

## Original Article

# Genetic polymorphism of human platelet antigens -1 to -17w in the multi-ethnic Chinese Guangxi population

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**Abstract:** Background and Objectives: Human platelet antigens (HPAs) are polymorphic structures associated with several platelet-specific alloimmune disorders. The frequencies of HPAs vary between different populations and ethnic groups. No data are available about the HPAs' frequencies among the Chinese Guangxi population. The purpose of this study was to investigate the allele frequencies of HPA-1 to -17w in healthy volunteer blood donors of the Guangxi population. Methods: The polymerase chain reaction-sequence specific primer (PCR-SSP) methods were used to determine the genotypes of HPA-1 to -17w systems in the four major ethnic groups (Han, Zhuang, Yao, and Miao) in the Guangxi population. A total of 400 random donor samples, 100 from each of the four ethnic groups, were genotyped. A DNA sequencing method was used to validate the results. Results: The three genotypes (aa, ab, and bb) were found in HPA-1, HPA-2, HPA-3, HPA-5, HPA-6w, and HPA-15 systems, and HPA-3 and HPA-15 systems exhibited the highest polymorphisms in our population. The frequency of aa, ab, and b/b genotypes was 0.40, 0.38, and 0.22 in the HPA-3 system and 0.77, 0.16, and 0.08 in the HPA-15 system, respectively. The allele frequencies of HPA in Han, Zhuang, Yao, and Miao ethnic groups displayed only a slight difference among them. Conclusions: This is the first study to investigate the HPA allele frequencies in the multi-ethnic Guangxi populations. These data may be useful for determining the distribution of HPA polymorphisms in this region and for future clinical research associated with platelet disorders in these groups.

**Keywords:** Human platelet antigens, polymorphism, Guangxi, minority

## Introduction

Human platelet antigens (HPAs) are polymorphic structures expressed on the membranes of platelets. Platelet membrane glycoproteins (GPs) are located in these polymorphic structures and are caused by single nucleotide polymorphisms (SNPs) in the genes that encode them [1]. The amino acid changes resulting from these SNPs induce changes in GP structure to form antigens that can elicit antibodies through exposure from transfusion or pregnancy [1]. Antibodies against HPAs can provoke an immune response causing several immune-mediated platelet disorders, such as fetal/neonatal alloimmune thrombocytopenia (FNAIT), post transfusion purpura (PTP), and platelet transfusion refractoriness (PTR) [2]. Therefore, data on the distribution of HPAs alleles in a given population are essential for the provision and diagnosis of HPA-matched blood compo-

nents for patients with those immune-mediated platelet disorders.

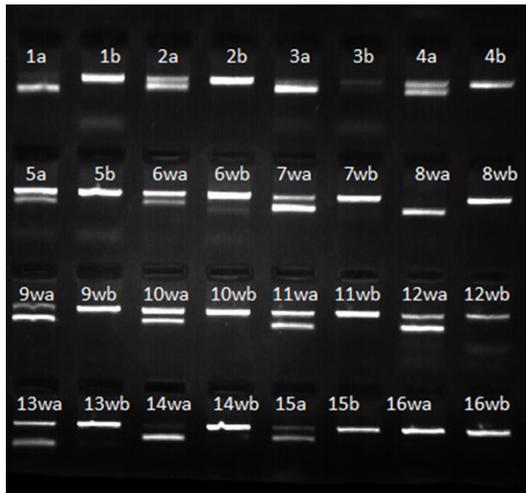
To date, 35 HPAs expressed on six different platelet GPs (GPIIIa, GPIIb/IIIa, GPIIb, GPIa, GPIIb/IIIa, and CD109) have been officially recognized ([http://www.ebi.ac.uk/ipd/hpa/](http://www.ebi.ac.uk/ipd/hpa/Table1.html)Table 1.html). Twelve HPAs are clustering into six biallelic groups (HPA-1, HPA-2, HPA-3, HPA-4, HPA-5 and HPA-15). HPAs are numbered in their order of discovery with the higher frequency antigen designated as "a" and the lower frequency antigen designated "b". In the remaining HPAs, for which alloantibodies against only one of the two antigens are labeled with a "w" for workshop (e.g., HPA-8w).

