Genotyping of Eight Human Platelet Antigen Systems in Serbian Blood Donors: Foundation for Platelet Apheresis Registry

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Keywords

Human platelet antigens · Plateletpheresis · Genotyping · Polymerase chain reaction · Donor registry

Abstract

Introduction: The aim of this study was to investigate the allele and genotype frequencies of 8 human platelet antigen (HPA) systems among blood donors from the Blood Transfusion Institute of Serbia and to compare them with published studies. These data would be useful to establish the basis for a platelet apheresis donor registry. **Material and Methods:** Seventy-two unrelated male platelet apheresis/blood donors from Serbia were typed for 8 HPA systems (HPA-1 to HPA-6, HPA-9, and HPA-15) via the FluoGene method, based on polymerase chain reaction-sequence-specific amplification (PCR-SSP; PCR using sequence-specific primers) with fluorometric signal detection. Allele and genotype frequencies were estimated by direct counting and compared to the expected genotype frequencies according to the Hardy-Weinberg principle. The transfusion mismatch probability was calculated for every HPA specificity. **Results:** The allele frequencies were: HPA-1a, 0.868; HPA-1b, 0.132; HPA-2a, 0.917; HPA-2b, 0.083; HPA-3a, 0.611; HPA-3b, 0.389; HPA-5a, 0.903; HPA-5b, 0.097; HPA-9a, 0.993; HPA-9b, 0.007; HPA-15a, 0.472; and HPA-15b, 0.528. For HPA-4 and HPA-6 only allele *a* was detected. **Discussion:** The HPA allele frequencies of European populations showed no significant differences in comparison with our results. Statistically significant differences were revealed in comparison with some populations

of non-European origin. In the tested donors no HPA-2 bb genotype was detected, but we found 1 donor with the rare HPA-9b allele. The biggest transfusion mismatch probability in the Serbian population is for systems HPA-15 (37.4%) and HPA-3 (36.2%), which means that more than a third of random transfusions could cause mismatch in these systems. This study was enabled by the introduction of molecular HPA typing, and it provides initial results of the HPA allele and genotype frequencies in the population of blood donors in Serbia. They will be used to provide a compatible blood supply on demand for treating patients with alloimmune thrombocytopenic disorders. The successful implementation of PCR-SSP with fluorometric signal detection could be further complemented in the future by the introduction of high-throughput methods, which will largely depend on the available financial resources.

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Introduction

The frequency of human platelet antigens (HPA) varies according to the ethnic distribution of HPA and among different geographical areas. This diversity influences the formation of different specific antiplatelet antibodies in individual populations, as well as the rate and severity of alloimmune thrombocytopenic (AIT) disorders, like posttransfusion purpura, fetal and neonatal alloimmune thrombocytopenia (FNAIT), and platelet transfusion refractoriness [1–3].

Table 1. Detected HPA specificities

HPA specificity	HPA-1	HPA-2	HPA-3	HPA-4	HPA-5	HPA-6	HPA-9	HPA-15
Glycoprotein	GPIIIa	GPIba	GPIIb	GPIIIa	GPIa	GPIIIa	GPIIb	CD109
Gene	<i>ITGB3</i>	<i>GP1BA</i>	<i>ITGA2B</i>	<i>ITGB3</i>	<i>ITGA2</i>	<i>ITGB3</i>	<i>ITGA2B</i>	<i>CD109</i>
SNP <i>a>b</i>	176 T>C	482 C>T	2621 T>G	506 G>A	1600 G>A	1544 G>A	2602 G>A	2108 C>A
Mature protein <i>a>b</i>	L33P	T145M	I843S	R143Q	E505K	R489Q	V837M	S682Y

The name assigned to the gene encoding each HPA specificity by the Human Genome Organisation (HUGO) Gene Nomenclature Committee is shown. SNP and nomenclature are according to Robinson et al. [13].

