Clinical research

P-selectin glycoprotein ligand-1 variable number of tandem repeats polymorphism in young myocardial infarction patients

Fatma Nihan Turhan Caglar[1], İsmail Biyik[2], Nilgun Isıksacan[3], Veysel Sabri Hançer[4], Ibrahim Faruk Akturk[1], Serkan Ciftci[1], Ismail Ungan[1], Alparslan Sahin[1], Fatih Uzun[5]

[1]Department of Cardiology, Bakirkoy Dr. Sadi Konuk Education and Research Hospital, Istanbul, Turkey
[2]Department of Cardiology, School of Medicine, Usak University, Usak, Turkey
[3]Department of Biochemistry, Mehmet Akif Ersoy Thoracic and Cardiovascular Surgery Training and Research Hospital, Istanbul, Turkey
[4]Department of Genetics, School of Medicine, Bilim University, Istanbul, Turkey
[5]Department of Cardiology, Istanbul Mehmet Akif Ersoy Education and Research Hospital, Istanbul, Turkey

Corresponding author:
İsmail Biyik
Department of Cardiology
School of Medicine
Usak University
64100 Usak, Turkey
Fax: +90 5424173209
E-mail: ismailbiyikmd@yahoo.com

Submitted: 17 October 2016
Accepted: 24 November 2016

Arch Med Sci Civil Dis 2016; 1: e112–e116
DOI: 10.5114/amscd.2016.64166

Abstract

Introduction: The prevalence of acute myocardial infarction (AMI) among young patients is increasing. Although the risk factors of AMI are well defined in the middle aged population, they are not clearly elucidated in young patients. Alternative risk factors such as genetic factors are being investigated. The P-selectin ligand gene, encoding P-selectin glycoprotein ligand-1 (PSGL-1), plays a role in atherosclerosis. In this study, we aimed to investigate whether there is an association between the PSGL-1 variable numbers of tandem repeats (VNTR) polymorphism and AMI in a young Turkish population.
Material and methods: We examined 119 young patients, less than 40 years old, presenting ST elevation AMI and undergoing percutaneous coronary intervention, and 53 sex and age matched controls with a normal coronary angiogram. Genomic DNAs were collected from whole blood samples by standard methods. The PSGL-1 was amplified by polymerase chain reaction.
Results: Ninety four (78.9%) showed AA, 15 (12.6%) showed AB, 4 (3.3%) showed BB, 4 (3.3%) showed AC and 2 (1.7%) showed BC genotype in the young AMI group, and 40 (75.4%) showed AA, 9 (16.9%) showed AB, 3 (5.6%) showed BB, 0 (0%) showed AC and 1 (1.8%) showed BC genotype in the control group. PSGL-1 VNTR genotype frequencies were similar between groups ($p = 0.64$). Smoking was significantly higher in the young AMI group ($p = 0.025$).
Conclusions: In this study, we found no significant difference between the PSGL-1 variable numbers of tandem repeats polymorphism and AMI in a young Turkish population. Further studies with additional polymorphisms are needed for further information about the genetic influences on premature myocardial infarction.

Key words: P-selectin, P-selectin glycoprotein ligand-1, premature myocardial infarction.

Introduction

Although age is a well-known risk factor for coronary artery disease (CAD) and acute myocardial infarction (AMI), the prevalence of CAD