The frequencies of HPAs vary between different populations and ethnic groups. Some investigations have reported the frequencies of HPA systems amongst various Caucasian [3-5], Asian

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**Table 1.** Characteristics of study subjects classified into four ethnic groups

Groups	Han (n=100)	Zhuang (n=100)	Yao (n=100)	Miao (n=100)
Gender (Male/Female)	60/40	59/41	62/38	57/43
Age (years) (Mean $\pm$ SD)	49.4 $\pm$ 5.3	48.3 $\pm$ 5.7	46.9 $\pm$ 4.6	50.3 $\pm$ 4.7



**Figure 1.** PCR-SSP and HPA genotyping assays. Ethidium bromide-stained gel showing PCR bands amplified using primers specific for the HPA systems polymorphisms in the patients DNA (PCR-SSP). The first row of bands is an internal PCR control amplification of the human growth hormone gene. Presence of a band in the second row indicates that the patient has the corresponding allele. 1a, 2a, 3a, 4a, 5a, 6wa, 6wb, 7wa, 8wa, 9wa, 10wa, 11wa, 12wa, 13wa 14wa 15a were positive.

[6-13], African [14, 15], and Amerindian [16, 17] populations. So far, the frequencies of HPAs have been most extensively studied in Caucasian populations. In Caucasians, there are higher gene frequencies for HPA-1b, 2b, and 5b than in other populations [5]. However, the information for distribution of HLA alleles in the Chinese population is rarely reported. Presently, only two data [11, 12] about the frequency of HPA-1 to 17w systems and one study [13] about the frequency of HPA-17w to -21w systems among Chinese individuals exist. All these studies focused on the Chinese Han population. Allele frequency data among the population of Chinese ethnic minority groups are, however, lacking. Guangxi is a multiethnic province in the south of China with a population of approximately 46 million. The majority of inhabitants are Han (58.8%), Zhuang (33%), Yao (3%), and Miao (1%).

Because distribution of HPA genotypes is geographically and ethnically restricted, and because information on the distribution of HPA genes in various ethnic groups of the Guangxi populations is scanty, we decided to determine the frequency of HPA-1 to -17w in the Han,

Zhuang, Yao, and Miao ethnic populations of Guangxi. We hope that this study can provide an informative background of HPA polymorphisms in Guangxi, establish the credible basis to screen compatible platelets for transfusing patients, and consequently enable risk assessment in multi-ethnic environments.

### Materials and methods

#### Study population

The participants were randomly recruited from volunteer blood donors of the Guangxi population. All participants in this study were unrelated and healthy individuals of Guangxi nationality and were grouped according to their self-reported ethnicities. A total of 400 peripheral blood samples were collected from the blood donors, equally divided between the four major ethnic groups (Han: 100; Zhuang: 100; Yao: 100; and Miao: 100). This research was approved by the ethics committee of the First Affiliated Hospital of Guangxi Medical University. All of the involved subjects provided written informed consent.