Currently, there are 41 antigens grouped in 35 HPA systems [4] labelled from HPA-1 to HPA-35. HPA antigens are expressed on different platelet glycoproteins, i.e., GPIIb, GPIIIa, GPIba, GPIb β , GPIa, GPIX, and CD109. The GPIIb-IIIa complex (integrin $\alpha_{IIb}\beta_3$), which acts as a fibrinogen receptor, carries the majority of the HPA systems and most of these systems are positioned on the GPIIIa glycoprotein [4, 5]. HPA-1, HPA-2, HPA-3, HPA-4, HPA-5, and HPA-15 represent 6 biallelic HPA systems and the other 29 systems are monoallelic. Most of the antigens are the product of single amino acid substitution caused by a single-nucleotide polymorphism (SNP) in the genes that encode them [4, 6, 7]. The only exception to this pattern is HPA-14bw, which is an in-frame triplet deletion of a codon for one amino acid (lysine) [2].

Antiplatelet antibodies related to an incompatibility between the platelet donor and the recipient, or between the mother and the fetus during pregnancy, could initiate platelet destruction, which may consequently result in the occurrence of severe hemorrhagic disorders [8]. In European populations, alloantibodies are most commonly developed against HPA-1, HPA-2, and HPA-5 antigens. Anti-HPA-1a antibody is the main cause of more than 80% of cases of FNAIT in ethnicities of European and African origin [3, 9]. In contrast, alloimmunization with anti-HPA-4b alloantibody is the predominant cause of FNAIT in Japan [3].

Investigation of the HPA antigen frequency in each population is important for understanding the association between alloimmunization to these antigens and its clinical implications. Until now, there was no published data of the HPA allele frequency in the Serbian population. The aim of our study was to investigate the allele and genotype frequencies of the 8 HPA systems (HPA-1 to HPA-6, HPA-9, and HPA-15) in the blood donor population of the Blood Transfusion Institute of Serbia (BTIS) from Belgrade, Serbia, and to compare data with already published studies of different populations.

Materials and Methods

DNA Samples from Blood Donors

This study was conducted in a cohort of 72 unrelated healthy male platelet apheresis/blood donors from the city of Belgrade, Serbia. It is expected that there are no differences in the frequencies of HPA between men and women; therefore, we considered this sample to represent the general population [10].

The samples were taken between 2015 and 2020 at the BTIS. Additionally, 3 or 9 mL EDTA whole blood samples were obtained from donors.

HPA Genotyping

DNA was extracted manually from 200 μ L EDTA whole blood at the Department of Molecular Testing of Blood Groups and Tissue Typing of the BTIS using a PureLink Genomic DNA Mini Kit (ThermoFisher Scientific, USA). The extracted DNA was subsequently quantified via the fluorometric method using a Qubit ds-DNA BR Assay Kit (ThermoFisher Scientific) according to the manufacturer's instructions.

Samples were tested using commercially available HPA-FluoGene kits (Inno-train Diagnostik, Germany) according to the manufacturer's instructions. The FluoGene method is based on polymerase chain reaction-sequence-specific amplification (PCR-SSP) [11, 12]. Detection of the PCR products was performed as an end-point method with fluorometric signal detection from modified TaqMan probes by using a FluoVista instrument (Inno-train Diagnostik). Data evaluation was performed automatically by FluoGene software (Inno-train Diagnostik).

The HPA specificities identified by the test system used, with their underlying SNP, genes, and glycoproteins [13], are displayed in Table 1.

Statistics

Gene frequencies and observed genotype frequencies were estimated by direct counting. Expected genotype frequencies were calculated according to the Hardy-Weinberg principle.

The Fisher exact test was used to evaluate whether the observed genotype frequencies were in agreement with the expected ones under Hardy-Weinberg equilibrium, except for HPA specificities where all of the variables were >5 (HPA-3 and HPA-15), where the Pearson χ^2 test was used instead to determine the *p* value (2 degrees of freedom). *p* < 0.05 was considered statistically significant.

The mismatch probability (MP) after random transfusions of blood or platelet concentrates for each of the evaluated HPA specificities was calculated using the following formula [14]: $MP = a^2(1 - a^2) + b^2(1 - b^2)$ or, shortened (considering $a + b = 1$), $MP = 2ab(1 - ab)$, where *a* and *b* are the frequencies of the respective allele of a certain HPA specificity.

