E-selectin gene haplotypes are associated with the risk of myocardial infarction

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Abstract

Introduction: Endothelial dysfunction is one of the most important factors implicated in the pathogenesis of coronary artery disease (CAD). The aim of this study was to investigate the association of the E-selectin gene (*SELE*) with CAD and CAD-related traits using tagging polymorphisms.
Material and methods: A total of 379 Polish patients who had undergone angiography were included: 261 patients with angiographically documented CAD, 202 CAD patients without myocardial infarction (CAD/MI(–) group) and 59 patients with myocardial infarction (CAD/MI(+) group) as well as 118 healthy control subjects (non-CAD). Eight tagging single nucleotide polymorphisms (SNPs) in the *SELE* gene were selected using genotype data from Hap-Map. Genotyping was performed using PCR-RFLP and PCR-DHPLC methods.
Results: The most common *SELE* haplotype in this analysis ([C;G;T;C;G;T], 31.2%) showed a negative association with myocardial infarction (MI) (CAD/MI(+) vs. non-CAD) under the additive ($p = 0.001$), dominant ($p = 0.006$) and recessive ($p = 0.012$) model. Two other haplotypes ([C;G;C;C;A;C], [C;A;C;A;G;T], 5.73% and 18.1%, respectively) were also negatively associated with MI under the additive and dominant model. We also found two haplotypes ([T;G;T;C;G;T], [C;G;C;C;A;T], 1.52% and 6.71%, respectively) associated with the risk for MI (CAD/MI(+) vs. CAD/MI(–)), acting in both additive ($p = 0.04$, $p = 0.007$, respectively) and dominant ($p = 0.04$, $p = 0.004$, respectively) manner. There was no association with either CAD/MI(–) or with severity of CAD expressed as the number of vessels involved.
Conclusions: Our results suggest that *SELE* is one of the independent genetic factors modifying the risk of myocardial infarction.

Key words: coronary artery disease, E-selectin gene, haplotypes, myocardial infarction, tagging single nucleotide polymorphisms.

Introduction

Cardiovascular diseases, including coronary artery disease (CAD) and myocardial infarction (MI), remain important mortality and morbidity factors worldwide. Coronary artery disease is one with a complicated background, resulting from traditional and genetic risk factors and their interaction [1–4]. Endothelial dysfunction is the most important factor in the pathogenesis of CAD. Leukocyte adhesion to the wall of the activated

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J. Gorący, M. Kaczmarczyk, A. Ciechanowicz, K. Safranow, J. Gorący, K. Jakubowska, D. Chlubek, I. Gorący

blood vessel endothelium has proved to be one of the initial features of arteriosclerosis. Proinflammatory adhesion molecules such as selectins, integrins, immunoglobulins and chemokines modulate this process [5]. Leukocytes are captured from the bloodstream and then rolled along the endothelial cell surface. E-selectin is expressed on the activated endothelium and plays the main role in monocyte migration as a rolling mediating factor. It is the first stage for endothelial monocyte and leukocyte adhesion and transmigration [6, 7].

The E-selectin gene (*SELE*) is located on chromosome 1q22-q25. Genetic polymorphisms at the *SELE* locus may regulate the gene expression levels and affect the biological function of the protein [8, 9]. The *SELE* gene was determined with many single nucleotide polymorphisms and several polymorphisms of the E-selectin gene have been mentioned to contribute to CAD, hypertension and ischemic cerebrovascular disease [10–12]. Most studies have extensively tested two polymorphisms in particular, A561C (rs5361) and G98T (rs1805193), which have been implicated in susceptibility to CAD [13, 14]. These functional variants seem to play an important role in modifying the secondary structure of E-selectin by exchanging amino acids and regulating cell-cell interactions [15, 16]. Other variations of *SELE* may also be associated with atherosclerosis or hypertension [17, 18]. We previously showed that the low frequency haplotypes of the E-selectin gene may reduce susceptibility to coronary artery diseases in the Polish population [19]. Although there are many reports confirming a correlation between genetic alterations of *SELE* and inter-individual variation in the soluble E-selectin (sE-selectin), the relationship between the two still remains controversial [20, 21].

Moreover, it has been established that *SELE* gene polymorphism, which predisposes to chronic inflammation development/progression, can affect the conditions with underlying inflammatory disorders [22]. According to *in vivo* studies, E-selectin expression can be elevated continuously as a result of chronic localized inflammation [23], whereas without coexisting inflammation, expression of E-selectin remains at negligible concentrations or it is indeterminable [24, 25]. The pivotal role in the inflammatory process and endothelial function indicate that the *SELE* gene is a plausible candidate in CAD development. It must be noted, however, that variability across the E-selectin gene and its association with coronary artery disease remain poorly investigated and the previous studies have mostly concentrated on single gene variants.