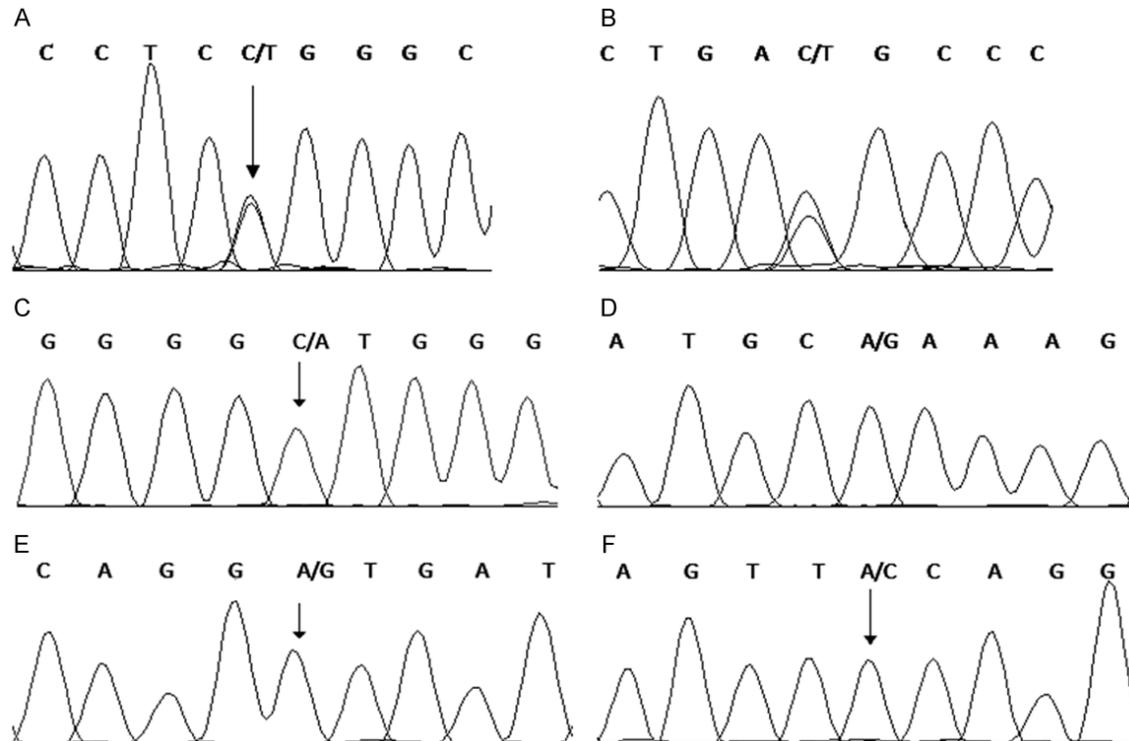
#### DNA extraction

Peripheral blood samples (2 mL) were collected from all of the subjects in ethylenediaminetetraacetic acid (EDTA) anticoagulant vacuum tubes and frozen at  $-20^{\circ}\text{C}$  until DNA extraction. Genomic DNA was isolated from whole blood samples using a QIAamp DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany) and processed according to the manufacturer's instructions. DNA concentration was determined spectrophotometrically.

#### HPA genotyping by PCR-SSP method

Genotyping of HPA was determined by polymerase chain reaction-sequence specific primer (PCR-SSP). PCR was performed in Gene Amp PCR system ABI 9700 (ABI Applied Biosystems, USA) with sequence-specific primers using the

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**Figure 2.** DNA sequencing chromatograms of HPA-1 to 5, and 15 systems. The arrows indicated the variant nucleotides HPA-1 to 5, and 15 systems, respectively. A. HPA-1ab. B. HPA-2ab. C. HPA-3aa. D. HPA-4aa. E. HPA-5aa. F. HPA-15aa.

commercial platelet SSP typing kit (Shanghai Sangon Biotech Co., Ltd., China). In brief, the thermocycler program consists of an initial step of 96°C for 10 min, followed by 5 cycles of 95°C for 25 s, 65°C for 50 s, 72°C for 30 s, then 30 cycles of 95°C for 30 s, 60°C for 45 s, 72°C for 30 s, and a final extension step of 72°C for 5 min.

### *Gel electrophoresis and gel interpretation*

The PCR products (7  $\mu$ l) were subjected to gel electrophoresis on standard 2.0% agarose gel containing 0.5  $\mu$ g/ml of ethidium bromide. The typing results were visualized by UV light transillumination. Results were recorded by ultraviolet photography (**Figure 1**). For each sample, it was possible to determine the absence or presence of one or two possible patterns: a and b genotype. Individuals are, therefore, genotypically classified as HPA aa, ab, or bb genotypes.