Table 2. Distribution of HPA-1, HPA-2, HPA-3, HPA-4, HPA-5, HPA-6, HPA-9, and HPA-15 alleles and HPA genotypes in Serbian blood donors, comparison to expected values from Hardy-Weinberg principle, and transfusion MP among HPA specificities

HPA specificity	HPA alleles (donors, <i>n</i> = 72)			HPA genotypes (donors, <i>n</i> = 72)						Transfusion MP ^e , %
	HPA allele	alleles ^a , <i>N</i>	HPA allele frequency, %	HPA genotype	observed, <i>n</i>	frequency observed, %	expected ^b , <i>n</i>	frequency expected ^c , %	<i>p</i> value ^d	
HPA-1	1a	125	86.8	1aa	54	75.0	54	75.4	1.000	20.3
	1b	19	13.2	1ab	17	23.6	16	22.9		
				1bb	1	1.4	1	1.7		
HPA-2	2a	132	91.7	2aa	60	83.3	61	84.0	1.000	14.1
	2b	12	8.3	2ab	12	16.7	11	15.3		
				2bb	0	0.0	1	0.7		
HPA-3	3a	88	61.1	3aa	29	40.3	27	37.3	0.7835	36.2
	3b	56	38.9	3ab	30	41.7	34	47.5		
				3bb	13	18.1	11	15.1		
HPA-4	4a	144	100.0	4aa	72	100.0	72	100.0	N/A	0.0
	4b	0	0.0	4ab	0	0.0	0	0.0		
				4bb	0	0.0	0	0.0		
HPA-5	5a	130	90.3	5aa	59	81.9	59	81.5	1.000	16.0
	5b	14	9.7	5ab	12	16.7	13	17.6		
				5bb	1	1.4	1	0.9		
HPA-6	6a	144	100.0	6aa	72	100.0	72	100.0	N/A	0.0
	6b	0	0.0	6ab	0	0.0	0	0.0		
				6bb	0	0.0	0	0.00		
HPA-9	9a	143	99.3	9aa	71	98.6	71	98.6	1.000	1.4
	9b	1	0.7	9ab	1	1.4	1	1.4		
				9bb	0	0.0	0	0.00		
HPA-15	15a	68	47.2	15aa	15	20.8	16	22.3	0.9456	37.4
	15b	76	52.8	15ab	38	52.8	36	49.8		
				15bb	19	26.4	20	27.9		

^a 2× the number of samples (*n* = 72). ^b Product of the expected genotype frequency and the number of samples (*n* = 72), rounded to the nearest integer. ^c As per the Hardy-Weinberg principle. ^d Fisher's exact test/Pearson's χ^2 test for comparison between expected and observed results – no significant deviation from Hardy-Weinberg equilibrium was observed (all *p* > 0.05). ^e $2ab(1 - ab)$, where *a* and *b* are the frequencies of the respective allele of a certain HPA specificity.

Results

HPA genotyping was performed on 72 male platelet apheresis/blood donors.

The frequencies of HPA alleles and HPA genotypes are displayed in Table 2. For HPA-4 and HPA-6 only allele *a* was detected. The biggest variability was found within the HPA-15 system, where HPA-15a and HPA-15b alleles were found with almost equal frequencies (i.e., 47.2 and 52.8%, respectively). One rare HPA-9b allele was found in our cohort.

Also, we demonstrated the consistency of the observed genotype frequencies with the expected genotype frequencies from Hardy-Weinberg equilibrium. No significant deviation from Hardy-Weinberg equilibrium was observed for any of the 8 HPA specificities (all *p* > 0.05).

The transfusion MP was calculated for every HPA specificity. The highest transfusion MP was detected in HPA-15 (37.4%).

Discussion

Research of HPA allele frequencies and study of HPA antigen distribution differences between various ethnic populations are conducted to better understand the association between alloimmunization to these antigens and their clinical importance [8]. There are a number of studies reporting distributions of HPA gene frequencies in worldwide populations, which exhibit great variations between different ethnic and geographic groups [10]. The HPA antigens are expressed not only on platelet glycoproteins but also on other cells such as endothelial cells

Table 3. HPA allele frequencies in Serbia and neighboring countries from Southeastern Europe as well as European populations

