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Low frequency haplotypes of E-selectin polymorphisms G2692A and C1901T give increased protection from coronary artery disease

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Background: E-selectin polymorphisms are an independent atherosclerosis and coronary artery disease (CAD) risk factor. This study aimed to investigate the link between the C1901T and G2692A E-selectin tagging SNPs and their haplotypes and the extent of coronary artery disease in Polish patients.

Material/Methods: For this study 321 patients were recruited CAD extent by coronary angiography and E selectin gene variant were investigated using HapMap, PCR/RFLP, multivariate logistic regression and haplotype analysis.

Results: Frequency distributions of the C1901T and G2692A polymorphisms were significantly different in CAD patients as compared to control subjects (p=0.037 and p=0.025, respectively). The C1901T polymorphism was found to be an independent genetic predictor of risk of CAD (OR=3.01) in a multivariate model adjusted for classic, environmental risk factors. The A-C and G-T haplotypes showed the strongest significant associations with CAD. The A-C haplotype proved to be significantly more common in controls (haplotype frequency 9.2%) than in CAD (5.7%, p=0.048); the G-T haplotype was not found among control subjects (0.0%) but was found in CAD (1.3%, p=0.0099).

Conclusions: Associations between the C1901T and G2692A E-selectin polymorphisms and CAD in the Polish population were found. Investigated variants correlated with the risk of coronary artery disease development but not with the extent of coronary artery vascular changes. In the haplotype analysis, 2 haplotypes influenced CAD – the A-C haplotype (7%) proved to exert a protective effect against CAD, while the effect of the less frequent G-T haplotype (1%) was associated with significant increase in CAD risk.

key words: coronary artery disease • E-selectin • gene polymorphism • arteriosclerosis

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Background

Cardiovascular diseases, including coronary artery disease (CAD), remain important mortality factors worldwide. Pathogenesis of CAD includes atherogenic processes with epithelial dysfunction and inflammation localized within the blood vessel wall [1,2]. Monocyte and leukocyte adhesion to the wall of activated blood vessel endothelium has proved to be one of the initial features of arteriosclerosis. This process is modulated and regulated by adhesion molecules including selectins, integrins, immunoglobulins and chemokines [3–5]. The family of selectins comprises -E, -P and -L molecules, which act in concert for the effective mediation of rolling and diapedesis, and are also involved in the promotion of atherogenesis [6]. E-selectin plays the key role in monocyte migration as a rolling mediating factor, and this phenomenon is the first step in endothelial monocyte adhesion and transmigration [7]. Increased expression of this selectin is observed under the influence of inflammatory mediators such as interleukin-1, TNF-α, LPS (Lipopolysaccharide), and is observed in tissue injury, but only low levels of this protein are constitutively secreted [7,8]. Development of arteriomatic lesions in the vessels might be promoted by this adhesion molecule [9,10].

Polymorphisms of E-selectin are currently considered to be an independent factor for atherosclerosis development, and an array of studies has identified multiple E-selectin gene variants associated with early CAD [11,12], ischemic cerebrovascular disease [13] and myocardial infarction [14]. It must be noted, however, that variability across the entire E-selectin gene and its association with coronary artery disease remain poorly studied thus far and previous studies have concentrated on 1 SNP, A561C (rs5361), which is associated with early arteriosclerosis and CAD development [15,16]. The mutation A561C modifies the secondary structure of E-selectin by exchanging amino acids S128R located in the EGF domain which plays a significant role in cell-cell interactions [17,18].

Additionally, it has been previously reported that other E-selectin polymorphisms modify the arteriosclerosis risk. The G98T variant was associated with early CAD [19] and C1839T was shown to significantly increase the speed of atherosclerotic plaque formation in carotid vessels in patients with end stage renal disease [20].

In our study, A561C did not perform as a tagging SNP. To our knowledge only 1 previous study has used similar techniques to identify 6 tagging SNPs [21] and also did not identify A561C as a tagging SNP. Two tagging SNPs identified by HapMap analysis were selected for further study (rs3917417 G2692A and rs3917454 C1901T). Both of them were in tight linkage disequilibrium with A561C (rs5361) and with each other (D'=1, r^2>0.3, according to HapMap).

These tagging SNPs C1901T and G2692A and reconstructed E-selectin haplotypes were investigated in a sample of Polish patients to determine the associations between these variants and coronary artery disease. The associations between the C1901T, G2692A and the extent of coronary vessel occlusion were also studied.

