Association of human platelet antigen polymorphisms with platelet count and mean platelet volume

Shihang Zhoua*, Xiaohua Lianga*, Ni Wanga, Linnan Shaoa, Weijian Yua and Ming Liub

aDalian Blood Center, Dalian, People’s Republic of China; bDepartment of Cell Biology, Dalian Medical University, Dalian, People’s Republic of China

ABSTRACT

Objectives: Although recent genome-wide association studies have identified a number of single nucleotide polymorphisms associated with platelet count and mean platelet volume (MPV), it is unclear whether polymorphisms in the human platelet antigens (HPA) genes are associated with platelet count and MPV. The aim of this study was to determine the association of the HPA-2, -3, -5 and -15 polymorphisms with platelet count and MPV.

Methods: The HPA were genotyped by 5ˊ-nuclease assay in 139 healthy Chinese Han individuals, while platelet count and MPV from the same samples were measured using an hematology cell analyzer.

Results: The platelet count was significantly lower in the individuals with the HPA-2aa genotype compared to those with HPA-2ab (P = 0.020), and significantly higher in individuals with HPA-5aa and HPA-15aa genotypes compared to those with HPA-5ab (P = 0.045) and HPA-15ab/bb (P = 0.032), respectively. On the other hand, platelet count of individuals with the HPA-3aa and HPA-3ab/bb genotypes did not differ significantly (P = 0.084). The MPV was significantly lower in individuals with HPA-5aa genotype compared to those with HPA-5ab (P = 0.001) but did not differ among the HPA-2, -3 and -15 genotypes. Furthermore, HPA-2, -5 and -15 polymorphisms were identified as independent factors for the platelet count and HPA-5 polymorphism was shown as an independent factor for MPV.

Conclusions: This study demonstrates that HPA-2, -5 and -15 polymorphisms are associated with the platelet count while HPA-5 polymorphism is associated with MPV. This finding will further our understanding of the association of HPA polymorphisms with platelet-related diseases.

KEYWORDS
Human platelet antigens; platelet count; mean platelet volume; single nucleotide polymorphism; genotype; platelet; genotyping

Introduction

Platelets are the enucleated fragments of cytoplasm derived from the bone marrow megakaryocytes and circulating platelets play a key role in blood hemostasis. Upon stimulation, the quiescent platelets are rapidly activated to release their granule contents and spread across injured tissues to create a physical barrier and prevent bleeding [1]. In addition, platelets also play important roles in cardiovascular disease, infectious diseases caused by bacteria, viruses and parasites, and cancer [2–9].

Platelet concentration is essential for the maintenance of its hemostatic function. Low platelet concentration, a condition known as thrombocytopenia, which is involved in numerous potential causes including decreased bone marrow platelet production, increased peripheral platelet destruction, increased splenic sequestration, and dilution [10]. Elevated platelet concentration, also called thrombocytosis, is involved in myeloproliferative diseases or reactive thrombocytosis [11]. Clinically, the platelet count has been used as a parameter that reflects the platelet concentration in vivo. Mean platelet volume (MPV) is a machine-calculated measurement of the average size of platelets. It is associated with cerebral infarction and cardiovascular diseases, and therefore, considered as an important marker for assessing the risk and prognosis of these diseases [12–15]. At present, platelet count and MPV are part of routine laboratory tests and have been widely used in the diagnosis of various diseases.

Previous twin heritability studies have reported that the variation in platelet count and MPV are largely genetically determined, [16,17]. Single nucleotide polymorphism (SNP) is the most common type of genetic variation in both coding and noncoding regions of genomes. To date, over 10 million human reference SNPs have been deposited into the NCBI’s public database dbSNP [18]. Recently, several genome-wide association studies for hematomal traits in humans showed a number of SNPs associated with platelet count and MPV [19–25]. Platelet membrane glycoproteins (GPs) express several polymorphic antigenic determinants on their surface, namely, the human platelet antigens (HPA) [26]. The polymorphism in most of these antigens is caused by the substitution

CONTACT Ming Liu liuminglinxi@163.com
*Shihang Zhou and Xiaohua Liang contributed equally to this work.
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of a single amino acid due to an SNP in the gene encoding the membrane protein [27]. However, little is known regarding the association of HPA polymorphisms with platelet count and MPV.