HPA specificity	HPA allele	HPA allele frequency				
		Serbia ^a	Slovenia [16]	North Macedonia [20]	Croatia [21, 22]	European non-Finnish ^b [23]
Study participants, <i>n</i> :		72	152	122	219 (HPA-1 to HPA-5) [21] 279 (HPA-15) [22]	
HPA-1	1a	0.868	0.809	0.865	0.854	
	1b	0.132	0.191	0.135	0.146	0.1550
HPA-2	2a	0.917	0.891	0.852	0.890	
	2b	0.083	0.109	0.148	0.110	0.0824
HPA-3	3a	0.611	0.591	0.578	0.575	
	3b	0.389	0.407	0.422	0.425	0.3883
HPA-4	4a	1.000	0.997			
	4b	0.000	0.003			0.0001
HPA-5	5a	0.903	0.934	0.909	0.895	
	5b	0.097	0.066	0.091	0.105	0.0912
HPA-6	6a	1.000				
	6b	0.000				0.0000
HPA-9	9a	0.993				
	9b	0.007				0.0039
HPA-15	15a	0.472			0.53	0.5038
	15b	0.528			0.47	

^a Results from this study. ^b Population frequencies for European non-Finnish populations from the gnomAD database [23] (<https://gnomad.broadinstitute.org/>).

and monocytes, and they probably behave as histocompatibility antigens in organ transplantation [15, 16]. Although HPA incompatibility between the donor and the recipient cannot be the cause of graft rejection, possible alloimmunization might influence the severity of transplant complications [17]. HPA genotyping could be very useful in decreasing the effects of alloimmune platelet disorders. Anti-HPA-1a and anti-HPA-5b account for about 90% of antiplatelet alloantibodies causing FNAIT [18]. For the best therapeutic effect of platelet transfusion therapy, ideally one would have to develop the donor platelet registry with the known distribution of HPA antigens among donors.

Platelet genotyping is currently considered the gold standard for HPA typing and it is useful when correlated with the results of antiplatelet antibody testing to determine alloimmunization and to measure the risk of AIT [10, 19].

The allele frequencies of investigated HPA blood donors in the Serbian population (Table 2) disclosed that allele *a* was more frequent than allele *b* in 7 of the 8 tested HPA systems. Only in HPA-15 was allele *b* slightly more frequent than allele *a* in our cohort.

Published data of other nations of Southeastern Europe reveal results similar to those of the Serbian population. HPA allele frequencies in populations of Slovenia (HPA-1 to HPA-5) [16], North Macedonia (HPA-1 to HPA-3 and HPA-5) [20], and Croatia (HPA-1 to HPA-3, HPA-5, and HPA-15) [21, 22], as shown in Table 3, all showed no significant differences in comparison with our results ($p > 0.05$ for all populations and HPA systems that were tested in these studies). Similarly, statistically significant differences were not encountered between the Serbian population and data from non-Finnish European populations from the gnomAD database [23], or other European populations [10]. There are, however, significant differences between the Serbian population and some populations of non-European origin, as exemplified by populations from Asia [10, 14], Africa [8, 10], and South America [10].

HPA-1 bb homozygotes are at the biggest risk of immunization, because they may form anti-HPA-1a antibodies which are, as previously mentioned, responsible for 80% of FNAIT cases [3, 9]. The frequency of the HPA-1 bb genotype is indicated to be 1.4% in the Serbian population, which correlates with its frequency in other populations of European origin [10, 16, 20, 21, 23].

In our study we could not find any sample with the HPA-2 *bb* genotype. We are aware that this study was performed in a relatively small sample. As per the Hardy-Weinberg principle, from which our sample group did not deviate in a statistically significant manner, we should have had around 0.7% of HPA-2 *bb* genotypes in our population (Table 2). This fact is one of the motives for expanding our research in the future. With this genotype, our registry would contain all possible HPA genotypes from the 4 clinically important HPA systems (HPA-1, HPA-2, HPA-3, and HPA-15).

As expected, the HPA-4b allele was not identified in our population in this study. It is already established that the HPA-4b allele is not common, if present at all, among populations of European or African origin, but it is more frequent among Asian populations [8, 14, 24]. According to the available published reports, populations are usually homozygous for allele *a* in HPA-4 and HPA-6 systems. When there is a *b* allele detected within these systems, usually heterozygotes are found, mainly in Asian populations [14, 25, 26]. In sporadic cases some *bb* homozygotes in these 2 systems have been caught [25, 26].

