

Distribution of human platelet antigen genotypes in Lithuanian blood donors

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Background - Human platelet antigens (HPAs) are polymorphic structures on platelet membrane glycoproteins that may induce alloimmune responses after transfusion or pregnancy. Knowledge of HPA distribution in different populations is essential for improving transfusion practice and managing platelet transfusion refractoriness.

Materials and methods - A total of 200 unrelated voluntary blood donors were included. Genomic DNA was extracted from EDTA-anticoagulated whole blood, and HPA genotyping was performed using polymerase chain reaction with sequence-specific primers. Genotype and allele frequencies were calculated, and Hardy-Weinberg equilibrium was assessed using the χ^2 test.

Results - The most frequent genotypes were HPA-1aa, HPA-2aa, HPA-3ab, HPA-5aa, and HPA-15ab. Allele frequencies were 0.833 (HPA-1a), 0.925 (HPA-2a), 0.578 (HPA-3a), 0.923 (HPA-5a), and 0.525 (HPA-15a). The HPA-6 and HPA-9 systems were monomorphic. No significant deviations from Hardy-Weinberg equilibrium or sex-related differences were observed.

Discussion - The distribution of HPA alleles in Lithuanian donors is comparable to other European populations. These findings are clinically relevant for improving donor-recipient compatibility and may support the development of HPA-typed donor registries, contributing to better management of platelet transfusion refractoriness.

Keywords: human platelet antigens, HPA genotyping, platelet transfusion refractoriness, blood donors, transfusion medicine.

INTRODUCTION

Human platelet antigens (HPAs) are polymorphic structures located on platelet membrane glycoproteins and are determined by single-nucleotide substitutions in genes encoding platelet surface proteins. These polymorphisms may lead to alloantibody formation after exposure to foreign antigens through transfusion or pregnancy. HPA incompatibility is clinically relevant in several immune-mediated conditions, including fetal and neonatal alloimmune thrombocytopenia, post-transfusion purpura, and platelet transfusion refractoriness^{1,2}.

The distribution of HPA alleles differs across ethnic and geographic populations, and these differences influence the likelihood of alloimmunization and the availability of compatible platelet donors. Therefore, determination of HPA genotype frequencies in a

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given population is important for diagnosing immune thrombocytopenic disorders and providing compatible platelet transfusions³⁻⁵.

Early investigations of HPA systems relied mainly on serological methods; however, these techniques have largely been replaced by molecular approaches. DNA-based genotyping enables accurate identification of platelet antigen polymorphisms and supports reliable population studies and large-scale donor screening programs^{2,6}. Molecular typing of platelet antigens is increasingly used in transfusion medicine to improve donor-recipient matching and to reduce the risk of alloimmune complications⁶.

Knowledge of population-specific HPA distributions is particularly important for establishing registries of genotyped donors. Such registries allow rapid identification of compatible platelet units for patients with suspected alloimmune thrombocytopenia or platelet transfusion refractoriness, and represent an important component of modern transfusion support^{3,7}. In addition, analysis of allele frequencies across populations contributes to our understanding of genetic variability between ethnic groups and may help predict the risk of alloimmunization^{8,9}.

Several studies have reported the distribution of HPA genotypes in European and non-European populations, demonstrating that allele frequencies may vary considerably between different regions^{5,7-9}. However, data on the distribution of human platelet antigens in the Lithuanian population are scarce and, to our knowledge, no comprehensive study of HPA genotype frequencies in Lithuanian blood donors has yet been published.

The aim of this study was to determine the genotype and allele frequencies of clinically relevant human platelet antigens in Lithuanian platelet donors using molecular methods, and to compare the results with those reported in other populations.

MATERIALS AND METHODS

Study population

The study included 200 unrelated voluntary blood donors recruited at the Blood Centre of the Hospital of the Lithuanian University of Health Sciences Kauno klinikos, Lithuania. Peripheral blood samples were collected into ethylenediaminetetraacetic acid (EDTA)-containing tubes

prior to routine laboratory testing. All laboratory analyses were performed at the Laboratory Medicine Clinic of the Hospital of the Lithuanian University of Health Sciences Kauno klinikos, Lithuania. All donors were healthy individuals eligible for blood donation according to national blood donation regulations. Only donors of Lithuanian origin were included in the study.

The study protocol was approved by the Kaunas Regional Biomedical Research Ethics Committee (No. BE-2-26, 11 August 2022), and all procedures were performed in accordance with the principles of the Declaration of Helsinki.

