

# Allelic and genotypic frequency of human platelet antigens.

## Frecuencia alélica y genotípica de antígenos plaquetarios humanos

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### Abstract

**BACKGROUND:** The frequency of human platelet alloantigens in a population determines the prevalence of diseases associated with alloimmunization, such as neonatal alloimmune fetal thrombocytopenia.

**OBJECTIVE:** To describe the allele frequencies of human platelet alloantigens in a sample of couples.

**MATERIAL AND METHOD:** A cross-sectional, observational and comparative study was made including male-female unrelated couples, selected from August 1<sup>st</sup> to December 31<sup>st</sup>, 2014. The human platelet alloantigens (HPA) alleles were determined by PCR-SSP that allows detection of HPA-1a/b, HPA-2a/b, HPA-3a/b, HPA-4a/b, HPA-5a/b, HPA-15a/b.

**RESULTS:** Fifteen male-female unrelated couples were selected. The HPA that showed highest heterozygosity were HPA-3, HPA-4 and HPA-15 with frequencies of 0.500, 0.534 and 0.466, respectively. The HPA-1, HPA-2 and HPA-5 systems showed the most frequent genotype a/a with 0.933, 0.800 and 0.767, respectively. The b/b genotype was identified in the HPA-3, HPA-4, and HPA-15 systems with frequency of 0.167, 0.033 and 0.167, respectively. In five couples (0.334), the incompatibility with platelet antigens was high risk for the development of neonatal alloimmune thrombocytopenia.

**CONCLUSIONS:** The allelic and genotypic frequencies agree with the reports made in other populations. The allelic incompatibility between couples can estimate a risk for development of fetal-neonatal alloimmune thrombocytopenia, pending to evaluate the presence of other concurrent variables.

**KEYWORDS:** Human platelet antigens; Genotype.

### Resumen

**ANTECEDENTES:** La frecuencia de aloantígenos plaquetarios humanos (HPA) en una población se relaciona estrechamente con la prevalencia de las enfermedades asociadas con la aloinmunización, como la trombocitopenia aloinmune fetal/neonatal.

**OBJETIVO:** Describir las frecuencias alélicas de los aloantígenos plaquetarios humanos en una muestra de parejas.

**MATERIAL Y MÉTODO:** Estudio transversal, observacional y comparativo que incluyó parejas hombre-mujer no relacionadas seleccionadas del 1 de agosto al 31 de diciembre de 2014. Se determinaron los alelos de los aloantígenos plaquetarios humanos (HPA) mediante PCR-SSP que permite la detección de los alelos HPA-1a/b, HPA-2a/b, HPA-3a/b, HPA-4a/b, HPA-5a/b, HPA-15a/b.

**RESULTADOS:** Se seleccionaron 15 parejas hombre-mujer no relacionadas. Los HPA que mostraron mayor heterocigosidad fueron los HPA-3, HPA-4 y HPA-15 con frecuencias de 0.500, 0.534 y 0.466, respectivamente. Los sistemas HPA-1, HPA-2 y HPA-5 mostraron el genotipo a/a más frecuente con 0.933, 0.800 y 0.767, respectivamente. El genotipo b/b se identificó en los sistemas HPA-3, HPA-4 y HPA-15 con frecuencia de 0.167, 0.033 y 0.167, respectivamente. En cinco parejas (0.334), la incompatibilidad a los antígenos plaquetarios fue de alto riesgo para la aparición de trombocitopenia neonatal aloinmunitaria.

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**CONCLUSIONES:** Las frecuencias alélicas y genotípicas concuerdan con los reportes realizados en otras poblaciones. La incompatibilidad alélica entre parejas puede estimar el riesgo de trombocitopenia aloinmunitaria fetal-neonatal, quedando pendiente de evaluar la existencia de otras variables concurrentes.