For the HPA-9 system, data are even more scarce [6, 14, 15, 27–29]. The HPA-9 system has not been studied often and, even when it has been, its *b* allele has usually not been detected, especially within European population studies. We were therefore pleasantly surprised when we found a participant in our study with repeatedly confirmed HPA-9b SNP. To our knowledge, this donor is of Serbian (European) origin. Considering that the HPA-9b allele results from a nucleotide substitution (2602G>A) in the HPA-3b allele, the finding of HPA-3b/3b and HPA-9a/9b genotypes suggests that this donor carries the ITGA2B*002 and ITGA2B*003 alleles that encode glycoproteins IIb expressing HPA-3b and HPA-3b-9bw epitopes, respectively [5, 6, 29].

The transfusion MP calculated for HPA specificities in our population confirms that there is a clinical need for developing an HPA genotyped platelet donor registry to provide matched transfusions to alloimmunized patients. Our estimations demonstrate that the biggest transfusion MP in the Serbian population, based on the observed allele frequencies, stands for systems HPA-15 (37.4%) and HPA-3 (36.2%), which means that more than a third of random transfusions could cause a mismatch within these 2 systems. This finding did not come as a surprise, because we detected more *bb* homozygous genotypes in these HPA systems than in any other. Therefore, there is a higher probability of a mismatch for both *aa* and *bb* homozygotes of HPA-15 and HPA-3 when they receive random non-HPA-matched transfusions. Our data show that HPA-1, HPA-5, and HPA-2 systems also have notably considerable transfusion MP of 20.3, 16.0, and 14.1%, respectively. All of these HPA systems are the ones whose

antigens have the highest immunization capacity [18]. Taking into account all these data, we can claim that alloimmunization against antigens belonging to the HPA-1, HPA-2, HPA-3, HPA-5, and HPA-15 systems should be considered the main cause of alloimmune thrombocytopenia in Serbia. Finally, we affirm that we have successfully implemented the PCR-SSP method with fluorometric signal detection (FluoGene), which detects 8 HPA systems (HPA-1 to HPA-6, HPA-9, and HPA-15) into our institute's routine HPA genotyping. We are aware that it could be further complemented by the introduction of high-throughput methods [29, 30], which are evidently useful for large donor pools but are also largely dependent on available financial resources. The greatest advantages of such methods would be the possibility to detect more HPA polymorphisms by typing more HPA systems and to simultaneously test more donors [30]. We additionally acknowledge that, for the best therapeutic effect of platelet transfusion therapy, it is important to develop the donor platelet registry with the known distribution of HPA antigens among donors within all ABO types, which is our intent for the future.

Conclusions

This is the first report of platelet genotyping and a population study of HPA allelic and genotype frequencies in a healthy donor population in Serbia. Although Serbia is a country in Southeastern Europe that has gone through various population migrations throughout the centuries, no significant differences in HPA allele and genotype frequencies versus other populations from Southeastern Europe were observed among the tested HPA systems. Nevertheless, this initial analysis of HPA allele and genotype frequencies can serve as the basis for a platelet apheresis donor registry. HPA genotyping can aid in the diagnostics of FNAIT, posttransfusion purpura, and platelet transfusion refractoriness and lead to a more accurate transfusion matching for patients with platelet alloimmune disorders.

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Statement of Ethics

The study protocol was approved by the Ethical Committee of the BTIS, and written consent was provided by each participant.

Conflict of Interest Statement

M.L. is employed as a scientific advisor by a local distributor of Inno-Train Diagnostik, which was not involved in this study in any way, particularly scientifically, financially, or in the writing of this paper. All other authors declare no conflict of interests.

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Author Contributions

S.J.S. and M.L.: substantial contributions to study conception and design, acquisition of data, analysis and interpretation of data, drafting of this paper, and critical revision for important intellectual content. N.V.: selection and collection of blood donors for molecular typing and critical revision of this work for important intellectual content. I.R.: acquisition, analysis, and interpretation of data, assistance with compilation of the results and critical revision of this work for important intellectual content. I.Dj.: assistance with compilation of the results and critical revision of this work for important intellectual content. All of the authors approved the final version of this paper for publication.