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Human platelet antigens are associated with febrile non-hemolytic transfusion reactions

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ABSTRACT

Background: Febrile non-hemolytic transfusion reaction (FNHTR) is the most common type of transfusion reactions, and it could be reduced by transfusing patients with leukocyte-poor blood products. However, FNHTR still occur in certain patients transfused with leukocyte-poor red blood cell (LPR) products. It is examined whether human platelet antigen (HPA) could be a potential membrane antigen that plays a role in FNHTR.
Methods: A total of 120 inpatient subjects who transfused with LPR (60 in FNHTR group, 60 in control group) were typed for HPA-2, HPA-3, and HPA-15 using sequence specific primer-polymerase chain reaction (SSP-PCR) and electrophoresis.
Results: HPA-2 unmatched rate between donors and patients in FNHTR group was 18%, and only 3% unmatched rate was observed in control group ($p = 0.0082$). FNHTR group was further classified according to the imputability. There was a significant difference ($p = 0.0041$) between FNHTR (probable imputability, infection) group and control group, and more significant difference ($p = 0.0008$) was seen between FNHTR (probable imputability, febrile neutropenia) group and control group.
Conclusions: Those results indicated that HPA-2 might play roles on inducing FNHTR in patients suffering from infectious diseases and febrile neutropenia. HPA-2 genotyping between donors and recipients might be worth integrating in pre-transfusion testing to increase transfusion safety.

1. Introduction

In transfusion medicine, pre-transfusion tests contain ABO typing, Rh typing, antibody screening and cross matching. Blood transfusion reactions occur frequently even after performing complete pre-transfusion tests [1–6]. Febrile non-hemolytic transfusion reaction (FNHTR) is the most common type of transfusion reactions and usually defined as a body temperature increase of ≧1 °C in a transfused patient with no other apparent alternative explanation for fever [7]. Hemovigilance guideline of national healthcare safety network (NHSN) in the U.S. defined definitive FNHTR as fever or chills occurring during or within 4 h of cessation of transfusion [8], and the transfusion reaction will be classified according to its severity and imputability.

In fact, FNHTR could be reduced by transfusing patients with leukocyte-poor blood products [9]. Although FNHTR is usually non-life-threatening and the fever and associated symptoms could be lessened by giving an anti-pyretic, it could cause patient discomfort and potentially exacerbate underlying disease [10–12]. Specifically, as fever associated with FNHTR may also be representative of serious health conditions, such as sepsis, infection, or febrile neutropenia, the life-saving blood transfusions are likely to be stopped until reaction investigation is completed [10–12].

However, FNHTR still occurs in certain patients transfused with leukocyte-poor blood products. Because platelet can't be completely reduced in leukocyte-poor red blood cell (LPR) products, we proposed that the human platelet antigen (HPA) could be a potential membrane antigen that plays a role in FNHTR.

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Table 1
Primers for HPA-2, -3 and -15 genotyping.

Primer code	Primer sequence	Glycoprotein	Gene	Size of PCR product (bp)
HPA-2a	CCC CCA GGG CTC CTG AC	GPIbα	GP1BA	241
HPA-2b	CCC CCA GGG CTC CTG AT			
HPA-2AS	GCC AGC GAC GAA AAT AGA GG			
HPA-3a	AAT GGG GGA GGG GCT GGG GA	GPIIb	ITGA2B	233
HPA-3b	GGG GGA GGG GCT GGG GC			
HPA-3AS	GAC CTG CTC TAC ATC CTG GA			
HPA-15a	AGT CTA CCT GTT TAC TAT CAA AG	CD109	CD109	510
HPA-15b	AGT CTA CCT GTT TAC TAT CAA AA			
HPA-15AS	CTC TCA TGG AAA ATG GCA GTA C			
HGH-S	TGC CTT CCC AAC CAT TCC CTT A			434
HGH-AS	CCA CTC ACG GAT TTC TGT TGT GTT TC			

2. Materials and methods

2.1. Study subjects and sample collection

The study was approved by the Ethics Committee of the CGMH, and all study subjects signed an informed consent. A total of 120 inpatient subjects who transfused with LPR were included between 1 January 2016 and 31 May 2017. Sixty study subjects developed FNHTR after being transfused with LPR (FNHTR group) and classified according to the hemovigilance guideline of NHSN, and another 60 patients didn't develop transfusion reaction (control group). Whole blood samples of study subjects and transfused LPR were collected.

2.2. DNA extraction, sequence specific primer-polymerase chain reaction (SSP-PCR) assay and electrophoresis

Genomic DNA was extracted from whole blood collected in EDTA-coated vacuum tubes using a QIAamp® DNA Mini kit and processed according to the manufacturer's instructions [13]. HPA-2, HPA-3 and HPA-15 were chosen for SSP-PCR assay.