Therefore, the purpose of this study was to investigate the association between *SELE* haplotype tagging polymorphisms and coronary artery disease with or without a history of MI, as well as severity of CAD based on the number of coronary vessels involved in a Polish angiographically characterized population.

Material and methods

The study was performed in a cohort of Polish subjects (*n* = 379) recruited randomly from the in-patient Cardiology Department, Pomeranian Medical University, Szczecin, Poland, including 261 patients with angiographically documented coronary artery disease (CAD) defined as ≥ 50% stenosis of at least one major coronary artery. There were 59 patients with a history of myocardial infarction (CAD/MI(+) group), diagnosed according to recommendations of the Joint European Society of Cardiology/American College of Cardiology Committee. The CAD patients without a history of myocardial infarction (CAD/MI(–), *n* = 202) were further divided into multi-vessel CAD (MCAD, *n* = 123) and single-vessel CAD (SCAD, *n* = 79) disease. The control group included 118 participants without CAD confirmed by coronary angiography (non-CAD group). Patients with clinical diagnosis of cardiomyopathy, coagulopathy, collagenosis and chronic inflammatory disease were excluded from the study. The protocol of the study was approved by the Ethics Committee of the Pomeranian Medical University, with formal informed consent signed by all the participants.

Demographic data were collected during the clinical trial. Hypertension, diabetes and smoking addiction were defined. Routine biochemical blood analyses were done including total cholesterol (TCH), triacylglycerol (TG), HDL cholesterol (HDL-C) and LDL cholesterol (LDL-C). Coronary angiography was performed according to standard procedures using Philips INTEGRIS HM 3000 (Philips, Netherlands) and Philips ALURA (Philips, Netherlands) devices. Significant stenosis was defined based on American Heart Association criteria as ≥ 50% stenosis of the coronary artery lumen.

The genotype data dump file for the CEU (Utah residents with northern and western European ancestry) population (chromosome 1: 166,423,439 – 166,434,836, HapMap release 21, NCBI Human Genome Build 35) was downloaded from the HapMap Genome Browser. The selection of tagging single nucleotide polymorphisms (SNPs) was conducted on HapMap data spanning a region of 11 kb using Haploview's Tagger in the pairwise mode, r^2 threshold of 0.8 and the minimum allele frequency of 5%. Eight markers were selected out of 30 SNPs by the tagging algorithm: C1901T (rs3917454, NM_000450.2:c.529+123C>T), A2252G (rs3917412, NM_000450.2:c.529+427A>G), A2692G (rs3917417, NM_000450.2:c.530-304G>A), C2935T (rs3917419,

NM_000450.2:c.530-61C>T), A3929C (rs1534904, NM_000450.2:c.716-11A>C), G5310A (rs1076637, NM_000450.2:c.1091-57G>A), T7836C (rs5356, NM_000450.2:c.1800T>C) and C8304T (rs3917438, NM_000450.2:c.*16-200C>T). Genomic DNA was extracted from peripheral blood leukocytes using the QIAamp DNA Mini Kit (QIAGEN, Germany). Seven tagging SNPs were genotyped using a polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) method (Table I). The products of digestion were separated in 3% agarose gels. The rs3917417 was genotyped using PCR-DHPLC (with primers 5′-AgACAgATgTgCAgcCATT-AgggTTTTA and 5′-TTCTACCCCTTTTCTTTTATTCAA). DHPLC analysis was performed using a Hewlett-Packard 1050/1100 chromatographic system (Agilent, Waldbronn, Germany).

Statistical analysis

Quantitative data (age, serum lipids) were presented as the mean and standard deviation or median and interquartile range depending on the distribution, which was assessed using the Kolmogorov-Smirnov test. Demographic, clinical and biochemical characteristics of patients were compared using the t-test or χ^2 test (Statistica, Dell Inc. (2016), version 13, software.dell.com). Agreement with Hardy-Weinberg expectations was checked using the χ^2 test with 1 degree of freedom. The haplotype analysis was conducted using the haplo.stats package in R (https://cran.r-project.org). The haplo.score function was used to compute both omnibus and haplotype-specific score statistics to test the association between *SELE* haplotypes and binary traits allowing for adjustment for traditional risk factors. As there was a correlation between serum lipids, only triacylglycerol (logarithm) was used as a covariate (in addition to other covariates).

Only haplotypes with estimated frequency of at least 1% were considered. Three haplotype effects were examined: additive (the number of copies of a particular haplotype), dominant (homozygotes and heterozygotes were assumed to have the same effect), recessive (only homozygotes for a particular haplotype have an effect). A $p < 0.05$ was considered significant.

