

HPA1-29w Genotyping and the Foundation for the Platelet Apheresis Registry in Jiangsu Province of China by MassARRAY Spectrometry

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Keywords

Human platelet alloantigen · Allele frequency · Chinese Han population · MassARRAY spectrometry · Platelet apheresis registry

Abstract

Introduction: This study aimed to investigate the allele frequencies of the human platelet antigens (HPA) HPA-1-29w system in Jiangsu (China) and establish the platelet apheresis registry in blood donors. **Methods:** HPA genotyping was performed using the MassARRAY iPLEX® platform. Allele and genotype frequencies were estimated by direct counting and tested for Hardy-Weinberg equilibrium. The transfusion mismatch probability was calculated for every HPA specificity. **Results:** The HPA allele frequencies in the Jiangsu Han population of HPA-1b, -2b, -3b, -4b, -5b, -6b, -11b, -15b, and -21b were 0.0055, 0.0530, 0.4116, 0.0015, 0.0155, 0.0162, 0.0003, 0.4683, and 0.0070, respectively, in which a heterozygote of HPA-11a/b was first detected in China. Only allele a was detected for HPA-7-10w, -12-14w, -16-20w, and -22-29w quasi-systems. The highest mismatch rate of HPA genes in 1,640 platelet donors was the HPA-15 system, followed by the HPA-3 system with a rate of 37.4% and 36.71%, respectively. **Conclusion:** China's largest-scale platelet registry of HPA-1-29w has been explored. The MassARRAY platform may help found the platelet apheresis registry which would be useful to provide matching platelets and lead to a more accurate, effective, and safe transfusion for patients with platelet therapy.

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Introduction

The human platelet alloantigen (HPA) systems have significance in clinical applications of the alloimmune platelet disorders including fetal and neonatal alloimmune thrombocytopenia, post-transfusion purpura, and platelet transfusion refractoriness (PTR) to random donor platelets [1–6]. To date, 6 HPA systems have been identified and officially nominated by the International Platelet Immunology Nomenclature Committee of the International Society of Blood Transfusion (ISBT), 41 HPAs that include 12 biallelic alloantigens (HPA-1, -2, -3, -4, -5, and -15) and 29 antithetical alloantigens have been described and were identified on six functionally important platelet glycoprotein complexes. Their underlying molecular basis has been resolved: most of these antigens result from single-nucleotide polymorphism (SNP) except a shift of 3 bases due to the deletion of the AAG triplet of HPA-14b, which provides a basis for the genotyping of known platelets. In recent years, several DNA-based methods have been developed for the detection of HPA polymorphisms: such as PCR sequence-specific primers, real-time PCR [7, 8], PCR sequence-based typing (PCR-SBT), next-generation sequencing [9, 10], BeadChip Microarray Technology [11, 12], and matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) MS analysis [13, 14].

Xin Dai, Chengcheng Liu, and Rong Chen contributed equally to this work.

Table 1. The distribution of HPA-1 to HPA-29b systems in the Chinese Han population ($n = 1,640$)