### *HPA genotyping by DNA sequencing*

In addition, 20 PCR amplification products were randomly selected and genotyped by DNA se-

quencing with an ABI Prism 3100. The direct sequencing tests were performed at Shanghai Sangon Biological Engineering Technology & Services Co., Ltd., People's Republic of China (**Figure 2**).

### *Statistical analysis*

The frequencies of HPA genotypes and alleles in different ethnic groups were calculated by direct counting. The gene frequencies were calculated by using the following formula: gene frequency = allele numbers/sample number. The consistency of the observed and expected genotype frequencies with the Hardy-Weinberg equilibrium was tested by using the  $\chi^2$  test. A two-tailed *P* value of less than 0.05 was considered statistically significant.

## **Results**

### *Characteristics of the study population*

The basic information of all participants is shown in **Table 1**. The mean ages ( $\pm$  standard deviation) of the Han, Zhuang, Yao, and Miao

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**Table 2.** Genotype and allele frequency of HPA in Guangxi population (n=400)

HPA system	Genotype frequencies			Allele frequencies		HWE	
	aa	ab	bb	a	b	$\chi^2$	P
HPA-1	395 (0.99)	4 (0.01)	1 (0.003)	794 (0.99)	6 (0.01)	43.11	<0.001
HPA-2	373 (0.93)	22 (0.06)	5 (0.01)	768 (0.96)	32 (0.04)	32.23	<0.001
HPA-3	163 (0.40)	150 (0.38)	87 (0.22)	476 (0.60)	324 (0.40)	19.7	<0.001
HPA-4	400 (1.00)	0 (0.00)	0 (0.00)	800 (1.00)	0 (0.00)	NA	NA
HPA-5	396 (0.99)	3 (0.01)	1 (0.003)	795 (0.99)	5 (0.01)	62.8	<0.001
HPA-6w	386 (0.97)	11 (0.03)	3 (0.01)	783 (0.98)	17 (0.02)	45.94	<0.001
HPA-7w	400 (1.00)	0 (0.00)	0 (0.00)	800 (1.00)	0 (0.00)	NA	NA
HPA-8w	400 (1.00)	0 (0.00)	0 (0.00)	800 (1.00)	0 (0.00)	NA	NA
HPA-9w	400 (1.00)	0 (0.00)	0 (0.00)	800 (1.00)	0 (0.00)	NA	NA
HPA-10w	400 (1.00)	0 (0.00)	0 (0.00)	800 (1.00)	0 (0.00)	NA	NA
HPA-11w	400 (1.00)	0 (0.00)	0 (0.00)	800 (1.00)	0 (0.00)	NA	NA
HPA-12w	400 (1.00)	0 (0.00)	0 (0.00)	800 (1.00)	0 (0.00)	NA	NA
HPA-13w	400 (1.00)	0 (0.00)	0 (0.00)	800 (1.00)	0 (0.00)	NA	NA
HPA-14w	400 (1.00)	0 (0.00)	0 (0.00)	800 (1.00)	0 (0.00)	NA	NA
HPA-15	307 (0.77)	62 (0.16)	31 (0.08)	676 (0.85)	124 (0.15)	66.68	<0.001
HPA-16w	399 (0.997)	1 (0.003)	0 (0.00)	799 (0.999)	1 (0.001)	0.00	0.98
HPA-17w	400 (1.00)	0 (0.00)	0 (0.00)	800 (1.00)	0 (0.00)	NA	NA

HWE, Hardy-Weinberg equilibrium; NA, No assay.

population groups were 49.4±5.3, 48.3±5.7, 46.9±4.6, and 50.3±4.7 years, respectively.

The results of DNA sequencing were 100% concordant.

The genotyping results obtained by PCR-SSP were validated using genotyping data by sequencing. The results of DNA sequencing were 100% concordant with the PCR-SSP typing for HPA-1 to 17w systems in 20 selected DNA samples, indicating the reliability of the PCR-SSP results.

