logistycznej wykazał, że wymienione kombinacje mogą być rozpatrywane jako niezależne czynniki ChNS u kobiet (p=0,007, OR=10,32 i p=0,005, OR=17,95, odpowiednio). Nosicielstwo kombinacji *MTHFR+ICAM1+IL-6* dowodzi nawet, że jest to silniejszy związek z chorobą u kobiet niż tradycyjne czynniki ryzyka ChNS, takie jak nikotynizm, podwyższone stężenie cholesterolu całkowitego (TC) lub wysoki wskaźnik masy ciała (BMI).

Wnioski: Badania wskazują, że jednoczesne nosicielstwo „promiażdżycowych" wariantów genów *MTHFR, IL-6* i *ICAM1* zwiększa ryzyko ChNS.

Słowa kluczowe: choroba niedokrwienna serca, polimorfizmy, MTHFR, IL-6, ICAM-1

Kardiol Pol 2008; 66: 1269-1277

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Praca wpłynęła: 22.07.2008. **Zaakceptowana do druku:** 01.10.2008.