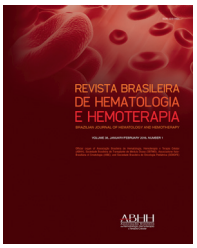
REV BRAS HEMATOL HEMOTER. 2017;xxx(xx):xxx–xxx

Revista Brasileira de Hematologia e Hemoterapia
Brazilian Journal of Hematology and Hemotherapy
www.rbhh.org



Original article

Molecular typing of human platelet antigens in immune thrombocytopenia patients in northern Brazil

Q1 *Julia Cavalcante do Carmo*[a], *Prissyla de Souza Klippel*[a], *Sabrine da Costa Cordeiro*[b], *Ângela Maria dos Santos Fernandes*[b], *Raquel Medeiros Pinto*[a], *Simone Schneider Weber*[c,d,*], *Cleiton Fantin*[a]

[a] *Universidade do Estado do Amazonas (UEA), Manaus, AM, Brazil*
[b] *Fundação de Hematologia e Hemoterapia do Amazonas (Hemoam), Manaus, AM, Brazil*
[c] *Universidade Federal do Amazonas, Itacoatiara (UFAM), AM, Brazil*
[d] *Universidade Federal do Mato Grosso do Sul (UFMS), Campo Grande, MS, Brazil*

ARTICLE INFO

Article history:
Received 26 October 2016
Accepted 5 January 2017
Available online xxx

Keywords:
Immune thrombocytopenia
Platelets
BeadCheap technology
Genotyping
HPA

ABSTRACT

Background: Immune thrombocytopenia is an immune disease characterized by thrombocytopenia and bleeding due to platelet antibodies against platelet membrane glycoproteins. Human platelet antigens are derived from polymorphisms of these glycoproteins. The aim of this study was investigate human platelet antigen frequencies in immune thrombocytopenia patients from the state of Amazonas, Brazil and investigate the potential association between specific antigens and risk for immune thrombocytopenia.

Method: Human platelet antigen typing was performed by BeadChip technology to determine allelic variants of 11 systems (HPA-1 to HPA-9, HPA-11 and HPA-15). Thirty-six patients (8 male and 28 female) were evaluated with a median age of 34 years (range: 9–69 years) and compared with data from Amazonas blood donors.

Results: Platelet counts varied from 3 to 98 $\times$ 10^9/L. The allele frequencies were 0.944 for HPA-1*a*, 0.056 for HPA-1*b*, 0.847 for HPA-2*a*, 0.153 for HPA-2*b*, 0.555 for HPA-3*a*, 0.444 for HPA-3*b*, 0.805 for HPA-5*a*, 0.222 for HPA-5*b*, 0.9975 for HPA-9*a*, 0.025 for HPA-9*b*, 0.486 for HPA-15*a* and 0.513 for HPA-15*b*. Among immune thrombocytopenia individuals, no *b* allele of the HPA-4, -6, -7, -8 and -11 were found.

Conclusions: The results suggest HPA-1a, HPA-3b and HPA-5b are immune thrombocytopenia-specific autoepitopes.

* *Corresponding author at:* Centro de Ciências Biológicas e da Saúde. Universidade Federal do Mato Grosso do Sul, Cidade Universitária S/N, Caixa Postal 549, 79070-900 Campo Grande, MS, Brazil.
E-mail address: weberblood@gmail.com (S.S. Weber).

http://dx.doi.org/10.1016/j.bjhh.2017.01.003

Introduction

Immune thrombocytopenia (ITP) is an immune-mediated acquired disease characterized by transient or persistent decrease affecting platelet numbers and, depending upon the degree of thrombocytopenia, increased risk of bleeding,[1] due to the presence of platelet autoantibodies. Platelet membrane glycoproteins (GPs) appear to be the principal binding sites of ITP serum antibodies.[2] The polymorphisms of the human platelet alloantigens occur due to single nucleotide substitutions that result in the substation of an amino acid.[3]