and AMI are increasing in the young population (< 40 years old) [1]. Six to ten percent of AMI cases are observed in patients younger than 45 years old [2, 3]. Coronary artery disease risk factors are well defined in middle and advanced aged patients. However, it is believed that different risk factors such as genetic predisposition may have relevance in the development of premature AMI [1–5]. Inflammation is a cornerstone of atherosclerosis [5]. Adhesion of circulating leukocytes to injured endothelium is the first step [5–7]. The selectin family plays a role in initiating this cascade [5–8]. P-selectin (PSEL), a member of the selectin family, initiates leukocyte rolling, and plays a role in leukocyte-endothelium, platelet-endothelium and leukocyte-platelet interaction [6–9]. In order to perform its actions, PSEL needs a counter-ligand [6–9]. The major ligand of PSEL is P-selectin glycoprotein ligand-1 (PSGL-1), expressed in myeloid cells and localized in the coding region of the gene; it controls the initial interaction between leukocytes, platelets and endothelium [7, 9–11]. Recently, studies have demonstrated the functional relevance of PSGL-1 polymorphism and different forms of cardiovascular diseases [9]. The most frequently studied polymorphism of PSGL-1 is the "variable number of tandem repeats" (VNTR) polymorphism [9, 11]. The extracellular domain length of PSGL-1 is altered by VNTR polymorphism that affects the binding efficacy of PSEL to PSGL-1 and leukocytes to platelets [6, 7, 9]. Activated platelets are less prone to bind to neutrophils carrying short alleles [6, 7, 9]. Therefore these alleles may be protective against thrombosis [6, 7, 9].

In this study, we aimed to investigate whether there is an association between PSGL-1 variable numbers of tandem repeats (VNTR) polymorphism and AMI in a young Turkish population.

Material and methods

Study population

In this case control study, we included 119 patients younger than 40 years old admitted to the tertiary heart center with the diagnosis of AMI and undergoing primary percutaneous coronary intervention (PPCI) between 2010 and 2013. The diagnosis of AMI was performed in the presence of detection of a rise and/or a fall of the cardiac biomarker troponin with at least one value above the 99th percentile upper reference limit together with at least one of the following: symptoms of myocardial ischemia, new or presumed new ST-segment changes or new left bundle branch block, pathological Q waves in the electrocardiogram (ECG), imaging evidence of new regional wall motion abnormality and/or loss of viable myocardium [10]. All of the patients received similar medical treatment. Primary percutaneous coronary intervention was performed to all patients. The control group consisted of 53 patients younger than 40 years old undergoing coronary angiography for any reason and having a normal invasive coronary angiogram (CAG) or multi-detector computed tomography coronary angiogram (MDCT). The CAG or MDCT was performed in those patients for preoperative evaluation for congenital heart defects or valvular heart disease because they had conventional CAD risk factors. Patients with unstable angina pectoris, recent myocardial infarction (MI), heart failure or left ventricular hypertrophy were excluded. Traditional cardiovascular risk factors, medical history and the clinical parameters were assessed according to the medical records. Blood pressure measurement more than systolic 140 or diastolic 90 mm Hg was defined as hypertension. Fasting blood glucose level of more than 126 mg/dl was defined as diabetes mellitus (DM). Total blood cholesterol level over 240 mg/dl or low-density lipoprotein (LDL) cholesterol level over 130 mg/dl was defined as hyperlipidemia. Patients previously diagnosed as hypertensive/diabetic/hyperlipidemic were also regarded as hypertensive/diabetic/hyperlipidemic. Body mass index (BMI) was defined as weight in kg divided by height in m^2. Family history was defined as CAD in a parent or sibling before 60 years of age. Subjects were defined as smokers if they were current smokers. The study protocol was approved by the local ethics committee, and written informed consent was taken from all patients. The study was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice (GCP) and International Conference on Harmonization (ICH) guidelines.

Genotyping and detection of P-selectin glycoprotein ligand-1 polymorphism

Two milliliters of venous blood samples were collected from all subjects into the EDTA-treated vacutainers. The genomic deoxyribonucleic acid (DNA) was extracted by using a spin column DNA isolation kit (Quick Blood DNA Isolation Kit, HibriGen, Istanbul, Turkey). Oligonucleotide primers specific for the PSGL-1 VNTR genomic regions listed below were used in this study (5′- CAT GAG AGA CAT CGC CTC TG-3′, 5′- GAC CTA ACA TGT TCT AGC CAG AAG- 3′).