Material and Methods

The study group

Protocol of the study was approved by the ethics committee of Pomeranian Medical University, with formal informed consent signed by all participants.

Patients were randomly recruited from the inpatient Cardiology Department, Pomeranian Medical University, Szczecin, Poland, with 321 individuals presenting with history of CAD and stenocardiac symptoms screened for this study. Of these, 202 patients with ⩾50% occlusion of the coronary artery lumen in angiography comprised the CAD group; and the control group consisted of 119 subjects without changes in coronary arteries. Age range of the enrolled subjects was 36-84 years for the entire group (mean 56.5±9.2), males (73.2%, age range 36–77 years, mean 55.9±8.9), females (age range 37–84 years, mean 57.9±9.9). The study excluded patients with a history of myocardial infarction, diagnosed according to recommendations of the Joint European Society of Cardiology/America College of Cardiology Committee [22]. Some patients were excluded from the study with clinical diagnosis of cardiomyopathy, coagulopathy, collagenosis and chronic inflammatory disease. Full medical history included arterial hypertension defined as systolic blood pressure exceeding 140 mmHg, and/or diastolic blood pressure greater than 90mmHg, or reported history data showing hypertension. Body mass index (BMI) was calculated as weight/height^2 and obesity was defined as BMI higher than 25 kg/m^2. Patients were classified as "current smokers" if they reported a daily rate of more than 5 cigarettes.

Laboratory data on the lipid profile, serum total cholesterol (CH), triglyceride (TG), HDL and LDL levels were recorded with classical coronary angiography performed in all subjects. The levels of CH, TG, HDL and LDL were measured using enzymatic methods (commercial kit, Roche Diagnostic, Poland).

Coronary angiography was performed according to standard procedures using Philips INTEGRIS HM 3000 (Philips, Netherlands) and Philips ALURA (Philips, Netherlands) devices.

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes with the QIAamp DNA Mini Kit (Quiagen, Hilden, Germany). For the analysis of E selectin C1901T gene polymorphism, a PCR/RFLP method was designed based on the E selectin gene reference sequence (GeneBank accession number NM 000450), with the following primer pair: forward: 5' gCA gAT ggT gTC ATA Tgg CgA T, reverse: 5' CgC Agg gAC ACA gAA TTA CAg TTT A (TIB MOL BIOL, Poznań, Poland). Amplification was performed in volumes of 20 µl containing 40ng genomic DNA, 0.2 µl of each primer, 10 µl 2xPCR Master Mix (Fermentas, Vilnius, Lithuania) in standard conditions using a Mastercycler gradient machine (Eppendorf, Germany). The resulting product of 236 base pair (bp) length was digested with the restriction enzyme Pvu I (MBI Fermentas, Vilnius, Lithuania). For the C1901 allele, restriction fragments of 214 and 22 bp were obtained, while for the T1901 allele, the product remained

undigested. Digestion products were separated in a 3% agarose gel, stained with ethidium bromide and recorded with a DS-34 Polaroid Instant Camera (Polaroid, Germany) under UV light (Transilluminator 4000, Stratagene). E selectin G2692A gene polymorphism was analyzed using a Sanger sequencing method. The initial PCR step design was based on the E selectin gene reference sequence (GeneBank accession number NM 000450) using the following primers: forward: 5' AgA CAg TgC AgC ATT Agg gTT TTA, reverse: 5' TTC ACC CCT TTT CTT TTA TTC AA (TIB MOL BIOL, Berlin. Germany); the reaction was performed using standard conditions. Purification of the PCR product was performed using the GenElute PCR Clean-Up Kit (Sigma-Aldrich, Milwaukee, USA); for sequencing the BigDye Terminator v3.1 Cycle Sequencing kit (AppliedBiosystems, Foster City, USA) was used. Sequencing reaction products were purified using the BigDye XTerminator™ Purification Kit, Applied Biosystems and analyzed using an ABIPRISM 3100-Avant analyzer (AppliedBiosystems, Foster City, USA). Mutation detection was performed using Sequencing Analysis software v 5.1 provided by the manufacturer of the analyzer.

