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BgI II Polymorhism of the α2β1 Integrin Gene in Macedonian Population

Marica Pavkovic[1], Aleksandar Petlichkovski[2], Aleksandar Stojanovic[1], Dejan Trajkov[2], Mirko Spiroski[2]

[1]Department of Hematology, Faculty of Medicine, University "Ss Kiril and Metodij", Skopje, Republic of Macedonia; [2]Institute for Immunobiology and Human Genetics, Faculty of Medicine, University "Ss Kiril and Metodij", Skopje, Republic of Macedonia

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Correspondence: Pavkovic Marica, MD, MSc. Department of Hematology, Faculty of Medicine, University "Ss Kiril and Metodij", Skopje, Republic of Macedonia. E-mail: pavkovicm@yahoo.com

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Abstract

Background. Glycoprotein (GP) Ia/IIa or α2β1 integrin is a platelet receptor for collagen and it mediates platelet adhesion to vascular subendothelium and is involved in thromb formation. Genetic polymorphism of α2β1 known as Bgl II affects the density of platelet GP Ia/IIa receptor on the platelet surface. Recent studies had shown relationship between this polymorphism and the risk of myocardial infarction, stroke, as well as diabetic retinopathy.

Aim. The aim of this study was to determine the frequency of this polymorphism in Macedonian healthy population.

Materials and Methods. We genotyped 217 healthy Macedonian individuals using the PCR and RFLP (restriction fragment length polymorphism) method.

Results. The allele frequencies in this study were 0.32 for *Bgl II (+)* allele and 0.67 for *Bgl II (-)*. Distribution of *Bgl II* genotypes in Macedonian population was *Bgl II (+/+) = 16/217 (7.3%), Bgl II (+/-)* =107/217 (49.3%) and *Bgl II (-/-)* = 94/217 (43.3%).

Conclusion. Our results showed a slightly lower proportion of the *Bgl II (+)* allele (0.32) in Macedonian population, but not significantly different from other Caucasian population.

Introduction

The integrin receptor for collagen/laminin, α2β1 integrin (also known as the platelet membrane glycoprotein (GP) Ia/IIa complex or very late activation antigen-2 VLA-2) is expressed on a wide variety of cell types, including megakaryocytes, platelets, fibroblast, endothelial cells and epithelial cells [1]. GP Ia/IIa mediates platelet adhesion to collagen and is involved in platelet activation and stable adhesion to exposed vascular subendothelium. Previous studies have shown that platelet levels of α2β1 vary significantly among normal individuals, whereas the levels of other integrins do not [2]. Thus, α2β1 integrin has the potential to contribute significantly to platelet function in vivo. Patients with quantitative abnormalities of platelet α2 present with prolonged bleeding times, chronic mucocuatneous bleeding, defective in vitro platelet adhesion to collagen and absent in vitro collagen induced aggregation [3,4]. The gene encoding α2 integrin has at least 8 polymorphisms, including two silent polymorphisms located within the I domain [5], *Phe (TTT/TTC)* due to a *T/*

C transition at nucleotide 807 (*807T/C*) and *Thr (ACA/ACG)* due to an *A/G* transition at nucleotide 873 (*873A/G*), and a *Bgl II (Bgl II +/-)* restriction length polymorphism within intron 7 [6,7]. These three polymorhisms are in linkage disequilibrium, the *Bgl II (+)* allele is linked to the *807T/873A* allele and *Bgl II (-)* is linked to *807C/873G* allele. Recently it was reported that this genetic variation affects the density of platelet GP Ia/IIa receptor on the platelet surface. The density of the receptor was higher in individuals with the *807T/873A* or *Bgl II (+)* homozygote than in individuals with *807C/873G* or *Bgl II (-)* homozygote [7-9]. Frequency of the *807T* allele in the healthy Caucasians, African Americans and Native Americans is 33.6%, 31.4% and 53.9% respectively [10].

Moreover, some recent studies had shown relationship between this polymorphism and the prevalence of myocardial infarction [11], stroke [12,13], as well as diabetic retinopathy [14]. There are also studies that have shown influence of this polymorphism on aspirin and clopidogrel efficacy [15]. The *807T* allele has been shown to be a risk factor for myocardial infarction, stroke and diabetic retinopathy, probable because of higher density of alpha2 integrin molecules on platelet surface. There are no such data for Macedonian population with occlusive artery or venous diseases which are the most common cause of morbidity and mortality in Macedonian population. There are studies, investigating the incidence of these disease and possible role of some other gene polymorphisms like Factor V Leiden with deep venous thrombosis [16], methylentetrahydrofolate reductase polymorphisms with occlusive artery disease [17], and polymorphisms in lipoprotein lipase gene with coronary artery disease [18] in Macedonian population. Results of this population studies could be useful in future studies investigating possible association of *Bgl II* polymorphisms with artery or venous occlusive disease in Macedonian population.

The aim of this study was to determine the frequency of this polymorphism in Macedonian healthy population.

Materials and Methods

In this study we genotyped 217 healthy Macedonian individuals (434 alleles), from the Macedonian Human DNA bank [19], collected at the Institute of Immunobiology and Human Genetics. Written consent was obtained from all participants in this study. Genotyping of *Bgl II* polymorphism was performed by the method of RFLP (restriction fragment length polymorphism).