Polymerase chain reaction (PCR) conditions (for 50 ml reaction)

Three milliliters of genomic DNA were added to the main mixture of 23 ml of sterilized water, 0.5 ml of primer-reverse, 0.5 ml of primer-forward, and 24 ml of 2X PCR master mix (PCR Master Kit, HibriGen, Istanbul, Turkey). Then, the mixture was

Table I. Demographic and clinical characteristics of the groups

Parameter	Young AMI (n = 119)		Control (n = 53)		P-value
Age [years]	35.71 ±4.27		36.74 ±4.27		0.55
Height [cm]	170.06 ±8.15		170.32 ±7.2		0.799
Weight [kg]	79.2 ±15.96		80.57 ±13.51		0.776
BMI	27.28 ±4.54		27.78 ±4.4		0.499
Gender:					
Male	103	86.55%	39	75.00%	0.647
Female	16	13.45%	13	25.00%	
Smoking	80	82.47%	26	49.06%	0.025
Diabetes	8	6.72%	2	3.77%	0.086
Hypertension	17	14.28%	7	13.20%	0.741
Family history	58	48.74%	22	41.51%	0.38
Creatinine [mg/dl]	0.8 (0.7–0.9)		0.9 (0.7–1)		0.224
LDL [mg/dl]	123.74 ±38.62		133.5 ±38.87		0.095

BMI – body mass index, AMI – acute myocardial infarction.

vortexed and loaded to a thermal cycler. A total of 35 cycles were performed for 3 min at 95°C, 25 s at 95°C, 45 s at 56°C and 45 s at 72°C. Extension time was adjusted to 10 min for the last cycle and then ended. Polymerase chain reaction products (8–10 ml) were collected and controlled on 1% agarose gel.

Interpretation of the results

A/A (homozygote) genotype showed 1 fragment of 558 bp, and A/B (heterozygote) genotype showed 2 fragments of 558 and 538 bp.

Statistical analysis

Number Cruncher Statistical System (NCSS) 2007 Statistical Software (NCSS Statistical Software, Utah, USA) was used for statistical assessment of this trial. Mean value, standard deviation, median and interquartile range were used for descriptive statistical evaluation. Normally distributed variables were compared between groups using the independent t-test, and non-normally distributed variables were compared using the Mann-Whitney U test. Qualitative data were evaluated by the χ^2 test. A p-value lower than 0.05 was considered statistically significant.

Table II. Allele frequencies of the groups

Allele	Young AMI (n = 119)	Control (n = 53)
A	86.97%	84.90%
B	10.50%	14.15%
C	2.52%	0.94%

AMI – acute myocardial infarction.

Results

Demographic characteristics of the groups are given in Table I. Mean age, sex, height, weight and BMI were similar among the groups (p > 0.05). Smoking was significantly higher in the young AMI group than the control group (80 (82.47%) vs. 26 (49.06%), p = 0.025). Diabetes and hypertension frequencies were similar among the groups (p = 0.08 and p = 0.74, respectively). Family history of CAD was similar among the groups (p = 0.38). The allele frequencies were 0.89 for the A allele, 0.08 for the B allele and 0.02 for the C allele in the young AMI group and 0.84 for the A allele, 0.14 for the B allele and 0.09 for the C allele in the control group (Table II). PSGL-1 gene VNTR polymorphism analyses of the groups are given in Table III.

Table III. PSGL-1 gene VNTR polymorphism analysis of the groups

PSGL-1 polymorphism	Young AMI (n = 119)	Control (n = 53)	P-value
AA	94 (78.99%)	40 (75.47%)	0.64
AB	15 (12.60%)	9 (16.98%)	
BB	4 (3.36%)	3 (5.66%)	
BC	2 (1.68%)	0 (0.00%)	
AC	4 (3.36%)	1 (1.89%)	

PSGL-1 – P-selectin glycoprotein ligand-1, VNTR – variable number of tandem repeats, AMI – acute myocardial infarction.

Ninety four (78.9%) showed AA, 15 (12.6%) showed AB, 4 (3.3%) showed BB, 4 (3.3%) showed AC and 2 (1.7%) showed BC genotype in the young AMI group, and 40 (75.4%) showed AA, 9 (16.9%) showed AB, 3 (5.6%) showed BB, 0 (0%) showed AC and 1 (1.8%) showed BC genotype in the control group. The genotype frequencies were not statistically different between the groups ($p = 0.64$).