DNA extraction

Genomic DNA was extracted from EDTA-anticoagulated whole blood samples obtained from 200 voluntary blood donors using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

HPA genotyping

Genotyping of HPAs was performed using commercially available HPA-FluoGene kits (Inno-Train Diagnostik, Kronberg, Germany) according to the manufacturer's instructions. The FluoGene assay is based on polymerase chain reaction with sequence-specific primers (PCR-SSP), allowing detection of single-nucleotide polymorphisms responsible for platelet antigen polymorphisms^{1,6}. Detection of amplification products was performed as an endpoint fluorometric method using the FluoVista analyzer (Inno-train Diagnostik), and genotype assignment was automatically carried out using FluoGene software (Inno-train Diagnostik).

HPA systems analyzed

The following HPA systems were analyzed: HPA-1, HPA-2, HPA-3, HPA-4, HPA-5, HPA-6, HPA-9, and HPA-15. These represent clinically relevant platelet alloantigen systems defined by single-nucleotide polymorphisms in genes encoding platelet membrane glycoproteins^{1,8}.

Statistical analysis

Genotype and allele frequencies were calculated by direct counting. Expected genotype frequencies were calculated according to the Hardy-Weinberg equilibrium, and deviations from equilibrium were evaluated using the χ^2 tests. Differences in genotype distribution between male and female donors were analyzed using the χ^2 tests.

Statistical analyses were performed using IBM SPSS Statistics software (version 30.0; IBM Corp., Armonk, NY, USA). A p value <0.05 was considered statistically significant.

RESULTS

A total of 200 voluntary blood donors were included in the study: 104 females and 96 males. The mean age of donors was 33.6 (± 12.1) years (range: 18-62 years); mean age was 33.1 (± 12.0) years for females and 34.1 (± 12.3) years for males.

All donors were successfully genotyped for human platelet antigen systems HPA-1, HPA-2, HPA-3, HPA-4, HPA-5, HPA-6, HPA-9, and HPA-15. Genotype frequencies for all analyzed systems are presented in **Table I**, and allele frequencies are shown in **Table II**.

Table I - Genotype frequencies of human platelet antigen systems in Lithuanian blood donors (No.=200)

HPA	Aa, No. (%)	Ab, No. (%)	Bb, No. (%)
HPA-1	138 (69.0)	57 (28.5)	5 (2.5)
HPA-2	171 (85.5)	28 (14.0)	1 (0.5)
HPA-3	64 (32.0)	103 (51.5)	33 (16.5)
HPA-4	199 (99.5)	1 (0.5)	0
HPA-5	169 (84.5)	31 (15.5)	0
HPA-6	200 (100)	0	0
HPA-9	200 (100)	0	0
HPA-15	59 (29.5)	92 (46.0)	49 (24.5)

HPA: human platelet antigen; No.: number.

Table II - Allele frequencies of human platelet antigen systems in Lithuanian blood donors

HPA	Allele a	Allele b
HPA-1	0.833	0.167
HPA-2	0.925	0.075
HPA-3	0.578	0.422
HPA-4	0.998	0.002
HPA-5	0.923	0.077
HPA-6	1.000	0.000
HPA-9	1.000	0.000
HPA-15	0.525	0.475

HPA: human platelet antigen

In the HPA-1 system, the most frequent genotype was HPA-1aa, observed in 69.0% of donors, followed by HPA-1ab (28.5%) and HPA-1bb (2.5%). In the HPA-2

system, the HPA-2aa genotype was detected in 85.5% of donors, heterozygotes in 14.0%, and only one donor (0.5%) was homozygous for HPA-2b. In the HPA-3 system, the heterozygous genotype HPA-3ab was the most frequent (51.5%), followed by HPA-3aa (32.0%) and HPA-3bb (16.5%). The HPA-4 system showed very low polymorphism, with 99.5% of donors being homozygous for HPA-4a and only one heterozygous individual detected. In the HPA-5 system, the HPA-5aa genotype was observed in 84.5% of donors, 15.5% were heterozygous, and no HPA-5bb genotype was found. All donors were homozygous for HPA-6a and HPA-9a alleles, indicating that, in the studied population, the HPA-6 and HPA-9 systems were monomorphic. In the HPA-15 system, genotype HPA-15ab was the most frequent (46.0%), followed by HPA-15aa (29.5%) and HPA-15bb (24.5%).

Allele frequency analysis showed that the most common alleles in the studied population were HPA-1a (0.833), HPA-2a (0.925), HPA-3a (0.578), HPA-5a (0.923), and HPA-15a (0.525), whereas the HPA-4b allele was detected at very low frequency. All individuals carried only the HPA-6a and HPA-9a alleles (**Table II**).

The observed genotype frequencies were consistent with the Hardy-Weinberg equilibrium for all polymorphic HPA systems analyzed (**Table III**). Expected and observed genotype frequencies are shown in **Table III**. The HPA-6 and HPA-9 systems were monomorphic in the studied population and were, therefore, excluded from the Hardy-Weinberg equilibrium analysis.