HPA system	N observed			Genotype frequency			Allele frequency		H-W equilibrium		MP
	aa	ab	bb	aa	ab	bb	a	b	χ^2	p value	
HPA-1	1,623	16	1	0.9896	0.0098	0.0006	0.9945	0.0055	18.4988	0.000	0.0109
HPA-2	1,478	150	12	0.9012	0.0915	0.0073	0.9470	0.0530	13.1772	0.000	0.0953
HPA-3	566	798	276	0.3451	0.4866	0.1683	0.5884	0.4116	0.0344	0.853	0.3671
HPA-4	1,635	5	0	0.9970	0.0030	0.0000	0.9985	0.0015	0.0038	0.951	0.0030
HPA-5	1,589	51	0	0.9689	0.0311	0.0000	0.9845	0.0155	0.4092	0.522	0.0301
HPA-6w	1,588	51	1	0.9683	0.0311	0.0006	0.9838	0.0162	0.7888	0.374	0.0314
HPA-7w	1,640	0	0	1.0000	0.0000	0.0000	1.0000	0.0000	NA	NA	0.0000
HPA-8w	1,640	0	0	1.0000	0.0000	0.0000	1.0000	0.0000	NA	NA	0.0000
HPA-9w	1,640	0	0	1.0000	0.0000	0.0000	1.0000	0.0000	NA	NA	0.0000
HPA-10w	1,640	0	0	1.0000	0.0000	0.0000	1.0000	0.0000	NA	NA	0.0000
HPA-11w	1,639	1	0	0.9994	0.0006	0.0000	0.9997	0.0003	0.0002	0.990	0.0006
HPA-12w	1,640	0	0	1.0000	0.0000	0.0000	1.0000	0.0000	NA	NA	0.0000
HPA-13w	1,640	0	0	1.0000	0.0000	0.0000	1.0000	0.0000	NA	NA	0.0000
HPA-14w	1,640	0	0	1.0000	0.0000	0.0000	1.0000	0.0000	NA	NA	0.0000
HPA-15	461	822	357	0.2811	0.5012	0.2177	0.5317	0.4683	0.0690	0.793	0.3740
HPA-16w	1,640	0	0	1.0000	0.0000	0.0000	1.0000	0.0000	NA	NA	0.0000
HPA-17w	1,640	0	0	1.0000	0.0000	0.0000	1.0000	0.0000	NA	NA	0.0000
HPA-18w	1,640	0	0	1.0000	0.0000	0.0000	1.0000	0.0000	NA	NA	0.0000
HPA-19w	1,640	0	0	1.0000	0.0000	0.0000	1.0000	0.0000	NA	NA	0.0000
HPA-20w	1,640	0	0	1.0000	0.0000	0.0000	1.0000	0.0000	NA	NA	0.0000
HPA-21w	1,617	23	0	0.9860	0.0140	0.0000	0.9930	0.0070	0.0818	0.775	0.0138
HPA-22w	1,640	0	0	1.0000	0.0000	0.0000	1.0000	0.0000	NA	NA	0.0000
HPA-23w	1,640	0	0	1.0000	0.0000	0.0000	1.0000	0.0000	NA	NA	0.0000
HPA-24w	1,640	0	0	1.0000	0.0000	0.0000	1.0000	0.0000	NA	NA	0.0000
HPA-25w	1,640	0	0	1.0000	0.0000	0.0000	1.0000	0.0000	NA	NA	0.0000
HPA-26w	1,640	0	0	1.0000	0.0000	0.0000	1.0000	0.0000	NA	NA	0.0000
HPA-27w	1,640	0	0	1.0000	0.0000	0.0000	1.0000	0.0000	NA	NA	0.0000
HPA-28w	1,640	0	0	1.0000	0.0000	0.0000	1.0000	0.0000	NA	NA	0.0000
HPA-29w	1,640	0	0	1.0000	0.0000	0.0000	1.0000	0.0000	NA	NA	0.0000

MP, mismatch probability; NA, no assay.

In our previous study [15], we established an SNP genotyping technology for genotyping HPA-1 to HPA-29w systems simultaneously, using the MALDI-TOF MS using the MassARRAY platform. This work is to explore the frequencies of genotypes and alleles of HPA-1 to -29w systems in the Jiangsu (China) Han population and found the platelet apheresis registry in blood donors, which may help assess the alloimmune platelet disorders and improve the platelet transfusion efficiency and safety.

Material and Methods

Blood Samples and DNA Extraction

A total of 1,640 peripheral blood samples of the Chinese Han population in Jiangsu Province were collected from regular platelet donors who have donated the platelet at least three times. Written informed consent was obtained from all healthy volunteers with the approval of the Ethical Scientific Committee of Jiangsu Province Blood Center (China) for blood samples. Genomic DNA was extracted from the blood samples using the automated nucleic acid extractor (E.HT01.Z0.0096, Zhongkebio med technol,

Nanjing, China) and his correlative magnetic nanoparticle kit (K.QX02.B0.1000) according to the manufacturer's instructions. The DNA concentration is adjusted to 20–200 ng/ μ L and the OD260/280 value is 1.7–1.9.