Discussion

The main finding of this study is that there is no significant difference in PSGL-1 VNTR polymorphism frequency between young MI patients and control patients with cardiovascular risk factors. This finding is in agreement with previous studies that also reported no association between VNTR polymorphism and different types of CAD. To our knowledge, although there are limited numbers of studies evaluating the role of PSGL polymorphism in premature MI, our study is the first with a control group which consisted of patients with cardiovascular risk factors and normal coronary arteries demonstrated via CAG or MDCT. In most of the studies evaluating PSGL polymorphism, control groups were composed of subjects who were considered to be healthy. We also demonstrated that smoking is the most significant risk factor for premature MI in the Turkish population. Previous studies have also demonstrated higher smoking prevalence in premature MI patients compared to elderly MI patients [1–3].

Atherosclerosis is a complex disease formed by interaction of genetic and environmental factors [1–7]. The genes involved in the inflammatory processes may also contribute to thrombotic processes [4, 5, 8]. Previous studies have revealed the role of PSEL and its ligand PSGL-1 expression in early atherosclerosis [7, 9, 11, 12]. Molenaar *et al.* showed the key role of PSEL in PSEL-deficient mice by demonstrating the correlation between PSEL expression and early and advanced atherosclerotic plaque development [12]. In the literature, the most frequently studied PSGL-1 polymorphism is the VNTR polymorphism [7]. There are 3 alleles of this gene: A, B and C. The most frequent allele is A, which has 16 repeating units, and the rare alleles B and C have 15 repeating units [8]. The length of PSGL-1 is modified in AA, BB and CC homozygotes, and the ligand-binding site configuration varies in AB, BC and AC heterozygotes, which affects their binding capacity to the activated platelets and development of atherosclerosis [6, 8, 9]. Hancer *et al.* determined the PSGL-1 VNTR polymorphism database of the Turkish population previously [13]. The A, B and C allele frequencies were 0.818, 0.16 and 0.02 respectively, which was similar to our findings [13]. The allele frequencies were 0.89 for the A allele, 0.08 for the B allele and 0.02 for the C allele in the young AMI group in our study, which were not different from the control group and similar to the general Turkish population.

Although it is not statistically significant, the C allele frequency was higher in the young AMI group than the control group in our study (2.52% vs. 9.94% respectively). Unlike us, Roldán *et al.* reported a possible protective role of shorter VNTR alleles (B and C) against premature MI [14]. The frequency of the shorter alleles was significantly lower in AMI patients younger than 45 years old compared to healthy controls in their study ($p = 0.012$) [14]. Bugert *et al.* performed two independent studies regarding the association between PSGL-1 VNTR polymorphism and CAD [8]. In the first trial, conducted on 678 patients, heterozygotes had higher CAD incidence, but in the other trial, conducted on 3662 patients, they did not find an association [8]. Therefore, similar to us, they concluded that PSGL-1 VNTR polymorphism may not be a risk factor for CAD [8]. Lozano *et al.* demonstrated an association between shorter VNTR alleles and cerebrovascular disease but not with CAD [15]. Ghazouani *et al.* were not able to find any significant difference among PSGL-1 VNTR allele variants in Tunisian patients with CAD [11]. Ozben *et al.* also did not reveal any association between PSGL-1 VNTR polymorphism and instent restenosis in a Turkish population [9]. They also detected the AA genotype as the most common PSGL-1 VNTR allele (64%), and they found similar allele frequencies in patients younger than 50 years old [9]. Similarly, we did not find any association between PSGL-1 VNTR polymorphism and premature MI in a young Turkish population.

Our study does have some limitations. The main limitations of our study were its single-centered basis and relatively small population size. Because of the comparative rarity of BB, BC and AC polymorphisms (< 4 individuals in each group), the results of our study were underpowered. However, we believe that our results reflect the general Turkish population. We did not look for any other polymorphisms suggested to be related to premature AMI. Another limitation is the cross-sectional design of our study, which allows us only to explore associations. Also there may have been survival bias because we only included patients surviving AMI, which may have led to polymorphism underestimation. We could have measured inflammatory markers while we were evaluating inflammation as the key mechanistic pathway. However, our aim was to explore the genetic factors, not the inflammatory markers that were previously studied. Despite its limitations, an important feature of the present study is the comparison of premature MI patients with the controls consisting of angiographically normal

subjects. We believe that positive findings in small cohort studies may be incidentally or ethnicity related, as others also suggested [6].