Results

The clinical and biochemical characteristics of the CAD ($n = 261$) and non-CAD patients ($n = 118$) are summarized in Table II. Two tag SNPs, c.530-304G>A and c.*16-200C>T, deviated significantly from HWE expectations in the non-CAD group and were excluded from further analyses (Table III). The results from the association analysis between the 6-marker *SELE* haplotypes (NM_000450.2:c.[529+123C>T; 529+474A>G; 530-61C>T; 716-11A>C; 1091-57G>A; 1800T>C]) and the CAD-related traits are presented in Tables IV–VIII. There were no differences in the *SELE* haplotype frequencies in the CAD group and in the CAD/MI(–) group as compared with non-CAD group (Tables VI and VII). Also, there was no association of the *SELE* haplotypes with severity of CAD in the CAD/MI(–) group expressed as the number of vessels involved (Table VIII).

We found an association of the *SELE* haplotypes with a history of myocardial infarction among CAD patients (CAD/MI(+) vs. non-CAD, CAD/MI(+) vs. CAD/MI(–) (Tables IV and V). Thirty-five haplotypes were reconstructed in 59 CAD/MI(+) and 118 non-CAD patients. Of these, 6 haplotypes had frequencies greater than 5% (accounting for 80.1%), whereas 10 inferred haplotypes had frequencies greater than 1% (87.6%) (Table IV). Among those 10 haplotypes with frequencies greater than 1%, three haplotypes ([C;G;T;C;G;T], [C;G;C;C;A;C],

Table I. Characteristics of primers, restriction enzymes and products (restriction fragments)

SNP	Primers	Restriction enzyme	Products [bp]
rs3917454	5′gCA gAT ggT gTC ATA Tgg CgA T 5′CgC Agg Gac ACA gAA TTA CAg TTT A	Pvu I	C: 214, 22 T: 236
rs3917412	5′AAg ACg TTg Tag AA TgA gT 5′TTg CAg gCT ggA ATA ggA g	Nmu C I	A: 607, 191, 101, 132 G: 607, 607, 132
rs3917419	5′AAg ACg TTg Tag AA TgA gT 5′TTg CAg gCT ggA ATA ggA g	Bse N I	C: 548, 240, 124, 120 T: 672, 240, 120
rs1534904	5′ CAA TgT ATA TTg CCA ACC CAg TA 5′ATT AgC TTg CCC ATT TCC AgT AT	Dra I	A: 363, 245, 271 C: 363, 516
rs1076637	5′gAA CTg ggT TCA CTC AAC AAg C 5′TTA AAA TAA AAT AAA AAC AAA GAC TgT ATgT A	Hin 6 I	G: 23, 274 A: 297
rs5356	5′gCT gCC AAA gCC TTg AAT CAC A 5′CTC CCC TgC TCC CTC CCT AAg AT	Hin 1 II	T: 24, 98, 58 C: 122, 58
rs3917438	5′ ACC TCC ACC TCC TgC gTT CAA 5′ gCC AgA gAC CCg Agg AgA gTT ATC	Ade I	C: 615, 271 T: 886

Restriction enzymes (MBI Fermentas, Vilnius, Lithuania).

J. Gorący, M. Kaczmarczyk, A. Ciechanowicz, K. Safranow, J. Gorący, K. Jakubowska, D. Chlubek, I. Gorący

Table II. Clinical and biochemical characteristics of patients with coronary artery disease and control individuals

Parameter	CAD (*n* = 261)	CAD/MI(–) (*n* = 202)	CAD/MI(+) (*n* = 59)	MCAD (*n* = 123)	SCAD (*n* = 79)	Non-CAD (*n* = 118)
Age [years]	56.4 ±9.3	57.2 ±9.0	53.7 ±9.5	58.7 ±9.4*	54.9 ±8.0	55.3 ±9.5
Sex, F/M	44/217* 16.9%/83.1%	36/166* 17.8%/82.2%	8/51* 13.6%/86.4%	21/102* 17.1%/82.9%	15/64* 19.0%/81.0%	49/69 41.5%/58.5%
BMI [kg/m²]	27.5 ±3.7	27.7 ±3.7*	26.6 ±3.6	27.7 ±3.6*	27.8 ±3.7	26.7 ±4.2
Smoking, Yes/no	111/150* 42.5%/57.5%	75/127* 37.1%/62.9%	36/23* 61.0%/39.0%	46/77* 37.4%/62.6%	29/50* 36.7%/63.3%	27/91 22.9%/77.1%
Diabetes mellitus, Yes/no	43/218 16.5%/83.5%	37/165* 18.3%/81.7%	6/53 10.2%/89.8%	28/95* 22.8%/77.2%	9/70* 11.4%/88.6%	11/107 9.3%/90.7%
Hypertension, Yes/No	152/109 58.2%/41.8%	124/78* 61.4%/38.6%	28/31 47.5%/52.5%	81/42 65.9%/34.1%	43/36* 54.4%/45.6%	59/59 50.0%/50%
Triacylglycerol [mg/dl]	174 (132–215)*	169 (123–216)*	180 (156–210)*	174 (120–217)*	165 (126–209)*	147.0 (103–197)
Total cholesterol [mg/dl]	225 ±40	225 ±42	226 ±33	227 ±43	222 ±41	220 ±37
Cholesterol LDL [mg/dl]	138 ±32	138 ±34	136 ±25	140 ±36*	135 ±31	132 ±29
Cholesterol HDL [mg/dl]	42.0 (37.0–47.0)*	42.0 (37.0–47.0)*	53.0 (47.0–62.0)*	42.0 (37.0–46.0)*	43.0 (38.0–49.0)*	47.0 (39.0–56.0)