Genotype distribution was also analyzed according to donor sex. No statistically significant differences in genotype distribution between male and female donors were observed for any of the HPA systems analyzed (**Table IV**). Although minor variations in genotype frequencies were noted between sexes, these differences were not statistically significant and are unlikely to have biological or clinical relevance. The overall similarity in genotype distribution between sexes suggests a homogeneous genetic structure of the studied population.

The allele frequencies observed in the Lithuanian donor population were compared with those reported in other European populations (**Table V**).

Table III - Hardy-Weinberg equilibrium analysis of human platelet antigen genotype frequencies

Genotypes	EN	ON	χ^2	p
HPA-1			0.096	0.757
aa	138	138.6		
ab	57	55.8		
bb	5	5.6		
HPA-2			0.016	0.899
aa	171	171.1		
ab	28	27.8		
bb	1	1.1		
HPA-3			0.613	0.434
aa	64	66.7		
ab	103	97.6		
bb	33	35.7		
HPA-4			0.001	0.972
aa	199	199.0		
ab	1	1.0		
bb	0	0.0		
HPA-5			1.412	0.235
aa	169	170.2		
ab	31	28.6		
bb	0	1.2		
HPA-6			-	-
aa	200	200		
ab	0	0		
bb	0	0		
HPA-9			-	-
aa	200	200		
ab	0	0		
bb	0	0		
HPA-15			1.207	0.272
aa	59	55.1		
ab	92	99.8		
bb	49	45.1		

EN: expected number; HPA: human platelet antigen; ON: observed number.

Table IV - Comparison of genotype distribution according to sex

Genotypes	Males No. (%)	Females No. (%)	χ^2	df	p
HPA-1			0.154	2	0.926
aa	67 (69.8)	71 (68.3)			
ab	27 (28.1)	30 (28.8)			
bb	2 (2.1)	3 (2.9)			
HPA-2			4.264	2	0.119
aa	86 (89.6)	85 (81.7)			
ab	9 (9.4)	19 (18.3)			
bb	1 (1.0)	0 (0.0)			
HPA-3			2.207	2	0.332
aa	26 (27.1)	38 (36.5)			
ab	54 (56.3)	49 (47.1)			
bb	16 (16.7)	17 (16.3)			
HPA-4			1.089	1	0.297
aa	95 (99.0)	104 (100.0)			
ab	1 (1.0)	0 (0.0)			
HPA-5			0.541	1	0.462
aa	83 (86.5)	86 (82.7)			
ab	13 (13.5)	18 (17.3)			
HPA-15			0.414	2	0.813
aa	29 (30.2)	30 (28.8)			
ab	42 (43.8)	50 (48.1)			
bb	25 (26.0)	24 (23.1)			

Percentages are presented within sex. The HPA-6 and HPA-9 systems were monomorphic and were, therefore, excluded from statistical analysis. df: degrees of freedom; No.: number.

Table V - Comparison of human platelet antigen allele frequencies in Lithuanian donors and selected European populations

Population	No.	HPA-1a	HPA-2a	HPA-3a	HPA-5a	HPA-15a	Ref.
Lithuania	200	0.833	0.925	0.578	0.923	0.525	Present study
Greece	1,000	0.820	0.910	0.560	0.910	0.470	Kaltsounis (2024) ¹⁰
Serbia	72	0.868	0.917	0.611	0.903	0.472	Jovanovic Srzentic (2021) ¹¹
Germany	206	0.850	0.900	0.600	0.930	0.510	Hauck-Dlimi (2012) ¹²
Poland	211	0.874	0.898	0.592	0.937	NR	Drzewek (1998) ¹³
Norway	105	0.867	0.943	0.471	0.929	NR	Randen (2003) ¹⁴
Slovenia	100	0.843	0.918	0.566	0.929	NR	Rozman (1999) ¹⁵
Macedonia	150	0.830	0.910	0.580	0.920	NR	Pavkovic (2006) ¹⁶

No.: number; NR: not reported; Ref.: reference.

DISCUSSION

The present study provides the first comprehensive analysis of HPA genotype and allele frequencies in Lithuanian blood donors. The results demonstrate that the distribution of HPA alleles in the Lithuanian population is generally comparable to that observed in other European populations, suggesting a similar genetic background across Caucasian populations.

The predominance of HPA-1a, HPA-2a, HPA-3a, HPA-5a and HPA-15a alleles identified in this study is consistent with previously reported data from European populations, including Greece, Germany and Serbia¹⁰⁻¹². In particular, the frequency of the HPA-1a allele in Lithuanian donors (0.833) closely resembles that reported in neighboring populations, supporting the reliability of the present findings and confirming regional genetic consistency.