### *HPA genotypes and allele frequencies in the Guangxi population*

**Table 2** shows the HPA genotypes and allele frequencies of 400 random Guangxi samples. The three genotypes (aa, ab, and bb) were found in HPA-1, HPA-2, HPA-3, HPA-5, HPA-6w, and HPA-15 systems. The aa and ab genotypes were found in HPA-16w system. Among these HPA systems, HPA-3 and HPA-15 systems exhibited the highest polymorphisms. The frequencies of aa, ab, and b/b genotypes were 0.40, 0.38, and 0.22 in the HPA-3 system and 0.77, 0.16, and 0.08 in the HPA-15 system, respectively. Only one individual with HPA-1bb and one with HPA-5bb had a "rare" genotype,

and no bb homozygote was found in the HPA-16w systems. The a allele was the most frequent and the b allele was infrequent in all the HPA systems analyzed. Complete aa homozygotes were found in HPA-4, HPA-7w, HPA-8w, HPA-9w, HPA-10w, HPA-11w, HPA-12w, HPA-13w, HPA-14w, and HPA-17w systems. There was no significant deviation from the Hardy-Weinberg equilibrium in HPA-16 systems, but the HPA-1, HPA-2, HPA-3, HPA-5, and HPA-6w systems were not in accordance with Hardy-Weinberg equilibrium in this population.

### *HPA genotypes and allele frequencies in different ethnic groups*

Results of genotyping for HPA-1 to -17w according to ethnic groups are shown in **Table 3**. The frequencies of all genotypes were similar for both studied ethnic groups. Similar to the overall results, the HPA-3 and HPA-15 systems are the most predominant polymorphisms for the Han, Zhuang, Yao, and Miao populations. The HPA-4, HPA-7w to -14w, and HPA-17w systems were observed only aa homozygosis in all studied ethnic populations (data not shown). For the Han and Zhuang populations, only aa homozygotes were found in the HPA-1 system. However, for the Yao and Miao populations, the b allele frequencies of

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**Table 3.** Genotype and allele frequency of HPA in different ethnic groups