Significant difference as compared with non-CAD patients, CAD – coronary artery disease, CAD/MI(+) – patients with a history of myocardial infarction, CAD/MI(–) – patients without a history of myocardial infarction, MCAD – multi-vessel coronary artery disease, SCAD – single-vessel coronary artery disease, non-CAD – absence of coronary artery disease; mean ± standard deviations or median with quartiles (in brackets). Arterial hypertension was defined as: systolic blood pressure exceeding 140 mm Hg, or diastolic blood pressure greater than 90 mm Hg; Diagnosed diabetes or fasting glucose ≥ 6.9 mmol/l; Smoking – patients were classified as "current smokers" if they reported a daily rate of more than five cigarettes, otherwise patients were classified as "non-smokers."

[C;A;C;A;G;T]) were associated (based on adjusted *p*-values) with a history of myocardial infarction under additive and dominant models and one haplotype [C;G;T;C;G;T] under a recessive model (Table IV). The most common *SELE* haplotype in this analysis ([C;G;T;C;G;T], 31.2%) was negatively associated with myocardial infarction under the assumption of additive, dominant and recessive effects. Its frequency in the patients with a history of myocardial infarction was 3.0 times lower than in non-CAD individuals (12.7% vs. 37.5%). Two other haplotypes ([C;G;C;C;A;C], [C;A;C;A;G;T]) showed a negative association effect under the assumption of additive and dominant models. The haplotype [C;G;C;C;A;C] was detected only in the non-CAD patients with a frequency of 8.81%.

Table III. Single nucleotide polymorphisms of the *SELE* gene included in the study

SNP (reference sequence NM_000450.2)	CAD (*n* = 261)	CAD/MI(–) (*n* = 202)	CAD/MI(+) (*n* = 59)	MCAD (*n* = 123)	SCAD (*n* = 79)	Non-CAD (*n* = 118)
c.529+123C>T, CC/CT/TT	219/40/2 $\chi^2 = 0.01$	180/22/0 $\chi^2 = 0.67$	39/18/2 $\chi^2 = 0$	111/12/0 $\chi^2 = 0.32$	69/10/0 $\chi^2 = 0.36$	113/5/0 $\chi^2 = 0.06$
c.529+474A>G, AA/AG/GG	16/106/139 $\chi^2 = 0.51$	11/87/104 $\chi^2 = 1.75$	5/19/35 $\chi^2 = 1.02$	7/50/66 $\chi^2 = 0.39$	4/37/38 $\chi^2 = 1.77$	9/40/69 $\chi^2 = 0.87$
c.530-304G>A, GG/GA/AA	210/51/0 $\chi^2 = 3.06$	162/40/0 $\chi^2 = 2.44$	48/11/0 $\chi^2 = 0.62$	99/24/0 $\chi^2 = 1.44$	63/16/0 $\chi^2 = 1$	95/19/4 $\chi^2 = 4.98^{‡}$
c.530-61C>T, CC/CT/TT	89/136/36 $\chi^2 = 1.97$	65/109/28 $\chi^2 = 2.75$	24/27/8 $\chi^2 = 0.01$	40/66/17 $\chi^2 = 1.54$	25/43/11 $\chi^2 = 1.21$	43/51/24 $\chi^2 = 1.5$
c.716-11A>C, AA/AC/CC	30/131/100 $\chi^2 = 1.74$	23/100/79 $\chi^2 = 1.06$	7/31/21 $\chi^2 = 0.76$	16/56/51 $\chi^2 = 0.01$	7/44/28 $\chi^2 = 3.12$	13/45/60 $\chi^2 = 1.03$
c. 1091-57G>A, GG/GA/AA	195/58/8 $\chi^2 = 1.96$	156/44/2 $\chi^2 = 0.33$	37/16/6 $\chi^2 = 3.71$	95/27/1 $\chi^2 = 0.38$	61/17/1 $\chi^2 = 0.02$	82/31/5 $\chi^2 = 0.85$
c.1800T>C, TT/TC/CC	211/46/4 $\chi^2 = 0.65$	168/32/2 $\chi^2 = 0.12$	43/14/2 $\chi^2 = 0.4$	102/21/0 $\chi^2 = 1.07$	66/11/2 $\chi^2 = 2.84$	95/21/2 $\chi^2 = 0.43$
c.*16-200C>T, CC/CT/TT	240/21/0 $\chi^2 = 0.46$	186/16/0 $\chi^2 = 0.34$	54/5/0 $\chi^2 = 0.12$	117/6/0 $\chi^2 = 0.08$	69/10/0 $\chi^2 = 0.36$	107/9/2 $\chi^2 = 8.43^{†}$