In conclusion, in this study, we found no significant difference between PSGL-1 VNTR polymorphism and young AMI in a Turkish population. The traditional cardiovascular risk factor profiles of young AMI patients were very similar to controls in our study. Larger randomized trials conducted on Turkish and different ethnic groups with additional polymorphisms are needed for further information about the genetic influences on premature myocardial infarction.

Conflict of interest

The authors declare no conflict of interest.

References

1. Jamil G, Jamil M, AlKhazraji H, et al. Risk factor assessment of young patients with acute myocardial infarction. Am J Cardiovasc Dis 2013; 3: 170-4.
2. Trzos E, Uznańska B, Rechciński T, et al. Myocardial infarction in young people. Cardiol J 2009; 16: 307-11.
3. Al-Murayeh MA, Al-Masswary AA, Dardir MD, et al. Clinical presentation and short-term outcome of acute coronary syndrome in native young Saudi population. J Saudi Heart Assoc 2012; 24: 169-75.
4. Incalcaterra E, Hoffmann E, Averna MR, Caimi G. Genetic risk factors in myocardial infarction at young age. Minerva Cardioangiol 2004; 52: 287-312.
5. Poledne R, Lorenzová A, Stávek P, et al. Proinflammatory status, genetics and atherosclerosis. Physiol Res 2009; 58 Suppl 2: S111-8.
6. Bugert P, Vosberg M, Entelmann M, et al. Polymorphisms in the P-selectin (CD62P) and P-selectin glycoprotein ligand-1 (PSGL-1) genes and coronary heart disease. Clin Chem Lab Med 2004; 42: 997-1004.
7. Volcik KA, Ballantyne CM, Coresh J, et al. Specific P-selectin and P-selectin glycoprotein ligand-1 genotypes/haplotypes are associated with risk of incident CHD and ischemic stroke: the Atherosclerosis Risk in Communities (ARIC) Study. Atherosclerosis 2007; 195: 76-82.
8. Bugert P, Hoffmann MM, Winkelmann BR, et al. The variable number of tandem repeat polymorphism in the P-selectin glycoprotein ligand-1 gene is not associated with coronary heart disease. J Mol Med 2003; 81: 495-501.
9. Ozben B, Diz-Kucukkaya R, Bilge AK, et al. The association of P-selectin glycoprotein ligand-1 VNTR polymorphisms with coronary stent restenosis. J Thromb Thrombolysis 2007; 23: 181-7.
10. Thygesen K, Alpert JS, Jaffe AS, et al. ESC/ACCF/AHA/WHF Task Force for the Universal Definition of Myocardial Infarction. Eur Heart J 2012; 33: 2551-67.
11. Ghazouani L, Abboud N, Khalifa SBH, et al. Contribution of SELP and PSGL-1 genotypes and haplotypes to the presence of coronary heart disease in Tunisians. Mol Biol Rep 2011; 38: 495-501.
12. Molenaar TJ, Twisk J, de Haas SA, et al. P-selectin as a candidate target in atherosclerosis. Biochem Pharmacol 2003; 66: 859-66.
13. Hancer VS, Diz-Kucukkaya R, Nalcaci M. Turkish population data on the factor XIII Val34Leu, glycoprotein (GP) Ib Kozak and P-selectin glycoprotein ligand 1 (PSGL-1) loci. Cell Biochem Funct 2005; 23: 55-8.
14. Roldán V, González-Conejero R, Marín F, Pineda J, Vicente V, Corral J. Short alleles of P-selectin glycoprotein ligand-1 protect against premature myocardial infarction. Am Heart J 2004; 148: 602-5.
15. Lozano ML, Gonzalez-Conejero R, Corral J, et al. Polymorphisms of P-selectin glycoprotein ligand-1 are associated with neutrophil-platelet adhesionand with ischaemic cerebrovascular disease. Br J Haematol 2001; 115: 969-76.