The aim of this study, therefore, was to determine the association of the HPA-2, -3, -5 and -15 polymorphisms (the HPA-1b and -4b alleles are rare in the Chinese population [28]) with platelet count and MPV in the Chinese Han population.

Materials and methods

Subjects

A total of 139 participants were recruited from randomly selected unrelated healthy blood donors of Chinese Han population in Dalian Blood Center. Signed informed consents were obtained in all participants and the ethics committee approval was obtained from the Local Ethics Committee. Inclusion criteria were: absence of any systemic disease, absence of any infections in the previous month, and none of the subjects was taking any medication or had any evidence of metabolic disease. Whole blood samples were drawn from all participants into 2 mL tubes that contained K$_2$-EDTA as an anticoagulant.

Platelet count and MPV measurements

Platelet count and MPV were measured within 30 min to 1 h of collection using an XT-1800i automated hematology cell analyzer (Sysmex, Kobe, Japan) following the standardized quality assurance procedure.

Extraction of DNA

Genomic DNA was extracted from whole blood samples using commercially available DNA isolation kit on MagCore® Automated Nucleic Acid Extractor (RBCBioscience, Taipei, Taiwan) according to the manufacturer’s instructions.

5’-NA with TaqMan minor groove binding probes for HPA-2, -3, -5 and -15 genotyping

Sequences of the primers and probes for the 5’-nuclease assay (NA) with TaqMan minor groove binding Probes were obtained from a previous study [29]. The primers and probes were synthesized by Takara Biotechnology and Applied Biosystems, respectively. The PCR mixtures included 1 µL of purified genomic DNA, 10 µL of 2× TaqMan Universal PCR Master Mix II (Applied Biosystems, Foster City, CA, USA), 0.9 µL of each primer (20 µM), 0.2 µL of each probe (20 µM) and 6.8 µL of distilled water in a final reaction volume of 20 µL. The 5’-NA was performed on the ABI Prism 7300 sequence detection system (Applied Biosystems) under the following conditions: pre-PCR heat step at 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and at 60°C for 1 min. After PCR was completed, results were analyzed on ABI Prism® 7300 sequence detection system with SDS software v1.4 (Applied Biosystems), using the allele discrimination function to detect the end-point fluorescent intensity of 6-carboxyfluorescein (FAM) and 4,7,2′-trichloro-7′-phenyl-6-carboxyfluorescein (VIC) in each well. The genotyping results were sorted into three distinct groups, corresponding to the three genotypes, homozygous aa, bb and heterozygous ab.

Statistical analysis

Statistical analyses were performed using SPSS 13.0 for windows package. The association analysis of the platelet traits with HPA genotypes were performed using independent-sample t test. A two-tailed P-value of less than 0.05 ($P < 0.05$) was considered statistically significant difference.

To evaluate the role of HPA genotypes in the platelet level and size, all the HPA polymorphisms along with sex and age were included in a multiple linear regression analysis model. The platelet count and MPV were defined as dependent variables, respectively.

Results

Characteristics of the study participants, including age, sex, platelet count, MPV and distribution of the HPA genotypes are summarized in Table 1. The HPA were genotyped successfully in 139 healthy Chinese Han individuals by 5’-NA following the quality control procedure. As shown in Table 2, the platelet count was significantly lower in individuals with HPA-2aa genotype compared to those with HPA-2ab ($P = 0.020$). Moreover, the platelet count was significantly higher in individuals with HPA-5aa genotype and HPA-15aa compared to those with HPA-5ab ($P = 0.045$) and HPA-15ab/bb ($P = 0.032$), respectively. However, there was no significant difference in platelet count between individuals with HPA-3aa and HPA-3ab/bb genotypes ($P = 0.084$). These results clearly indicate that the platelet count indeed varies with HPA polymorphisms.

We also explored the association of HPA genotypes with the MPV. As shown in Table 2, the MPV was significantly lower in individuals with HPA-5aa genotype compared to those with HPA-5ab genotype ($P = 0.001$). The MPV did not differ among the HPA-2, HPA-3 and HPA-15 genotypes.