Significant deviation from HWE expectations as tested using χ^2 with 1 degree of freedom: †p = 0.004, ‡p = 0.03, CAD – coronary artery disease, CAD/MI(+) – patients with a history of myocardial infarction, CAD/MI(–) – patients without a history of myocardial infarction, MCAD – multi-vessel coronary artery disease, SCAD – single-vessel coronary artery disease, non-CAD – absence of coronary artery disease.

Table IV. *SELE* haplotype (NM_000450.2:c.[529+123C>T; 529+474A>G; 530-61C>T; 716-11A>C; 1091-57G>A; 1800T>C]) association analysis in patients with a history of CAD/MI(+) (*n* = 59) and non-CAD control individuals (*n* = 118)

Haplotype	Frequency (%)			Additive		Dominant		Recessive	
	Total[‡]	CAD/MI(+)	Non-CAD	Score	P[†] < 0.001*	Score	P[†] < 0.001*	Score	P[†] 0.007*
[C;G;T;C;G;T]	31.2	12.7	37.5	–3.46	0.001	–2.82	0.006	–3.06	0.012
[C;G;C;C;A;C]	5.73	0	8.81	–3.18	0.0007	–3.18	0.0007	–	–
[C;A;C;A;G;T]	18.1	6.72	23.0	–2.98	0.01	–2.73	0.02	–2.05	0.10
[C;G;T;C;A;T]	1.81	0	2.25	–0.63	0.48	–0.47	0.32	–	–
[C;G;C;C;G;T]	10.3	9.31	10.7	–0.49	0.92	–0.56	0.74	–	–
[C;G;T;C;G;C]	1.38	2.54	1.24	0.53	0.37	NA	–	–	–
[T;G;C;C;G;T]	2.62	4.58	2.12	1.30	0.38	1.30	0.38	–	–
[C;G;C;C;A;T]	7.65	11.6	6.32	1.39	0.14	1.39	0.14	–	–
[C;G;C;A;G;T]	7.12	12.9	5.65	1.66	0.17	1.44	0.27	–	–
[C;A;C;C;G;T]	1.74	3.48	0.90	2.33	0.13	2.33	0.13	–	–

[†]*P-value adjusted for sex, age, BMI, smoking, diabetes mellitus, hypertension, triacylglycerol (logarithm), *omnibus haplotype test statistic p-value, NA – not applicable. [‡]Total – CAD/MI(+) group and non-CAD group, CAD/MI(+) – patients with a history of myocardial infarction, non-CAD – absence of coronary artery disease.*

Thirty-seven *SELE* haplotypes were reconstructed in 59 CAD/MI(+) and 202 CAD/MI(–) patients. Of these, 5 haplotypes with frequencies greater than 5% accounted for 77.4% of all haplotypes, whereas 12 haplotypes with frequencies above 1% accounted for 92.9% (Table V). Similarly to a previous analysis, we identified three negatively associated haplotypes [C;G;T;C;G;T], [C;A;C;A;G;T], [C;G;C;C;A;C] that overlapped with haplotypes inferred for CAD/MI(+) and non-CAD patients (Table IV). The most common negatively associated haplotype [C;G;T;C;G;T] was 2.9 times less frequent in

Table V. *SELE* haplotype (NM_000450.2:c.[529+123C>T; 529+474A>G; 530-61C>T; 716-11A>C; 1091-57G>A; 1800T>C]) association analysis in CAD/MI(+) (*n* = 59) and CAD/MI(–) (*n* = 202)