Statistical analysis

Clinical and laboratory parameters were compared using Student's t or χ^2 tests. Hardy-Weinberg equilibrium was evaluated for each polymorphism using Fischer's exact test. The association between a pair of analyzed *loci* (linkage disequilibrium, LD) was tested using a χ^2 test with the parameters D' and correlation coefficient r. Hardy-Weinberg equilibrium and LD were analyzed using the 'Genetics' package. Differences in genotype frequencies between groups were tested for statistical significance using a χ^2 test. Genotype frequencies between groups were then compared by logistic regression, non-adjusted (univariate) and adjusted (multivariate) for demographic and environmental factors. In haplotype analysis we used 'haplo.score' and 'haplo.glm' functions from the 'haplo.stats' package [23] to test the effect of haplotypes. A positive Hap.Score value implies that the haplotype occurs more frequently in the CAD (or CAD_{2+3}) groups than control subjects (or CAD_1), whereas a negative Hap.Score indicates that the haplotype occurs more frequently in control subjects/CAD_1. We also checked for possible selectin haplotype interactions with environmental covariates (gene – environment interactions). Only two-way interactions were tested and only for those covariates that were significant in the main GLM model (without interactions). 'Genetics' and 'haplo.stats' are packages for R – a free software environment for statistical analysis (ver. 2.11.1, R Foundation for Statistical Computing, Vienna, Austria, *http://www.R-project.org*). The remaining calculations were performed using the STATISTICA 8.0 program for Windows. P<0.05 was considered statistically significant.

RESULTS

Clinical and laboratory parameters in the patient and control group

Study participants were divided into the following groups: control group (controls) consisted of 119 subjects: 69 males (57.98%) and 50 females (42.02%), age range 36–75 years, mean 55.3±9.5. The coronary artery disease (CAD) group included 202 subjects with coronary artery occlusions: 166 males (82.18%), 36 females (17.82%), age range 38–84 years, mean 57.2±9.0 and was subdivided into the CAD_1 subgroup (79 individuals with single vessel occlusion: 64 males (81.01%) and 15 females (18.99%) aged from 38 to 79 years, mean 54.9±8.0, and the CAD_{2+3} subgroup which included 123 individuals with 2 or 3 significant occlusions in the coronary vessels: 102 males (82.93%) and 21 females (17.07%), aged 41–84 years, mean 58.7±9.4.

Baseline characteristics of patients and control subjects are shown in Table 1. Differences between patients with single (CAD_1) and multivessel (CAD_{2+3}) disease are also presented in this table. Frequency of male gender and BMI proved to be significantly higher in the CAD group when compared to controls (p=0.0001 and p=0.02, respectively). Similarly, smoking and diabetes were more common among CAD patients than controls (p=0.007 and p=0.02, respectively). Lipid profile tests revealed that serum HDL was notably lower in the CAD group (p=0.00004). LDL, triglyceride levels and total cholesterol levels did not differ significantly between the CAD and controls. The extent of atherosclerosis in the coronary vessels was also investigated. A significant difference in the clinical and biochemical characteristics between patients with single and multivessel atherosclerosis was noted only for diabetes (Table 1).

E-selectin genotype distribution

E-selectin gene C1901T and G2692A polymorphism were found to be in Hardy-Weinberg equilibrium both in CAD group and controls. Next, the *loci* were examined for the level of LD in the entire group (n=321). The estimated values of D' and r were 0.78 and 0.48, respectively (p<0.001). Genotype counts and frequencies were computed in CAD patients, control subjects and individuals classified into CAD_1 and CAD_{2+3} groups. For the C1901T only 2 E-selectin genotypes, CC and CT, were observed.

Frequency distribution of the C1901T and G2692A polymorphisms were significantly different in CAD patients as compared to control subjects (p=0.037 and p=0.025, respectively). For C1901T in the CAD group: CC 180 (89.1%), CT 22 (10.9%), in control subjects: CC 114 (95.8%), CT 5 (4.2%). For G2692A in CAD group: GG 162 (80.2%), GA 40 (19.8%) AA 0 (0%) in control subjects: GG 96 (80.7%), GA 19 (16.0%) AA 4 (3.4%).

Lastly, the C1901T and G2692A genotype and allele frequencies did not differ significantly between CAD_1 and CAD_{2+3} groups (p=0.518 and p=0.897, respectively). For C1901T in the CAD_{2+3} group: CC 111 (90.2%), CT 12 (9.8%) in the CAD_1 group: CC 69 (87.3%), CT 10 (12.7%). For G2692A in the CAD_{2+3} group: GG 99 (80.5%), GA 24 (19.5%), AA 0 (0%) in the CAD_1 group: GG 63 (79.8%), GA 16 (20.2%), AA 0 (0%).