To evaluate whether HPA polymorphism is an independent factor that is related to platelet level and size, we included all the HPA polymorphisms along with sex and age in a multiple linear regression analysis model.
and took platelet count and MPV as the dependent variable, respectively. HPA-2 (\( P = 0.012 \)), -5 (\( P = 0.029 \)), and -15 (\( P = 0.009 \)) polymorphisms and sex (\( P = 0.003 \)) were independent variables for platelet count (Table 3), whereas HPA-5 polymorphism was an independent variable for MPV (\( P = 0.002 \)). For both platelet count and MPV, the results obtained by multiple linear regression analysis were similar to those obtained by independent-sample t test.

### Discussion

To date, 33 HPAs expressed on six different platelet GPs, GPIIb, GPIIIa, GPIb\(\alpha\), GPIb\(\beta\), GPIa and CD109 have been reported, 12 of which are clustered into six biallelic systems (HPA-1, -2, -3, -4, -5 and -15) [26]. Clinically, alloimmunization against HPA may result in three clinical conditions: neonatal alloimmune thrombocytopenia, post-transfusion purpura and platelet transfusion refractoriness [30]. Furthermore, previous studies have shown that HPA polymorphisms are associated with hepatitis C virus infection, progression of fibrosis in chronic hepatitis C, idiopathic thrombocytopenic purpura and periprocedural myocardial infarction [5,6,31–33]. In the present study, we explored the correlation of HPA genotypes with the platelet count and MPV. To our knowledge, this is the first report to evaluate the association of HPA polymorphisms with the platelet count and MPV in healthy Chinese individuals.

Our study shows that the HPA-2, -5 and -15 polymorphisms are associated with the platelet count. Using a multiple linear regression, we further verify that HPA-2, -5 and -15 polymorphisms are independent factors for the platelet count. Gieger et al. [25] found that HPA-2 polymorphism was associated with platelet count in individuals of European ancestry. Gentile et al. [34] reported HPA-3aa as an independent factor for the low platelet count in patients with chronic Hepatitis C virus (HCV) infection regardless of disease stage. The polymorphic antigenic determinants of HPA-3 are located on GPIIb [27]. A study of 50 HCV-positive patients found that alloantibodies generated against GPIIb and other human platelet GPs induced thrombocytopenia during chronic HCV infections [35]. These studies suggest that HPA polymorphisms are likely associated with different rates of immune-mediated platelet clearance, which occurs in the spleens of patients with HCV infection. In this study, however, HPA-3 polymorphism was not associated with the platelet count. A possible explanation could be that the samples used in this study were collected from healthy donors and alloantibodies against human

### Table 1. Laboratory results and distribution of the HPA genotypes and HPA combined genotypes (\( n = 139 \)).

| Features | Values |
|----------|--------|
| Age (years) | 40.7 (19–60) |
| Sex | 
| Male | 70.5% |
| Female | 29.5% |
| Platelets count (\( \times 10^3/\mu L \)) | 237.39 ± 50.29 |
| MPV (fL) | 10.05 ± 0.76 |
| HPA-2 genotypes | 
| aa | 127 (91.4%) |
| ab | 12 (8.6%) |
| bb | 0 (0%) |
| HPA-3 genotypes | 
| aa | 45 (32.4%) |
| ab | 73 (52.5%) |
| bb | 21 (15.1%) |
| HPA-5 genotypes | 
| aa | 132 (95.0%) |
| ab | 7 (5.0%) |
| bb | 0 (0%) |
| HPA-15 genotypes | 
| aa | 43 (30.9%) |
| ab | 65 (46.8%) |
| bb | 31 (22.3%) |
| HPA combined genotypes | 
| 2aa/3ab/5aa/15ab | 29 (20.9%) |
| 2aa/3ab/5aa/15aa | 23 (16.5%) |
| 2aa/3ab/5aab/15ab | 15 (10.8%) |
| 2aa/3ab/5aab/15bb | 12 (8.6%) |
| 2aa/3bb/5aab/15bb | 12 (8.6%) |
| 2aa/3aab/5aab/15ab | 11 (7.9%) |
| 2aa/3aab/5aab/15bb | 10 (7.2%) |
| 2ab/3aab/5aab/15ab | 6 (4.3%) |
| 2aab/3aab/5aab/15ab | 5 (3.6%) |
| 2aab/3aab/5aab/15aa | 3 (2.2%) |
| 2aab/3aab/5aab/15ab | 3 (2.2%) |
| 2aab/3aab/5aab/15bb | 2 (1.4%) |
| 2aab/3aab/5aab/15aa | 2 (1.4%) |
| 2aab/3aab/5aab/15bb | 1 (0.7%) |
| 2aab/3aab/5aab/15aa | 1 (0.7%) |
| 2aab/3aab/5aab/15ab | 10 (7.0%) |
| 2aab/3aab/5aab/15bb | 10 (7.0%) |
| 2aab/3aab/5aab/15bb | 10 (7.0%) |
| 2aab/3aab/5aab/15bb | 10 (7.0%) |
| 2aab/3aab/5aab/15ab | 10 (7.0%) |
| 2aab/3aab/5aab/15bb | 10 (7.0%) |
| 2aab/3aab/5aab/15bb | 10 (7.0%) |