Haplotype	Frequency (%)			Additive		Dominant		Recessive	
	Total‡	CAD/MI(+)	CAD/MI(–)	Score	P[†] < 0.001*	Score	P[†] < 0.001*	Score	P[†] 0.02*
[C;G;T;C;G;T]	31.8	12.7	36.3	–4.05	< 0.001	–4.02	< 0.001	–2.19	0.02
[C;A;C;A;G;T]	20.3	6.72	24.1	–3.60	0.001	–3.53	0.002	–1.65	0.11
[C;G;C;C;A;C]	4.81	0	6.41	–2.75	0.006	–2.75	0.006	NA	–
[T;G;C;C;G;T]	3.81	4.58	4.04	–0.14	0.93	–0.14	0.93	NA	–
[C;G;C;A;G;T]	10.4	12.9	10.1	0.32	0.59	0.32	0.68	NA	–
[C;A;T;A;G;T]	1.46	0.81	1.49	0.39	0.75	0.77	0.67	NA	–
[C;G;C;C;G;T]	8.16	9.30	7.48	0.67	0.68	0.62	0.71	NA	–
[C;G;T;C;G;C]	1.21	2.54	0.88	0.87	0.32	0.87	0.32	NA	–
[C;A;T;C;G;T]	1.39	4.46	1.06	1.01	0.84	1.01	0.84	NA	–
[C;G;C;C;G;C]	1.33	2.01	0.96	1.14	0.44	1.14	0.44	NA	–
[T;G;T;C;G;T]	1.52	5.24	1.16	2.42	0.04	2.42	0.04	NA	–
[C;G;C;C;A;T]	6.71	11.6	5.19	2.60	0.007	2.86	0.004	NA	–

[†]*P-value adjusted for sex, age, BMI, smoking, diabetes mellitus, hypertension, triacylglycerol (logarithm), *omnibus haplotype test statistic p-value, NA – not applicable. [‡]Total – CAD/MI(+) group and CAD/MI(–) group, CAD/MI(+) – patients with a history of myocardial infarction, CAD/MI(–) – patients without a history of myocardial infarction.*

J. Gorący, M. Kaczmarczyk, A. Ciechanowicz, K. Safranow, J. Gorący, K. Jakubowska, D. Chlubek, I. Gorący

the CAD/MI(+) group compared with CAD/MI(–) patients (12.7% vs. 36.3%), while [C;G;C;C;A;C] occurred only in CAD/MI(–) patients (0% vs. 6.41%). In addition, we found two haplotypes associated with a history of myocardial infarction ([T;G;T;C;G;T], [C;G;C;C;A;T]) acting in both additive and dominant manner (Table V).

Discussion

In the current study we conducted a haplotype-based analysis of the *SELE* gene in 261 patients with coronary artery disease (including 59 with myocardial infarction) and 118 control in-

dividuals with no significant coronary stenosis. To the best of our knowledge this is the first study investigating the association of the *SELE* gene with the risk of coronary artery disease and related phenotypes that uses a tagging SNPs approach for the selection of genetic variants.

Our main findings are as follows:
• We identified two *SELE* haplotypes ([T;G;T;C;G;T] and [C;G;C;C;A;T]) that were significantly more common in patients with a history of myocardial infarction than in non-MI CAD patients, thereby possibly increasing the risk of MI among patients with coronary artery disease.

Table VI. *SELE* haplotype (NM_000450.2:c.[529+123C>T; 529+474A>G; 530-61C>T; 716-11A>C; 1091-57G>A; 1800T>C]) association analysis in patients with CAD (*n* = 261) and non-CAD control individuals (*n* = 118)

Haplotype	Frequency (%)			Additive		Dominant		Recessive	
	Total[‡]	CAD	Non-CAD	Score	P[†] 0.02*	Score	P[†] 0.17*	Score	P[†] 0.22*
[C;G;C;C;A;C]	6.13	4.73	8.81	−1.85	0.07	−1.85	0.07	NA	–
[C;G;T;C;G;T]	33.8	31.7	37.5	−1.52	0.09	−0.40	0.33	−2.53	0.05
[C;G;C;C;G;T]	9.01	8.14	10.7	−1.08	0.22	−1.09	0.19	−0.43	0.80
[C;A;C;A;G;T]	21.3	20.5	23.0	−0.56	0.91	−0.04	0.75	−1.45	0.69
[C;G;C;C;A;T]	6.17	6.73	6.32	−0.27	0.93	−0.40	0.71	NA	–
[C;G;T;C;G;C]	1.29	1.43	1.24	0.38	0.67	0.38	0.67	NA	–
[C;A;T;A;G;T]	1.19	1.41	0.66	0.54	0.82	0.39	0.96	NA	–
[C;G;C;C;G;C]	1.06	1.34	0.12	1.07	0.10	1.07	0.10	NA	–
[T;G;C;C;G;T]	3.20	3.78	2.12	1.31	0.15	1.31	0.15	NA	–
[C;G;C;A;G;T]	8.80	10.39	5.65	1.92	0.14	1.74	0.19	NA	–

[†]P-value adjusted for sex, age, BMI, smoking, diabetes mellitus, hypertension, triacylglycerol (logarithm), *omnibus haplotype test statistic p-value, NA – not applicable. [‡]Total – CAD group and non-CAD group, CAD – coronary artery disease, non-CAD – absence of coronary artery disease.