Univariate and multivariate logistic regression analyses

Table 2 presents univariate and multivariate analyses of CAD. CAD severity and calculated odds ratios with confidence intervals (ORs, 95% CIs) for the 2 polymorphisms evaluated in the study. For G2692A, different modes of inheritance additive, dominant, or recessive were investigated.

The C1901T polymorphism was found to be an independent genetic predictor of risk of CAD (OR=3.01) in the

Table 1. Baseline clinical and biochemical characteristics of patients with coronary artery disease (CAD), advanced coronary artery disease (CAD_{2+3}) and control subjects (Controls).

	Controls (n=119)	CAD (n=202)	p CAD *vs.* controls	CAD_{2+3}	CAD_1	p CAD_{2+3} *vs.* CAD_1
Gender (Male/Female)	69/50	166/36	0.0001	102/21	64/15	0.729
BMI (kg/m ^{2})	26.7±4.1	27.7±3.6	0.020	27.7±3.6	27.7±3.7	0.993
Smoking status (Yes/No)	27/92	75/127	0.007	46/77	29/50	0.921
Diabetes (Yes/No)	11/108	37/165	0.020	28/95	9/70	0.041
Hypertension (Yes/No)	60/59	124/78	0.055	81/42	43/36	0.104
Triglycerides (mg/dl)	161±87	177±79	0.100	174±67	181±96	0.503
Cholesterol (mg/dl)	219±37	225±41	0.240	227±42	222±40	0.452
HDL (mg/dl)	49±13	43±9	0.00004	42±9	43±9	0.324
LDL (mg/dl)	131±29	138±33	0.100	140±35	134±31	0.292

Table 2. Estimated effects for E-selectin C1901T and G2692A variants in univariate and multivariate analyses of CAD and CAD severity.

			CAD *vs.* Controls			CAD_{2+3} *vs.* CAD_1		
			OR	95%CI	p	OR	95%CI	p
C1901T	Univariate		2.79	1.02–7.60	0.044	0.75	0.30–1.83	0.519
CT *vs.* CC	Multivariate *		3.01	1.05–8.65	0.040	0.72	0.28–1.90	0.510
G2692A	Univariate	Dominant	1.03	0.58–1.83	0.917	0.95	0.47–1.94	0.897
		Recessive	–	–	–	–	–	–
		Additive	0.86	0.51–1.44	0.567	0.95	0.47–1.94	0.897
	Multivariate*	Dominant	0.91	0.49–1.68	0.751	0.94	0.44–2.00	0.876
		Recessive	–	–	–	–	–	–
		Additive	0.75	0.43–1.30	0.303	0.94	0.44–2.00	0.876

* With an adjustment for age, gender, BMI, smoking, diabetes mellitus and arterial hypertension; Dominant AA+ GA *vs.* GG; Recessive AA *vs.* GA+GG; Additive AA *vs.* GA *vs.* GG; CAD – coronary artery disease, CAD_{2+3} – CAD with two or three significant occlusions, CAD_1 – single vessel disease.

multivariate model adjusted for "classic", environmental risk factors: age, sex, BMI, smoking, diabetes mellitus and arterial hypertension. However, none of the studied polymorphisms in any pattern of inheritance was identified as a risk factor for advanced, multivessel coronary artery disease.

E-selectin haplotype analysis

The association of selectin gene haplotypes with CAD and CAD severity were evaluated by score tests (global score and haplotype-specific statistics) adjusted for environmental risk factors (Tables 3, 4). Four haplotypes represented 2 alleles at each of the 2 *loci* G2692A and C1901T: A-C, G-C, A-T and G-T. The haplotype G-C was markedly more frequent than the others (estimated frequency, 0.88728) while the G-T haplotype had an estimated frequency below 1% (0.00836). The models were constructed using selectin gene haplotypes and the following covariates of CAD risk factor: age, sex, BMI, smoking, diabetes mellitus and arterial hypertension. The A-C and G-T haplotypes showed the strongest (hap. score –1.97 and 2.58, respectively) and significant association with CAD. The A-C haplotype proved to be significantly more common in controls (5.7% *vs.* 9.2%, p=0.048); the G-T haplotype was not found among control subjects (haplotype frequency of 1.3% *vs.* 0.0%, p=0.0099). The global statistic score was 11.39 and the p value was 0.0098, showing a significant difference in overall selectin gene haplotype profile between CAD and control subjects. The severity of CAD was not associated with E-selectin haplotypes, neither in the global score test (global score statistics was 1.58, p= 0.6643), nor in the individual haplotype score tests.