Ethnic groups	HPA system	Genotype frequency			Allele frequency		HWE	
		aa	ab	bb	a	b	$\chi^2$	P
Han	HPA-1	100 (1.00)	0 (0.00)	0 (0.00)	200 (1.00)	0 (0.00)	NA	NA
	HPA-2	92 (0.92)	7 (0.07)	1 (0.01)	191 (0.94)	9 (0.05)	3.44	0.063
	HPA-3	36 (0.36)	48 (0.48)	16 (0.16)	120 (0.60)	80 (0.40)	0.00	1.000
	HPA-5	96 (0.96)	3 (0.03)	1 (0.01)	195 (0.97)	5 (0.03)	14.79	<0.001
	HPA-6w	98 (0.98)	1 (0.01)	1 (0.01)	197 (0.98)	3 (0.02)	43.77	<0.001
	HPA-15	21 (0.21)	38 (0.38)	41 (0.41)	80 (0.40)	120 (0.60)	4.34	0.037
	HPA-16w	99 (0.99)	1 (0.01)	0 (0.00)	199 (0.99)	1 (0.01)	0.003	0.960
Zhuang	HPA-1	100 (1.00)	0 (0.00)	0 (0.00)	200 (1.00)	0 (0.00)	NA	NA
	HPA-2	98 (0.98)	2 (0.02)	0 (0.00)	198 (0.99)	2 (0.01)	0.01	0.920
	HPA-3	22 (0.22)	48 (0.48)	30 (0.30)	92 (0.46)	108 (0.54)	0.11	0.735
	HPA-5	100 (1.00)	0 (0.00)	0 (0.00)	200 (1.00)	0 (0.00)	NA	NA
	HPA-6w	98 (0.98)	2 (0.02)	0 (0.00)	198 (0.99)	2 (0.10)	0.01	0.920
	HPA-15	25 (0.25)	54 (0.54)	21 (0.21)	108 (0.52)	96 (0.48)	0.67	0.414
	HPA-16w	100 (1.00)	0 (0.00)	0 (0.00)	200 (1.00)	0 (0.00)	NA	NA
Yao	HPA-1	97 (0.97)	3 (0.03)	0 (0.00)	197 (0.98)	3 (0.02)	14.95	<0.001
	HPA-2	96 (0.96)	3 (0.03)	1 (0.01)	195 (0.97)	5 (0.03)	14.79	<0.001
	HPA-3	42 (0.42)	44 (0.44)	14 (0.14)	128 (0.64)	72 (0.36)	0.20	0.652
	HPA-5	100 (1.00)	0 (0.00)	0 (0.00)	200 (1.00)	0 (0.00)	NA	NA
	HPA-6w	96 (0.96)	3 (0.03)	1 (0.01)	195 (0.97)	5 (0.03)	14.79	<0.001
	HPA-15	40 (0.40)	39 (0.39)	31 (0.31)	119 (0.59)	101 (0.41)	9.01	0.003
	HPA-16w	100 (1.00)	0 (0.00)	0 (0.00)	200 (1.00)	0 (0.00)	NA	NA
Miao	HPA-1	98 (0.98)	1 (0.01)	1 (0.01)	197 (0.98)	3 (0.02)	43.77	<0.001
	HPA-2	87 (0.87)	10 (0.10)	3 (0.03)	184 (0.92)	16 (0.08)	10.28	0.001
	HPA-3	25 (0.25)	48 (0.48)	27 (0.27)	98 (0.49)	102 (0.51)	0.16	0.692
	HPA-5	100 (1.00)	0 (0.00)	0 (0.00)	200 (1.00)	0 (0.00)	NA	NA
	HPA-6w	94 (0.94)	5 (0.05)	1 (0.01)	193 (0.96)	7 (0.04)	6.75	0.009
	HPA-15	24 (0.24)	62 (0.62)	14 (0.14)	110 (0.55)	90 (0.45)	6.38	0.012
	HPA-16w	100 (1.00)	0 (0.00)	0 (0.00)	200 (1.00)	0 (0.00)	NA	NA

HPA-1 were both 0.02. For the HPA-5 system, three individuals with an HPA-5ab and one with an HPA-5bb genotype had the "rare" genotypes in Han population, but only aa homozygosis was found in the Zhuang, Yao, and Miao populations. For the Han population, the allele frequency of the b allele was higher than that of the a allele in the HPA-15 system. For the Zhuang and Miao populations, the allele frequency of the b allele was higher than that of the a allele in the HPA-3 system. For the remaining HPAs systems, the a allele was the most frequent, and the b allele was infrequent.

### Discussion

Human platelet antigens (HPA) are found on platelet membrane glycoproteins. To date, 35 HPAs expressed on six different platelet glyco-

proteins: GPIIIa, GPIb $\alpha$ , GPIIb, GPIa, GPIb $\beta$ , and CD109 have been described. For a current list, see <http://www.ebi.ac.uk/ipd/hpa/> **Table 1.html**). Antibodies that form against HPAs are clinically important in several immune platelet disorders, including FNAIT, PTP, and MPR [1]. Platelet membrane GPs are expressed in polymorphic forms caused by SNPs in the genes that encode them. HPA genotyping of donor blood is important for managing platelet-specific alloimmunization and facilitating the selection of matching HPA types for patients who possess rare alleles among different ethnic groups [7].

The distribution frequency of HPA varies among populations worldwide. In this study, we used the recommended PCR-SSP method to determine the genotypes of HPA-1 to -17w systems in

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regular blood donors, and we also used a DNA sequencing method to validate the genotype. The results of these two methods were 100% concordant. The b allele generally presented at low frequencies (<0.10) in all HPA systems studied except for HPA-3 and -15. We could detect the bb homozygous in six HPA systems (HPA-1, HPA-2, HPA-3, HPA-4, HPA-5, and HPA-15), and several ab heterozygous in HPA-1, HPA-5, and HPA-16w systems. The frequencies of all genotypes were similar for both studied ethnic groups. However, the HPA-1b allele frequency among the Yao and Miao populations was 0.02, but among the Han and Zhuang populations, it was 0.00. The HPA-5 and HPA-16w b allele frequencies among the Han population were 0.03 and 0.01, respectively, but those among the Zhuang, Yao, and Miao populations were all 0.00. These results illustrate the heterogeneous composition of the Guangxi population.