Table VII. *SELE* haplotype (NM_000450.2:c.[529+123C>T; 529+474A>G; 530-61C>T; 716-11A>C; 1091-57G>A; 1800T>C]) association analysis in CAD/MI(–) (*n* = 202) and non-CAD control individuals (*n* = 118)

Haplotype	Frequency (%)			Additive		Dominant		Recessive	
	Total[‡]	CAD/MI(–)	Non-CAD	Score	P[†] 0.35*	Score	P[†] 0.41*	Score	P[†] 0.42*
[C;G;C;C;G;T]	8.96	7.48	10.7	−1.52	0.10	−1.53	0.09	NA	–
[C;G;C;C;A;C]	7.05	6.41	8.81	−1.00	0.29	−1.00	0.29	NA	–
[C;G;C;C;A;T]	5.39	5.19	6.32	−0.63	0.64	−0.80	0.42	NA	–
[C;G;T;C;G;T]	36.9	36.3	37.5	−0.32	0.54	0.80	0.93	−1.75	0.19
[C;A;T;A;G;T]	1.36	1.49	0.66	0.45	0.99	0.24	0.85	NA	–
[C;A;C;A;G;T]	23.6	24.1	23.0	0.46	0.35	0.97	0.28	−0.89	0.90
[T;G;C;C;G;T]	3.31	4.04	2.12	1.36	0.10	1.36	0.10	NA	–
[C;G;C;A;G;T]	8.49	10.1	5.65	1.71	0.25	1.51	0.32	NA	–

[†]P-value adjusted for sex, age, BMI, smoking, diabetes mellitus, hypertension, triacylglycerol (logarithm), *omnibus haplotype test statistic p-value, NA – not applicable. [‡]Total – CAD/MI(–) group and non-CAD group, CAD/MI(–) – patients without a history of myocardial infarction, non-CAD – absence of coronary artery disease.

Table VIII. *SELE* haplotype (NM_000450.2:c.[529+123C>T; 529+474A>G; 530-61C>T; 716-11A>C; 1091-57G>A; 1800T>C]) analysis in CAD/MI(–) patients with multi-vessel disease (MCAD, *n* = 123) and single-vessel disease (SCAD, *n* = 79)

Haplotype	Frequency (%)			Additive		Dominant		Recessive	
	Total‡	MCAD	SCAD	Score	*P*† 0.99*	Score	*P*† 0.98*	Score	*P*† 0.71*
[C;A;C;A;G;T]	24.1	22.0	25.8	−0.57	0.70	−0.83	0.41	0.36	0.41
[T;G;C;C;G;T]	4.04	3.74	4.28	−0.46	0.76	−0.46	0.76	NA	−
[C;G;C;C;A;C]	6.41	5.69	6.38	−0.32	0.78	−0.32	0.78	NA	−
[T;G;T;C;G;T]	1.16	0	0.84	−0.28	0.95	NA	−	NA	−
[C;G;T;C;G;T]	36.3	38.0	38.2	−0.003	0.87	0.12	0.89	−0.18	0.90
[C;A;T;A;G;T]	1.49	1.55	0.85	0.02	0.85	−0.37	0.63	NA	−
[C;G;C;C;A;T]	5.19	5.41	4.48	0.26	0.94	0.58	0.76	NA	−
[C;A;T;C;G;T]	1.06	1.10	0	0.47	0.79	NA	−	NA	−
[C;G;C;A;G;T]	10.1	10.6	8.41	0.54	0.71	0.54	0.66	NA	−
[C;G;C;C;G;T]	7.48	8.32	5.89	0.91	0.20	0.68	0.29	NA	−

†*P-value adjusted for sex, age, BMI, smoking, diabetes mellitus, hypertension, triacylglycerol (logarithm), *omnibus haplotype test statistic p-value, NA – not applicable. ‡Total – MCAD group and SCAD group, MCAD – multi-vessel coronary artery disease, SCAD – single-vessel coronary artery disease.*

- We identified three negatively associated (possibly protective) *SELE* haplotypes against MI ([C;G;T;C;G;T], [C;A;C;A;G;T] and [C;G;C;C;A;C]) with significantly lower frequency in patients with a history of MI than in non-CAD or no-MI CAD patients. It is worth mentioning that those risk-decreasing or risk-increasing haplotype effects for MI were independent of traditional modifiable and non-modifiable risk factors.