PCR primers and mixtures for HPA-2, -3 and -15 are shown in Table 1, respectively. Genotyping for each HPA was carried out by using three detection primers in 2 PCR amplification reactions that regularly contained an internal control for the validation of the PCR [13]. Primers amplifying a fragment of the human growth hormone gene served as internal positive controls (Table 1) [14]. Primer mixes for the HPA alleles contained each of the internal control primers and each of 2 primers amplifying one HPA allele [14]. For HPA-2 and -3 PCR cycling was performed as follows. After denaturation for 11 min at 95 °C, samples were subjected to 32 cycles of PCR in a peltier thermal cycler (PTC-200 thermal cyclers, BioRad). Each PCR cycle comprised denaturation (95 °C, 30 s), annealing (61 °C, 50 s), and extension (72 °C, 30 s), and finally soaking at 10 °C forever [13]. On the other hand, HPA-15 PCR cycling was performed as follows. Initial denaturation was carried out at 95 °C for 10 min, amplification for 35 cycles at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min and extension at 72 °C for 7 min and finally soaking at 10 °C forever [15].

For gel electrophoresis visualization, 5 μl of the PCR products was pipetted onto a 2.5% agarose gel [13–15]. Finally, HPA-2 and -3 PCR products run at 100 V for 5 min and HPA-15 PCR products run at 100 V for 30 min, visualized under UV illumination, and documented by photography [13–15]. In addition to PCR-SSP, sequence based typing (SBT) was used to confirm that PCR products' sequence was completely accurate. The amplified PCR products were purified using 0.05 μl of shrimp alkaline phosphatase and 2.5 μl of exonuclease (BioLabs). The PCR cycle was started at 37 °C for 30 min, 80 °C for 15 min and subsequent storage at 10 °C.

2.3. Statistical analysis

Genotype and gene frequencies were determined by direct counting. The allelic and genotypic frequencies were evaluated by the Pearson's chi-squared test for goodness of fit (a $p < 0.05$ was considered statistically significant) [16].

3. Results

Through the SSP-PCR and gel electrophoresis visualization, representative results of HPA-2, HPA-3, and HPA-15 genotyping of 8 samples are shown in Fig. 1. The genotyping results obtained by SSP-PCR were validated using genotyping data by SBT. No discrepancies were observed between the SSP-PCR and SBT typing for HPA-2, HPA-3, and HPA-15 systems in 240 selected DNA samples, indicating the reliability of the SSP-PCR results.

HPA-2 unmatched rate between donors and patients in FNHTR group was 18% (Table 2), and only 3% unmatched rate was observed in control group. There was a significant difference between 2 groups ($p = 0.0082$). However, there was no significant difference between 2 groups in HPA-3 and HPA-15 unmatched rate. These results revealed that HPA-2 might play roles in FNHTR.

FNHTR group was further classified according to the imputability defined by hemovigilance guideline of NHSN. Eighteen FNHTR cases were classified as definite imputability (Table 2) because there were no other conditions that could explain fever during or within 4 h of cessation of transfusion. Twenty-one cases were suffering from infectious diseases on transfusion, so that condition could explain fever. Therefore, those cases were classified as probable imputability. Febrile

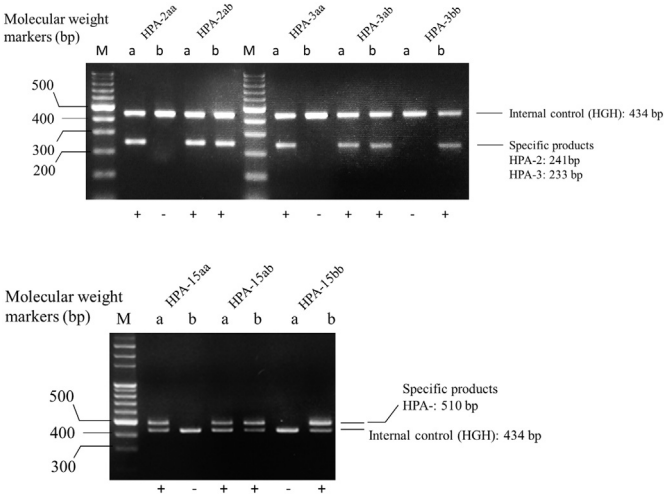

Fig. 1. A typical case for simultaneous HPA-2, -3 and -15 genotyping, whose HPA allele was confirmed by PCR-SSP. The 434-bp amplification product of the HGH control primers is present in all lanes, which shows that amplification has occurred optimally. A positive specific amplification demonstrated the presence of an HPA allele. Absence of an allele was accepted only in case of the positivity of the internal control. M = 100- to 3000-bp DNA markers.