SNP Selection and Amplification

HPA-1 to -29w polymorphism nucleotide sequences and nucleotide change of single SNPs were obtained from the HPA Gene Database (online suppl. Table S1; for all online suppl. material, see <https://doi.org/10.1159/000535653>) [16]. Amplification and extension primers flanking the gene region of the SNPs were designed by Sequenom's MassARRAY Designer software (<http://agenacx.com/online-tools>) (Table S2). The first PCR on 384 multi-titration PCR plate was performed using forward and reverse primer pool with initial denaturing at 95°C for 2 min followed by 45 cycles of 30 s at 95°C, 30 s at 56°C and 1 min at 72°C, plus a final extension at 72°C for 5 min and 4°C forever; the shrimp alkaline phosphatase was then used to disgust unincorporated dNTPs from the first PCR reaction (37°C 40 min; 85°C 5 min; 12°C, Hold). iPLEX extension reaction was performed using pool single base extension primers with initial denaturing at 94°C for 30 s followed by 40 cycles of 5 cycles of 5 s denaturing at 94°C, for 5 s annealing at 52°C, 5 s extension at 80°C, plus a final extension at 72°C for 3 min, and 12°C forever.

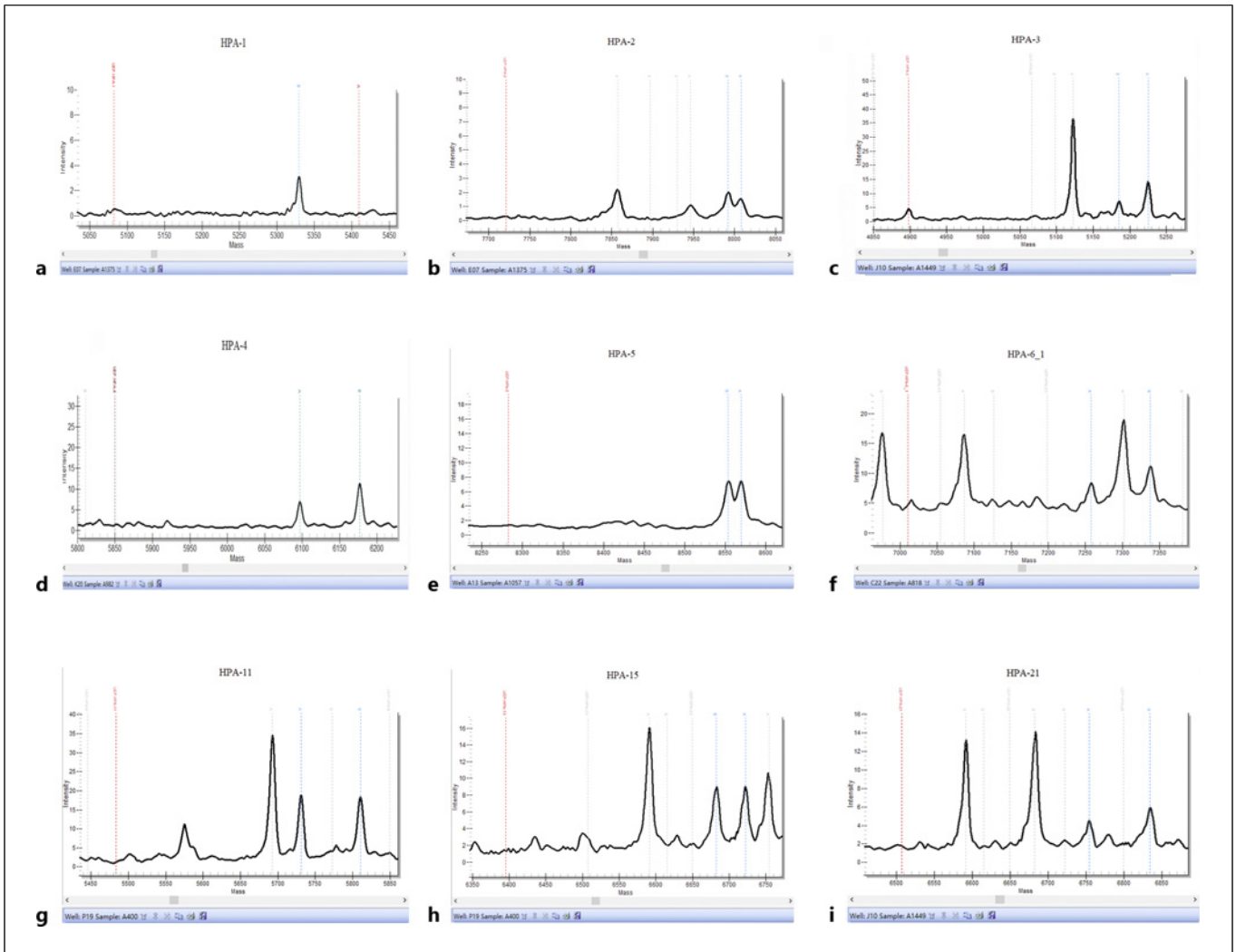


Fig. 1. Mass spectrometry diagram of allele b for HPA variants of this study. MassARRAY spectrum. The peaks corresponding to the HPA alleles are indicated with the color blue. HPA-1b/b (a), HPA-2a/b (b), HPA-3a/b (c), HPA-4a/b (d), HPA-5a/b (e), HPA-6a/b (f), HPA-11a/b (g), HPA-15a/b (h), HPA-21a/b (i).

Genotyping by MassARRAY Spectrometry

The samples were genotyped in two wells with different group primer pairs, using the Sequenom MassARRAY® iPLEX platform. The whole process was performed according to the manufacturer's instructions for the multiplex reactions, including the PCR amplification, the Shrimp Alkaline Phosphatase (SAP) treatment, the iPLEX extension reactions, and the desalting of the amplified products. 