So far, mainly two presumably functional *SELE* polymorphisms, A561C (rs5361) and G98T (rs1805193), have been investigated in different ethnic groups with respect to CAD [13, 14, 26]. However, the haplotype-based analysis may offer greater power in association studies than the analysis of one SNP at a time, especially when none of the investigated SNPs is a causative marker [27]. Considering the lack of association with increased risk of CAD among Caucasians for G98T in a recent meta-analysis [13], our decision to use tag SNPs in an attempt to capture a high degree of the known common variability, instead of the standard approach to testing individual candidate SNPs, seems well justified.

Our results suggest that E-selectin gene variability may, independently of conventional risk factors, modify the risk of myocardial infarction. However, there was no clear evidence for an association in patients with CAD in whom MI did not occur. Increased levels of E-selectin were reported in both coronary artery disease [28] and myocardial infarction [29]. Atalar *et al*. [30] found increased levels of selectins, including E-selectin, in patients with unstable angina compared with those with stable angina or without angiographically visible occlusions. Interestingly, sE-selectin levels were higher in patients with acute myocardial infarction preceded by unstable angina compared with sudden onset of infarction [31]. Since at least part of the sE-selectin level in recent years [32], despite variation, can be attributed to *SELE* gene polymorphism (a borderline association of the 7-marker haplotype with soluble E-selectin [33]), it seems reasonable to propose that the risk-decreasing and risk-increasing *SELE* haplotype effects for MI possibly reflect extreme areas of the soluble E-selectin level continuum.

Nonetheless, it is unclear why there was no association between *SELE* haplotypes and the risk of coronary artery disease. None of the *SELE* haplotypes, except for [C;G;T;C;G;T] under the recessive model (Table VI), were associated with CAD with or without a history of MI (the association of the [C;G;T;C;G;T] haplotype is likely generated by MI patients as it disappears in the no-MI CAD patients, Table VII). It must be noted that traditional risk factors of atherosclerosis are deeply rooted in the Polish population although it should be emphasized that there has been a slight improvement which may lessen the impact of genetic factors. Indeed, the frequency of classic risk factors such as arterial hypertension, smoking, diabetes and BMI differed significantly between CAD and control patients (Table II). The other possibility is the presence of an interaction with other systems that may modify the impact of *SELE* gene haplotypes on CAD susceptibility. These mechanisms, however, have not been

J. Gorący, M. Kaczmarczyk, A. Ciechanowicz, K. Safranow, J. Gorący, K. Jakubowska, D. Chlubek, I. Gorący

fully explained yet. For example, Wu *et al.* [33] reported that genotypes/haplotypes of the *SELE* gene in Taiwanese individuals are independently associated with E-selectin and matrix metalloproteinase 9 (MMP9) levels. Metalloproteinase 9 plays an important role in the destabilization of atherosclerotic plaque [34], but a direct mechanism linking E-selectin and the MMP9 gene is not clear, and further studies are needed to clarify this relationship. It is also possible that the lack of association with susceptibility to CAD may be related to ethnic differences in the social, environmental and genetic conditions contributing towards the development of CAD [35].

However, our study has several limitations. It is important to emphasize that this is the first report demonstrating that the haplotypes capturing the majority of the E-selectin gene variation are correlated with occurrence of MI , but there is still a lack of studies showing this association in other countries and nations. Additionally it is a relatively small sample size, so the observation need to be followed up with a much larger sample size to make a causal inference. The strength of our study lies in the clinically well characterized and homogeneous cohort of patients who had undergone coronary angiography. Homogeneity is a crucial aspect in genetic association studies as population stratification may lead to spurious findings. The geographic area from which the subjects came coincides with the West Pomerania province in Poland and its genetic structure appears to be extremely homogeneous [36]. However, we did not measure the level of E-selectin, and this is a major limitation of our study. Thus, it is not clear whether the MI risk-modifying effect of the *SELE* haplotypes is mediated through an effect on the serum E-selectin level.

In conclusion, using a tagging SNPs approach we identified both negatively associated and myocardial infarction risk-enhancing common *SELE* haplotypes in a homogeneous Polish angiographic cohort. However, there was no evidence of an association between the *SELE* gene and susceptibility to coronary artery disease without a history of MI. Our results suggest that *SELE* is one of the independent genetic factors modulating the risk of developing myocardial infarction.

Acknowledgments

Conflict of interest

The authors declare no conflict of interest.

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