Model with gene-environment interactions

No significant two-way interactions between selectin gene haplotypes and each significant non-genetic factor (age, sex, smoking) were observed, either in the model with CAD, nor in the model with CAD severity (CAD_{2+3} *vs.* CAD_1) (Table 5).

Table 3. Haplotype frequency estimates of the selectin gene and score tests after adjustment for conventional CAD risk factors (CAD *vs.* controls).

G2692A	C1901T	Haplotype frequency			Hap.Score	p
		CAD+Controls	CAD	Controls		
A	C	0.07067	0.05779	0.09244	–1.97457	0.048
G	C	0.88728	0.88775	0.88655	0.21381	0.830
A	T	0.03369	0.04122	0.02101	1.10908	0.267
G	T	0.00836	0.01324	0.00000	2.57617	0.009
Global score statistics 11.39, d. f.=3, p=0.0098						

With an adjustment for age, gender, BMI, smoking, diabetes mellitus and arterial hypertension; CAD – coronary artery disease.

Table 4. Haplotype frequency estimates of the selectin gene and score tests after adjustment for conventional CAD risk factors (CAD_{2+3} *vs.* CAD_1).

G2692A	C1901T	Haplotype frequency			Hap.Score	p
		$CAD_{2+3}CAD_1$	CAD_{2+3}	CAD_1		
A	T	0.04122	0.03567	0.04985	–0.90006	0.368
G	C	0.88775	0.88933	0.88530	–0.20159	0.840
G	T	0.01324	0.01311	0.01344	0.55110	0.581
A	C	0.05779	0.06189	0.05141	0.73595	0.461
Global score statistics 1.58, d. f.=3, p=0.6643						

With an adjustment for age, gender, BMI, smoking, diabetes mellitus and arterial hypertension; CAD – coronary artery disease, CAD_{2+3} – CAD with two or three significant occlusions, CAD_1 – single vessel disease.

Table 5. Two-way interactions and regression parameters including non-genetic factors and haplotypes of the selectin E gene.

Variables	Coef.	S.E.	OR (95%CI)	p
CAD *vs.* controls				
Age* x haplotypes				
Age x AC (0.0706)	–0.0349	0.0397	0.97 (0.89–1.04)	0.379
Gender* x haplotypes				
Gender x AC (0.0706)	–0.1720	0.8190	0.84 (0.17–4.19)	0.834
Smoking* x haplotypes				
Smoking x AC (0.0706)	1.4550	0.8970	4.28 (0.74–24.84)	0.105
CAD2+3 *vs.* CAD1				
Age* x haplotypes				
Age x AC (0.0578)	–0.1123	0.0643	0.89 (0.79–1.01)	0.082

* Significant based on haplo.glm main model (without interactions); Coef. – regression coefficient, S.E. – standard error. CAD – coronary artery disease, CAD_{2+3} – CAD with two or three significant occlusions, CAD_1 – single vessel disease.

Discussion

Additional factors regulating and mediating inflammatory processes within the arterial vessel wall have remained in the research spotlight and are regarded as factors of primary importance in the promotion of arteriosclerosis (eg, adhesion molecules, including E-selectin which stimulates leukocyte rolling and early atherogenic plaque formation) [24–26]. Genetic variants influencing expression of this selectin might significantly alter disease progression [15,27].

In this study, data on the association between the C1901T and the G2692A E-selectin gene polymorphisms and risk of coronary artery disease and its severity in the Polish population is presented. The study group included patients with only a single type of vascular disorder, namely coronary artery disease, in order to maximize the sample's homogeneity, which is desirable in terms of increasing the strength of association.

It must be noted that for the E-selectin gene, only functional variants have been studied previously (e.g., the A561C). In the Saudi population, an association between A561C polymorphism and CAD was confirmed, while in European Caucasians an increase in early atherosclerosis risk was described [11,12]. A discordant result was obtained by Hamid et al in the Egyptian population, with no association between the E-selectin A561C variant and CAD observed [28]. However the study group was very small, which might have influenced final conclusions. The estimated values of D' and r in our group were 0.78 and 0.48, respectively, contrasting with the 1 and 0.36 values obtained from HapMap. The fact that our study has managed to dissect out a low frequency haplotype with a protective association with CAD, as well as another giving increased risk, gives some justification to choosing these tagging SNPs, and this could be contrasted with simple analyses of 1 polymorphism (e.g., A561C) in which only 1 increased risk allele was identified.