*The mean ± standard deviation.

| Platelet count and MPV in the study participants according to HPA genotype.

| Platelet count (\( \times 10^3/\mu L \)) | HPA-2 | HPA-3 | HPA-5 | HPA-15 |
|---------------------------------------|-------|-------|-------|-------|
| ab or bb | 234.35 ± 50.10 | 226.73 ± 51.64 | 239.36 ± 49.81 | 251.00 ± 58.41 |
| P-value | 0.020 | 0.084 | 0.045 | 0.032 |
| MPV (fL) | 10.07 ± 0.73 | 10.19 ± 0.76 | 10.01 ± 0.72 | 10.02 ± 0.72 |
| P-value | 0.352 | 0.148 | 0.001 | 0.715 |

Note: Data are given as the mean ± standard deviation.
platelet GPs are rare in healthy individuals. Therefore, an alternative mechanism in place of alloantibodies-mediated platelet clearance may exist and needs to be investigated. The HPA-2 and -5 polymorphic antigenic determinants are located on GPIba and GPIa, respectively [26]. GPIba is essential for membrane development and distribution in maturing megakaryocytes, and GPIa might negatively regulate proplatelet formation [36,37]. Taken together, these HPA polymorphisms might play important roles in megakaryopoiesis and proplatelet formation. In addition, their roles in non-alloantibodies-mediated platelet clearance should also be studied.

Previous studies have shown the association of a number of human SNPs with MPV [19–22,38]. For example, Meisinger et al. [38] found a strong association of MPV with three common SNPs: rs7961894 (intron 3 of WDR66), rs12485738 (upstream of ARHGEF3) and rs2138852 (upstream of TAOK1). Our study shows an association of the HPA-5 but not of the HPA-2, -3 and -15 genotypes with MPV. Using a multiple linear regression, we further identify HPA-5 polymorphism as an independent factor for MPV. As mentioned above, the HPA-5 polymorphic antigenic determinants are located on GPIa. A recent study showed that rs28095 in GPIa was correlated with MPV, and allelic differences in rs28095 were the most important factor regulating integrin α2β1 levels [39]. The authors concluded that expression levels of integrin α2β1 might be associated with the regulation of platelet size and rs28095 likely affected MPV via the influence of integrin α2β1 level on megakaryocyte maturation. It is possible that HPA-5 polymorphism also affects MPV via a similar mechanism. Therefore, it will be of interest to further investigate the association between HPA-5 polymorphism and expression levels of integrin α2β1.

A previous study showed differences in the association of SNPs with hematological trait variation among different ethnic groups [20]. Our study indicates an association of the HPA polymorphisms with platelet count and MPV in a healthy Chinese Han population. Whether this association is also applicable in other ethnic groups needs further elucidation.

In conclusion, we demonstrate that HPA-2, -5 and -15 polymorphisms are associated with the platelet count and HPA-5 polymorphism is associated with MPV. This finding is useful for further exploring the association of HPA polymorphisms with platelet-related diseases, such as infarction and cardiovascular diseases.

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Disclosure statement
No potential conflict of interest was reported by the authors.

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