**PALABRAS CLAVE:** Antígenos plaquetarios humanos; genotipo.

## INTRODUCTION

Platelets participate in different physiological processes, not only in primary hemostasis through adhesion and platelet aggregation or in secondary hemostasis favoring generation of thrombin and formation of fibrin clot.<sup>1,2</sup> In addition, they play a role in inflammation,<sup>3</sup> innate and adaptive immunity,<sup>4</sup> vascular functionality, autoimmunity and even cancer.<sup>3</sup> All these functions are carried out through junctions between ligands and receptors that express on their surface known as glycoproteins, present in polymorphic forms caused by mutations in a single nucleotide (SNPs or single nucleotide polymorphism) in the genes that encode them. New antigens that can induce antibodies when exposed during pregnancy or platelet transfusion, these are called human platelet alloantigens and are capable of generating an immune response in susceptible individuals. Antibodies formed against human platelet alloantigen are important in several platelet immune diseases such as fetal-neonatal alloimmune thrombocytopenia,<sup>5</sup> postransfusional thrombocytopenia,<sup>6</sup> or platelet refractoriness.<sup>7</sup>

The human platelet antigens (HPA) are named using the HPA system by number in the order in which they were first described; six are grouped into biallelic systems (HPA-1, -2, -3, -4, -5 and -15). Within this nomenclature are described according to the frequency in which they occur

within the population, designating the letter “a” to those with high frequency and “b” to which they have low frequency.<sup>8</sup> The inheritance of HPA is autosomal co-dominant and its frequency varies depending on the population under study, in Caucasians HPA-1a is the antigen mostly involved in alloimmunization followed by HPA-5b and finally HPA-3a,<sup>9,10</sup> population genetic studies show diverse heterogeneity among different HPA genotypes around the world.<sup>11,12</sup>

The importance of establishing the frequency of platelet alloantigens in a population lies in its close relationship with the prevalence of clinical entities related to alloimmunization such as fetal or neonatal alloimmune thrombocytopenia. The aim of this report is to describe the allele frequencies of PAHs in a group of couples, to compare the results obtained with those described in other populations and the possibility of developing maternal-fetal alloimmunization to platelet antigens.

## MATERIAL AND METHOD

The study was conducted in perinatal hematology laboratory from National Institute of Perinatology in Mexico City between 1<sup>st</sup> August through 31<sup>st</sup> December 2014. In this cross-sectional, observational and comparative study, a sample of unrelated male-female couples was selected. Peripheral blood samples were obtained and the leukocyte DNA was extracted. The HPA

alleles were determined using the PCR-SSP by Gene HPA-ready commercial kit by inno-train Germany, that allows the detection of the HPA-1a/b, HPA-2a/b, HPA-3a/b, HPA-4a/b, HPA-5a/b, HPA-15a/b. The allelic and gene frequencies of the HPA genes are presented.

The research protocol was approved by the Institutional Research and Ethics Committee, and the informed consent of the participating subjects was obtained.

### RESULTS

Fifteen male-female couples were included. The most common allelic combination was a/a in all HPA systems. The HPA that showed the highest heterozygosity were the HPA-3, HPA-4, and HPA-15 with frequencies of 0.500, 0.534 and 0.466 respectively. The HPA-1, HPA-2, and HPA-5 systems showed the most frequent genotype a/a, the b/b genotype was identified in the HPA-3, HPA-4 and HPA-15 systems (Table 1).

Taking into account the presence of the “a” allele or the “b” allele between both couples, the risk of developing fetal and/or neonatal alloimmune thrombocytopenia was estimated. In three couples no incompatibility was found, in the rest of the couples three were found with one incompatibility, five with two incompatibilities and four with three incompatibilities. Seven

**Table 1.** Genotypic and allelic frequency of human platelet alloantigens (HPA) in 30 unrelated subjects

	Genotype			Alleles	
	a/a	a/b	b/b	a	b
HPA-1	0.933	0.067	0	0.967	0.033
HPA-2	0.800	0.200	0	0.900	0.100
HPA-3	0.333	0.50	0.167	0.550	0.450
HPA-4	0.433	0.534	0.033	0.700	0.300
HPA-5	0.767	0.233	0	0.883	0.117
HPA-15	0.367	0.466	0.167	0.600	0.400

couples had a single incompatibility; four had two incompatibilities and only one couple had three incompatibilities. The allele b of the HPA-3 and HPA-15 systems were the most incompatible between the pairs with six and five cases, respectively.