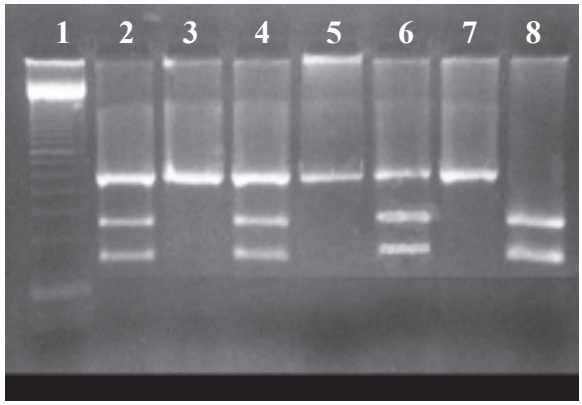

Figure 1. Electrophoresis of digested PCR products. Lane 1: 100 bp ladder, Lane 2,4,6: Bgl II +/- genotype, Lane 3,5,7: Bgl II -/- genotype, Lane 8: Bgl II +/+ genotype.

Genomic DNA was isolated from peripheral blood leukocytes by the standard phenol: chloroform extraction method [20]. Genotyping of this polymorphism was performed by PCR amplification of the target sequence of *GP Ia* gene that contains the *Bgl II* polymorphic site as described by Matsubara Y et al. [14]. Amplification of the target DNA sequence was carried out using the specific primers 5'-GAT TTA ACT TTC CCG ACT GCC TTC-3' and 5'-CAT AGG TTT TTG GGG AAC AGG TGG-3' The thermal cycler profile consisted of 45 cycles of 95°C for 1 min, 65°C for 1 min, and 72°C for 1min and 30 sec. The amplified product was 582 bp in length and was digested with a restriction enzyme Bgl II at 37°C over night and analyzed by 2% agarose gel electrophoresis [14] . The PCR products containing *Bgl II (+)* allele would be cut into two fragments of 241 bp and 341 bp, whereas those containing *Bgl II (-)* allele would not be cut (Figure 1).

Results

Distribution of *Bgl II* genotypes in Macedonian population in our study was *Bgl II (+/+)* = 16/217 (7.3%), *Bgl II (+/-)* = 107/217 (49.3%) and *Bgl II (-/-)* = 94/217

Table 1: Genotype distribution and allele frequency of the Bgl II gene polymorphism in healthy control subjects.

	Healthy Controls (n=217)
Genotype	
Bgl II +/+, +/-	16 (7.3%) / 107 (49.3%)
Bgl II -/-	94 (43.3)
Allele	
Bgl II (+)	139 (32%)
Bgl II (-)	295 (68%)

(43.3%). We analyzed 434 alleles in total and the allele frequencies were 0.32 (139/434) for *Bgl II (+)* allele and 0.68 (295/434) for *Bgl II (-)* (Table 1). Our population displayed a slightly lower proportion of the *Bgl II (+)* allele (0.32), but not significantly different from other Caucasian population, Germany 0.39, Spain 0.35, USA 0.41 (Table 2) [21,22].

Table 2. *Bgl II* allele frequencies in Macedonian and other population.

	Number of subjects	Allele frequeecies *Bgl II (+)*	*Bgl II (-)*
Macedonia	n=217	139/434 (**0.32**)	295/434 (**0.68**)
USA [10]	n=65	0.41	0.59
China [21]	n=217	0.27	0.73
Spain [22]	n=284	0.35	0.65
Germany [12]	n=184	0.39	0.61

Discussion

Human platelet glycoproteins play a major role in platelet adhesion and aggregation, which are the key events in the development of thrombosis and hemostasis. Thus, any variation in platelet GP density could become a risk factor for hemostatic abnormalities [2,4].

The expression of alpha2beta1 by platelets is critical in promoting platelet adhesion to the subendothelium [7-9]. Adhesion of platelets to collagen is critical for normal platelet activity, in hemostasis and in wound repair. Hereditary variation in platelet levels of alpha2beta1alphaintegrin [8], defined by the existence of multiple alleles of the alpha2 gene that are associated with variable alpha2beta1 expression levels, could therefore have a significant impact on platelet function, contributing to an increased risk of thrombosis or bleeding in relevant disease states.

Kunicki et al. [8] identified that polymorphisms in *GP Ia* gene are associated with variations in platelet α2β1 expression levels. Platelets from individuals with *807T* allele express higher levels of α2β1, where individuals with *807C* exhibit a lower density of α2β1 integrins. Interestingly, high levels of GP Ia/IIa only depend on the presence of the *807T* allele and heterozygous individuals express almost similar number of GP Ia copies as individuals homozygous for *807T* [7]. Platelets derived from *807T* donors adhere significantly faster than platelets from *807C* donors [8].

These findings were tested in few recent clinical studies that investigated possible association of these polymorphisms with myocardial infarction and stroke [11-13] . Carlsson et al. [12] and Nikolopoulos et al. [13] observed association of stroke in younger individuals with *807T* allele. Similar were the results of Santoso et al. [11] in younger patient with myocardial infarction. Our study investigates the distribution of these linked polymorphisms in Macedonian population in order to obtain population genetics data and to compare them with data from other populations. Future studies should explore the hypothesis of association of this polymorphism with some disorders in our patients.

In summary, the allele frequencies in this study were 0.32 for *Bgl II (+)* allele and 0.67 for *Bgl II (-)*. Distribution of *Bgl II* genotypes in Macedonian population was 7.3% for *Bgl II (+/+)* genotype, 49.3 % for *Bgl II (+/-)* genotype and 43.3% for *Bgl II (-/-)* genotype. Our results showed a slightly lower proportion of the *Bgl II (+)* allele (0.32) in Macedonian population, but not significantly different from other Caucasian population.

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