2 μ L of DNA sample with a concentration of 20–50 ng/ μ L was used with volume 5 μ L for the locus-specific PCR reaction. The amplified products were desalted using SpectroCLEAN resin following protocol [17]. The cleaned extension products were transferred from 384 multi-titration PCR plate to 384 SpectroCHIP by RS100 nanodispenser and the chip was placed in Agena MassARRAY compact mass spectrometer and the mass signals for the different alleles were captured with high accuracy by MALDI-TOF MS. Agena MassARRAY Typer V4.0.5 (Sequenom Inc., San Diego, USA) was used to obtain and process the raw data from the assays.

Statistical Analysis

Allele frequencies were calculated by the gene counting method. The validity of the Hardy-Weinberg Equilibrium (HWE) was calculated by the classical χ^2 test and a p value <0.05 was

considered a departure from HWE. The mismatch probability (MP) after random transfusions of platelet concentrate for each of the evaluated HPA specificities was tested 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. HPA variant frequencies observed in Jiangsu were compared with different ethnic groups retrieved from the 1000 Genomes Project database by the χ^2 test or Fisher's exact test. Differences were considered significant when p values <0.05.

Results

The Distribution of HPA-1 to 29w Systems by MassARRAY Spectrometry

Frequencies of HPA variants were analyzed in a total of 1,640 platelet donors using matrix-assisted laser desorption ionization mass spectrometry. The frequencies

Table 2. Comparison of HPA allele "a" frequencies between Jiangsu Han Chinese and global subpopulations in 1000 Genomes projects

HPA systems	HPA-1		HPA-2		HPA-3		HPA-4		HPA-5		HPA-6b		HPA-11b		HPA-15		HPA-21b		
	T	C	C	T	T	G	G	A	G	A	G	A	G	A	C	A	C	A	
nucleotide change																			
AFR	1,201**	121	990**	332	749	573	1,322	0	1,053**	269	1,321**	1	16,247	1	866**	456	1,322*	0	
AMR	623**	71	587**	107	441*	253	686**	8	648**	46	694**	0	34,566	6	352	342	694*	0	
EUR	873**	133	918**	88	615	391	1,006	0	917**	89	1,003**	3	135,354	10	498*	508	1,006*	0	
EAS	999	9	937*	71	548*	460	1,004	4	976*	32	993	15	18,394	0	531	477	1,008	3	
SAS	867**	111	917	61	650**	328	977	1	892**	86	978**	0	30,602	0	335**	643	978*	0	
CHB	204	2	193	13	112	94	205	1	202	4	201	5	/	/	104	102	206	0	
CHS	209	1	203	7	121	89	210	0	204	6	209	1	/	/	120	90	210	1	
CJS	3,262	18	3,106	174	1,930	1,350	3,275	5	3,229	51	3,227	53	3,279	1	1,744	1,536	3,257	23	