Considering the alleles present among the couples, the pairs were grouped into three risk groups for the development of FNAIT (Table 2).

### DISCUSSION

The allele frequencies are similar to those reported in other studies, for both alleles “a” and allele “b”. However, the “a” allele of the HPA-3 and HPA-4 systems shows a lower frequency compared with the other populations. With respect to the allele “b”, our population shows a lower frequency with respect to the other reports,<sup>1</sup> even these allelic differences contrast with another study conducted in Mexico in a sample of donors.<sup>13</sup>

**Table 2.** Risk group according to alleles present and absent between the couples

Group	Couple number	Alleles of couple	Level risk (proportion)
I	Couple 4 Couple 6 Couple 12	They did not present incompatible alleles	Null (0.200)
II	Couple 1 Couple 5 Couple 7 Couple 8 Couple 11 Couple 14 Couple 15	HPA 5b + HPA 2b + HPA 1b + HPA 2b +, HPA 3a + HPA 3b + HPA 2b + HPA 3b +	Low (0.466)
III	Couple 2 Couple 3 Couple 9 Couple 10 Couple 13	HPA 15b + HPA 4b +, HPA 15b + HPA 15b + HPA 5b +, HPA 15b + HPA 15b +	High (0.334)

Systems indicate that population studied is in equilibrium and is not affected by selection or migration.

Antibodies against HPA-1a are responsible in most cases of fetal-neonatal alloimmune thrombocytopenia (FNAIT) in about 85% of cases. The frequency of alloimmunization in Caucasians is lower than expected considering that 75% of men are homozygous (HPA-1a/1a) and 25% are heterozygous, despite these numbers the alloimmunization in women only occurs in about 10% of HPA-1a negative, this may be related to the presence or absence of specific HLA-2 antigens, in addition other factors such as HLA type have been demonstrated; in this regard, an increase in risk of alloimmunization has been observed in mothers who express HLA-B8, HLA-DR3, and HLA-DR52a. In women with allele HLA-DRB3\*0101, DQB1\*0201 and HLA-DRB4\*01:01<sup>14</sup> with negative HPA-1a increases 140-fold the risk of alloimmunization.<sup>15,16</sup> HPA-1 has been identified as a hereditary risk factor for coronary thrombosis.<sup>17,18</sup> The polymorphism of the HPA 1a/1b allele 1b of the GP IIIa gene of HPA 1a/1b is common.<sup>19</sup> This and other polymorphisms have been widely investigated as a risk factor for arterial thrombosis.<sup>20</sup>

Regarding the obstetric risk for the development of FNAIT, seven couples were classified as low risk since they had incompatibility in at least one HPA system; they were considered low risk since said incompatibility was different from the antigenic systems frequently reported in said pathology (HPA-1a, HPA-5b, HPA-15b), five pairs were classified as high risk. This risk was only established according to the incompatibility, however this incompatibility is not enough to develop alloantibodies,<sup>21-24</sup> other factors such as peptides of HPA-1a antigen that requires HLA class II DRB\*01:01 to be presented to the maternal T cells, this characteristic being necessary in

the mother to be able to produce alloantibodies. In other cases, although the mother produces alloantibodies not always cause pathology in the fetus and / or newborn, factors such as the type and subclass of immunoglobulin generated and the HLA class I in the FcRn receptor of the placenta a,<sup>21</sup> where the binding of IgG to FcRN is a prerequisite for its transport through the placenta playing a central role in the release of IgG to the fetus.

## CONCLUSION

The allelic incompatibility between couples can estimate the risk for development of fetal-neonatal alloimmune thrombocytopenia, pending to evaluate the presence of other concurrent variables.

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