The Immuno Polymorphism Database (IPD) of human platelet alloantigens (HPA) lists 35 platelet alloantigens, which are located in GPs (platelet receptors).[4] The three major platelet receptors are GPIIb-IIIa, GPIb-IX-V and GPIa-IIa.[3,5,6] GPIIb/IIIa is the most polymorphic complex and carries 19 antigens[5]: HPA-1 (176T>C); HPA-3 (2621T>G); HPA-4 (506G>A); HPA-6 (1544G>A); HPA-7w (1297C>G); HPA-8w (1984C>T); HPA-9w (2602G>A); HPA-10w (263G>A); HPA-11w (1976G>A); HPA-14w (1909_1911delAAG); HPA-16w (497C>T); HPA-17w (662C>T); HPA-19 (487A>C); HPA-20w (1949C>T); HPA-21w (1960G>A); HPA-22w (584A>C); HPA-23w (1942C>T); HPA-24w (1508G>A) and HPA-26w (1818G>T). The von Willebrand factor (vWF) receptor GPIb/IX carries two antigens HPA-2 (482C>T) and HPA-12w (119G>A). In addition, the GPIa/IIa complex carries the HPA-5 (1600G>A), HPA-13w (2483C>T), HPA-18w (2235G>T) and HPA-25w (3347C>T) polymorphic systems.[7,8] Moreover, *the* HPA-15 (Gov) polymorphism is located in the CD109 molecule and its alleles differ at a single nucleotide polymorphism (C2108A) that causes a Tyr682Ser amino acid substitution.[9,10] These polymorphisms can be recognized as alloantigens or autoantigens and trigger the clearance of opsonized platelets by phagocytes in the reticuloendothelial system or inhibition of platelet production.[11] Several groups worldwide have tried to establish a possible association between HPA polymorphisms and ITP.[12–15] Some ITP-specific autoepitopes have been suggested, such as HPA-2a in German patients with chronic refractory ITP[14] and HPA-2b in Macedonian patients with ITP.[15] In addition, Castro et al.,[12] suggested the presence of HPA-5b in Brazilian patients with increased the risk for acute ITP, while the HPA-5a has been implicated in Korean patients.[13] However, the results have been unclear, and studies exploring these hypotheses are still lacking.

The aim of this study was to analyze the frequencies of human platelet antigens, grouped as 11 biallelic HPA systems (HPA-1 to HPA-9, HPA-11 and HPA-15) in patients from Amazonas State with primary ITP, and to investigate the potential association between specific HPA polymorphisms and risk for ITP.

Methods

Study site

The Fundação Hospitalar de Hematologia e Hemoterapia do Amazonas (HEMOAM) is a referral center for the diagnosis, treatment and monitoring of hematological diseases in the northern region of Brazil. The service receives approximately 100 new cases of ITP annually. This study was approved by the institution's Ethics Committee (CEP/HEMOAM #803.634/2014).

Sample definitions

In total, 36 unrelated sequential ITP patients treated in HEMOAM participated in the study between October 2014 and April 2015. Informed consent was obtained from all enrolled patients. All patients in this study had chronic primary ITP, which by definition meant the disease lasted for more than 12 months after the initial treatment. The criterion for primary ITP was the presence of isolated thrombocytopenia (peripheral blood platelet count $<100 \times 10^9$/L) in the absence of other causes or disorders that might be associated with this low platelet count, in accordance with the standardization of ITP diagnosis established by an international ITP working group.[16] Thus, the diagnosis of primary ITP was achieved by exclusion. The sample definition for HPA genotyping of blood donors excluded samples with platelet counts less than 150×10^9/L.

Genomic DNA extraction

Genomic DNA samples were obtained from EDTA-preserved whole blood using the QIAamp DNA Blood kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The automated epMotion 5075 system (Eppendorf, Hamburg, Germany) was adapted. The DNA concentration and quality were evaluated spectrophotometrically using NanoDrop technology (Thermo Fisher Scientific, Massachusetts, USA). The samples were stored at −80 °C until use.

Platelet genotyping by BeadChip microarray technology

Platelet genotyping was performed using a BeadChip assay.[17,18] The BeadChip microarray method is capable of determining 22 allelic variants of 11 HPA systems (HPA-1 to HPA-9, HPA-11 and HPA-15). DNA amplification and post-polymerase chain reaction steps were performed according to the manufacturer's instructions. The BeadChip slides were analyzed in a fluorescent system using the Bioarray Solutions software (Immucor, Warren, NJ) in the HEMOAM genomic laboratory.

Statistical analysis

The genotype and allele frequencies were estimated by direct counting, and the results were compared individually with the values published for healthy individuals from Amazonas.[19] The 95% confidence interval (CI), chi square (X^2) test or Fisher's exact test were used for comparative analysis. The Hardy–Weinberg equilibrium of HPA system genotypes was evaluated using the Hardy–Weinberg calculator.[20] *p*-Values lower than 0.05 were considered significant in all statistical analyses.

Results

The clinical data and allele frequencies obtained from the microarray for HPA-1 to HPA-9, HPA-11 and HPA-15 in chronic

Please cite this article in press as: Carmo JC, et al. Molecular typing of human platelet antigens in immune thrombocytopenia patients in northern Brazil. Rev Bras Hematol Hemoter. 2017. http://dx.doi.org/10.1016/j.bjhh.2017.01.003