The populations listed above are as follows: AFR, African; AMR, American; EUR, European; EAS, East Asian; SAS, South Asian; CHB, Han Chinese in Beijing, China; CHS, Southern Han Chinese, China; CJS, Jiangsu Han Chinese, China. *p* values: * <0.05 , ** <0.001 .

of HPA alleles and HPA genotypes were displayed in Table 1 which summarized the observed frequencies of HPA variants along with the counts of samples carrying the variant in homozygous and heterozygous states. Among the 29 HPA systems, only HPA-1 to 6w, HPA-11w, HPA-15, and HPA-21w systems showed polymorphism, the bigger variabilities were found within the HPA-3 and HPA-15 systems, the homozygote for the HPA-1b, -2b, and -6b alleles were detected at low frequencies and complete a/a homozygotes were found in the remaining 20 HPA quasi-systems, while HPA-11a/b was the first detected heterozygote in the Chinese Han population. The partial sequencing chromatograms are shown in Figure 1.

HWE and Mismatch Probabilities

Among the 9 heterozygous HPA variants, 2 cases (HPA-1 and HPA-2) deviated from the HWE ($p < 0.05$), and these deviated loci showed significant heterozygote deficiencies in 1,640 platelet donors. No significant deviation from HWE was observed for any of the 8 HPA specificities (HPA-3, -4, -5, -6, -11, -15, and HPA-21). HPA mismatch probabilities have only existed in HPA-1 to 6w, HPA-11w, HPA-15, and HPA-21w systems in the Jiangsu Han population. The highest mismatch rate of HPA genes in 1,640 platelet donors was the HPA-15 system, followed by the HPA-3 system with a rate of 37.4% and 36.71%, respectively. However, the probabilities of HPA mismatch were lower in the HPA-1, HPA-4, HPA-5, HPA-6w, HPA-11w, and HPA-21w systems because most individuals were a/a homozygote in the Chinese Han population (Table 1).

Statistic of Heterozygous HPA Frequencies between Jiangsu and the Subpopulations of 1000 Genomes Project

Two-tailed significance tests for 2×2 contingency tables were used to compare the allele counts and allele numbers observed in the study dataset with the subpopulations of the 1000 Genomes Project. It was found that frequencies of HPA-1a, 2a, -5a, -6a, and 21a were found significantly distinct from more than African, American, and European subpopulations. Jiangsu frequencies had no statistically significant differences with Han Chinese in Beijing (China) and Southern Han Chinese, but a few differences with East or South Asian populations (Table 2).

Discussion

This study provides the first comprehensive analysis of HPA1-29w alleles and genotype frequency data of the Chinese Han population using MassARRAY

spectrometry. Our results showed that the HPA1-29 a/a genotype was present in the Jiangsu population, with a frequency range from 53.17% to 100%, but only allele a was detected for HPA-7-10w,-12-14w,-16-20w, and -22-29w systems, the HPA-8b and HPA-10b with low frequency [18, 19], which have also been reported in the Chinese population have not been observed in this work. Interestingly, a heterozygote of HPA-11a/b, which was the first case reported and identified by SBT (online suppl. Fig. S1) in the Chinese population has been found in the Jiangsu Han population in this study. HPA-1 and HPA-2 showed significant disequilibrium in the Jiangsu Han population, the possible cause might be that the test specimens came from platelet donors and were not randomly sampled due to the exclusion of some ineligible donors. Our data supported that the frequency and distribution of HPA systems have regional characteristics and would be useful to establish the basis for a platelet apheresis donor registry. Therefore, it should focus on the involvement of these HPA systems in alloimmune platelet disorders in the Jiangsu Han population.