A number of studies were performed previously to investigate the distribution of the HPA system in different populations, such as in Caucasians [3, 4], Africans [15], South Americans [17], and Asians [7, 11, 12]. In Caucasians, there are higher gene frequencies for HPA-1b, 2b, and 5b than in other populations [5]. In addition, HPA-1 is known to be the most important antigen system involved in antiplatelet alloimmunity in Caucasians [18]. In Africans, the gene frequencies for HPA-1b, 2b, and 3b are higher than in Asians [15]. Halle et al. studied four different sub-Saharan African populations (Beninese, Cameroonians, Congolese, and Pygmies), and the allele HPA-1b was reported to be somewhat low by contrast to other HPA systems (HPA-2, -3, -4, -5, -15) [19]. In South Americans, the frequency of the b allele was higher in HPA-1 and -15 systems and lower in HPA-2 and -5 systems in comparison with sub-Saharan Africans [16]. Seo et al. described that HPA-3b can be found significantly more often among Koreans than among whites [8]. The HPA-4b allele, commonly associated with the Japanese [20] and Korean populations [8], has not been observed in our studied population or in other South-East Asian populations, such as Indonesian [9], Vietnamese [19], and Thai [10]. Apart from HPA-4b, HPA-6b is also nearly exclusively found within the Asian population [6].

Presently, only two data [11, 12] about the frequency of HPA-1 to 17w systems and one study

[13] about the frequency of HPA-17w to -21w systems among Chinese individuals exist. However, both of these two studies focused on the Chinese Han population. Guangxi is a multi-ethnic province in the south of China. Likewise, HPA-3 and HPA-15 systems exhibited the highest polymorphisms in these two studies. In addition, the allele frequencies of HPA-3a and -3b in this study are 0.60 and 0.40, respectively, almost equivalent to that reported by the Chinese studies [11, 12]. However, in the present study, the allele frequency of HPA-15b is 0.15, significantly lower than that reported by the two Chinese studies (0.418 and 0.432, respectively). These results illustrate the heterogeneous composition of the Chinese population. This is the first study to evaluate the allele frequency of the HPA-1 to -17w system in the Guangxi population. The allele frequencies of the Guangxi population, composed mainly of Han, Zhuang, Yao, and Miao in Southern China, is somewhat different from those of other Chinese populations because of the low frequency of the HPA-15b allele in our overall group.

The HPA type of an individual is determined by the analysis of that person's DNA for the individual allelic forms of the genes that encode the various HPAs [1]. Genotyping of DNA is the gold standard HPA typing method in use today. A variety of different platforms have been applied to HPA genotyping throughout the years [21-23], such as PCR-SSP, restriction fragment length polymorphism (RFLP), real-time PCR, 5'nuclease, microarray, hybridization, mass spectroscopy, and so on. However, the most popular method is PCR-SSP with a gel end-point [24]. Recently, advances in technology have made it possible to use noninvasive techniques to obtain fetal DNA from maternal plasma for genotyping [25]. Finally, use of the PCR technique combined with allele-specific primers is suitable for accurate large-scale typing of platelet donors, which may be useful in special clinical settings. Here, we set up a PCR-SSP method for HPA-1 to -17w alleles. Our results show specific single PCR products, and excellent sequences were obtained in the present study.

In summary, our data provide information on HPA allele frequency in the Guangxi population and establish the platelet donor bank to provide HPA-matched platelets. In addition, our

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results may be helpful for the laboratory diagnosis of FNAIT, PTP, and PTR.

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### Disclosure of conflict of interest

None.

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