Both E-selectin polymorphisms studied here are non-functional variants which do not alter amino-acid sequence and might be used as markers for genetic variability in this region. However, the major haplotype GC is in fairly tight linkage with the A561C variant, which was shown to facilitate leukocyte adherence to activated endothelium, especially in atherosclerotic vessels, and might also be associated with the protein conformational changes influencing and modulating the inflammatory response.

In our present study we found significant differences in genotype frequencies of T1901C and G2692A polymorphisms of the E-selectin gene between CAD and healthy subjects (p=0.037 and p=0.025, respectively) but not between CAD_{2+3} and CAD_1 groups. The frequency of the CT genotype in patients with coronary vessel occlusions was around 2.5 times higher than in individuals without lesions in the coronary artery wall. Moreover, this polymorphism retained its significance after adjustment for the well-known demographic and clinical factors (e.g., age, sex, BMI, smoking, diabetes mellitus, arterial hypertension) of CAD. Endler et al. [29] failed to demonstrate an association between A561C polymorphism and CAD in patients with diabetes mellitus type 2. In our group, the T1901C polymorphism, linked with A561C, remained significant even after control for various factors including diabetes mellitus type 2.

As for the G2692A, although the distribution of genotype frequencies were statistically different among CAD and controls – GA (19.8% *vs.* 16.0%) and AA (0% *vs.* 3.4%) – no significant differences in terms of allele distribution were noted (data not shown).

It must also be observed that when the extent of the disease is considered, the influence of E-selectin genetic variant became insignificant. Neither in univariate nor multivariate analysis was a notable difference in investigated E-selectin genotypes detected between patients with multivessel and single vessel lesions. This might suggest that the degree of coronary vessel occlusion is promoted in a different way, with environmental factors possibly being stronger than the genetic influence.

Since only single variants have been studied so far, this paper is, to our best knowledge, the first attempt to estimate the effect of E-selectin haplotypes on the risk of CAD and its severity. Such an approach has been adopted before with other genes – previous reports have described an association between angiotensinogen gene haplotypes and CAD in the female population [30]. A haplotype-based approach can significantly improve the power of association-mapping studies as compared to single locus tests, and this approach can be more informative than a single point analysis, even if the phase information is recovered by statistical methods [31]. We used a method of testing the statistical association between haplotypes and the trait that allows adjustment for nongenetic covariates, which are of critical importance when analyzing such genetically complex traits as coronary artery disease [32]. In the haplotype analysis, the A-C haplotype proved to exert a protective effect against CAD, while the effect of the less frequent G-T haplotype was associated with significant increase in the risk of CAD. It must be emphasized that the haplotypes showing a significant effect on CAD were relatively rare A-C with a frequency of 7% and the G-T haplotype with a frequency below 1% of the whole group. Therefore, further studies with more SNPs could be used to cover a wider range of E-selectin gene diversity, and the negative and/or protective impact of E-selectin gene variability on CAD could be confirmed.

The frequency of classical risk factors such as arterial hypertension, smoking, diabetes and unfavorable lipid profile differed significantly between CAD individuals and controls, which confirms their influence for this group. Despite the development of public health programs aimed at lifestyle changes, "classical" factors remain common in the Polish population, with a possibility that combined genetic and environmental pressures exert a strong effect in promoting atherosclerotic processes. The inclusion of gene and environment interaction in association analyses may further improve the power to detect genetic effects, and may contribute to the identification of important environmental effect modifiers [32]. Therefore, we also assessed whether the level of interaction between E-selectin haplotypes and environmental factors such as age, sex, BMI, smoking and arterial hypertension could contribute to coronary vessel lesions. We found no significant interactions between CAD and selectin haplotypes.

Our study has the limitation that it applies only to the Polish population, and further studies from other geographical location would be needed to extend its applicability.

Conclusions

In this study, associations between the 2 E-selectin polymorphisms (C1901T and G2692A), its haplotypes and CAD in the Polish population were found. Variants studied here correlated with the risk of coronary artery disease development but not to the extent of coronary artery vascular

changes. Additionally, using multivariable analysis E-selectin haplotypes, both beneficial and negative E- selectin haplotypes are reported.

Conflict of interest

The authors declare no conflict of interest with respect to this research.

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