It is very well established that the frequency distribution of HPAs differed among various human ethnic groups of different geographical regions. The 1000 Genomes project is a large-scale effort to catalog genetic variations in human populations from around the world, which provides a reliable source of information about human genetic variation generated based on whole genome sequencing data from more than 2,500 individuals across 26 populations from five continental groups [20], so we compared the HPA allele frequencies in Jiangsu with those from other ethnic groups using this publicly available dataset. The comparison indicates that, in general, the frequencies of HPA-1a, 2a, -5a, -6a, and -21a in Jiangsu Han population differ from those of the African, American, and European subpopulations, but with a few differences from East or South Asian populations. It is observed that the Jiangsu Han population presented variation for the frequencies of HPA-1a, -2a, -3a, -4a, -5a, -6a, -15a, and -21a comparable to those of the Chinese in North and South China Han populations, differ subtly from East Asian populations in HPA--2a, -3a, -5a, and be distinguished from South Asian populations in HPA-1a, -3a, -5a, -6a, -15a, and -21a. Our study showed polymorphism in the Jiangsu Han population and the similarities and differences in the allelic profiles with those of other global ethnic groups.

Providing HPA-matched donors for platelet transfusion is the optimal strategy for patients with platelet antibodies or for patients who do not yet have platelet antibodies but are expected to require multiple platelet transfusions in the future, to reduce the risk of alloimmune thrombocytopenia and refractoriness to platelet transfusion [21, 22]. Therefore, genotyping of HPA and establishing population-specific donor registries

to serve donor selection and administration of platelet-specific immunization have been suggested but cannot be easily performed without a registry with a reasonable donor pool size and composition. Nevertheless, developing a large donor pool registry is dependent on available genotyping methods as well as on great financial resources. MassARRAY has the advantages of high throughput, high-cost performance, high sensitivity, and high flexibility [23]. This technology is much less expensive than PCR-SBT and next-generation sequencing, while the throughput is much higher than the above two sequencing technologies, and the customization is flexible, allowing the free selection of SNP loci of interest for design. In the MassARRAY method, a chip has 384 wells, which can detect up to 384 samples at the same time. In addition, up to 40 SNPs can be detected in one well, and it only needs 10 ng of DNA to test, which can shorten the time and make good use of the precious samples [24]. Therefore, the MassArray platform is the most suitable typing technology for platelet donor registry from the viewpoint of technical performance and health economics.

To date, no uniform standard has been established for the platelet donor registry in China, and each region selected the genotyping technology and the HPA-typed system according to their local situation. The technologies used included PCR-restriction fragment length polymorphism, PCR--allele-specific oligonucleotide, PCR-SBT, etc., and the mainly HPA-typed patterns were detecting HPA-1~6w, -15, -21w pattern, HPA-1~17w pattern, HPA-1~21w pattern or other patterns [25]. To study the distribution of HPA1-29w polymorphism in the Chinese population and to meet the long-term clinical outcome of patients, we used the HPA1-29w typing pattern in our registry and submitted 1,640 cases of HPA1-29w genotyping data into the Chinese platelet donor registry, which are the largest amount of HPA1-29w systems genotyping data in China.

In summary, this study provides a comprehensive catalog of HPA allele frequencies in the Jiangsu population by MassARRAY spectrometry and the frequencies of genotypes/alleles of HPA-1 to -29w systems were first obtained in the Chinese population. Our results could be helpful to establish a useful HPA-matched plateletpheresis donor registry, provide support in platelet transfusion, and minimize the risks associated with HPA alloimmunization.

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Statement of Ethics

The study protocol was approved by the Ethics Committee of Jiangsu Province Blood Center (APPROVAL REFERENCE NUMBER:20180312). All donors provided written informed consent for their donations and data collection. The study was performed following the principles of the Declaration of Helsinki.

Conflict of Interest Statement

The authors declare no competing interests.

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Author Contributions

W.L. and H.L. contributed to the conception and design of the study and drafting of this paper. X.D., R.C., T.J., and R.Z. selection and collection of blood donors, as well as a foundation for platelet apheresis registry. C.L. genotyping the donor samples. C.F. and T.L. acquisition, analysis, and interpretation of data. All authors provided critical feedback and helped shape the research, analysis, and manuscript, and approved the final version of this paper for publication.

Data Availability Statement

All data generated or analyzed during this study are included in this article and its online supplementary material files. Further inquiries can be directed to the corresponding author.