The HPA-3 system showed a relatively balanced distribution of alleles, with a predominance of the heterozygous genotype. Similar patterns have been described in other European populations, including German and Polish cohorts^{12,13}. This relatively balanced distribution reflects the highly polymorphic nature of the HPA-3 system and may have clinical relevance, as polymorphic antigens are more likely to be involved in alloimmune responses following platelet transfusion or pregnancy. In contrast, studies conducted in non-European populations have demonstrated different patterns of HPA allele distribution, reflecting ethnic and genetic diversity⁶.

In the present study, the HPA-5 system demonstrated a predominance of the HPA-5a allele, with no HPA-5bb genotype observed. Comparable findings have been reported in several European populations, including those from Norway and Slovenia, where the HPA-5b allele is relatively rare^{14,15}. The low frequency of the HPA-5b allele may influence both the risk of alloimmunization and the availability of compatible platelet donors in clinical practice.

The HPA-15 system showed a relatively balanced distribution of alleles in the Lithuanian population, which agrees with findings from other European populations, including Germany and Serbia^{11,12}. This finding is clinically relevant, as antibodies directed against HPA-15 antigens have been implicated in platelet transfusion refractoriness and other alloimmune conditions.

Notably, the HPA-6 and HPA-9 systems were monomorphic

in the Lithuanian donor population, with all individuals carrying the HPA-6a and HPA-9a alleles. Similar observations have been reported in other European populations, where these systems exhibit very low polymorphism or are completely monomorphic^{11,12}. The absence of variability in these systems suggests limited clinical relevance in the context of alloimmunization within this population.

From the perspective of transfusion medicine, the identification of population-specific HPA frequencies is essential for improving donor-recipient compatibility. The predominance of certain alleles, such as HPA-1a and HPA-5a, together with the low frequency of alternative alleles, may influence the risk of alloimmunization and the availability of compatible platelet units. Individuals carrying rare genotypes may be at increased risk of developing alloantibodies, highlighting the importance of identifying such donors and incorporating them into targeted donor registries^{14,16}.

These findings are highly relevant to the management of immune-mediated platelet transfusion refractoriness. In clinical practice, patients who develop alloantibodies against HPA antigens often require antigen-negative platelet transfusions. The availability of population-based HPA genotype data facilitates the identification of compatible donors and supports the implementation of more personalized transfusion strategies. Establishing a registry of HPA-typed donors in Lithuania could significantly improve transfusion support for alloimmunized patients and reduce delays in providing compatible platelet components¹⁴⁻¹⁶. Although HPA typing represents an important component of donor selection for patients with immune-mediated platelet refractoriness, HLA compatibility should also be considered in the development of donor registries. In the present study, donors were not HLA typed, and, therefore, the proposed registry would currently be limited to information on HPA. Nevertheless, integration of both HPA and HLA typing in future donor registries would provide a more comprehensive approach to identifying compatible platelet donors and improving transfusion support for alloimmunized patients.

Overall, the comparison with other European populations indicates that the Lithuanian donor population shares a common genetic background with neighboring and other

Caucasian populations. At the same time, the availability of national data is essential for optimizing transfusion strategies at the local level and for integration into broader European transfusion networks.

This study has several limitations. The sample size, although sufficient for estimating allele frequencies, is relatively small, and the study population only included healthy blood donors. In addition, clinical data on alloimmunization and transfusion outcomes were not available, and, therefore, the direct impact of HPA variability on platelet transfusion refractoriness could not be assessed. Future studies including patient cohorts, antibody screening, and HLA typing are needed to further clarify the clinical significance of HPA incompatibility and to support the development of comprehensive donor registries for personalized transfusion support.

CONCLUSIONS

In conclusion, this study provides the first comprehensive data on the distribution of human platelet antigen genotypes and allele frequencies in Lithuanian blood donors. The findings demonstrate that the Lithuanian population exhibits a pattern of HPA distribution comparable to that observed in other European populations. From a clinical perspective, the identification of population-specific HPA frequencies is essential for improving platelet donor-recipient compatibility and for optimizing transfusion strategies. These data are particularly relevant for the management of immune-mediated platelet transfusion refractoriness, where the availability of antigen-compatible platelet units is critical. The results of this study support the potential development of HPA-typed donor registries in Lithuania. Future incorporation of HLA typing may further enhance donor selection and facilitate the implementation of more comprehensive and personalized transfusion strategies. Finally, further studies including larger cohorts and patient populations are warranted to better define the clinical impact of HPA incompatibility and to support the implementation of personalized transfusion approaches.

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Authorship contributions

AS: study design, data analysis, manuscript writing. AV: supervision and critical revision. DR: data collection and methodology. ES: laboratory analysis and data interpretation.

The Authors declare no conflicts of interest.

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