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ORIGINAL ARTICLE

Methodological Study on the Establishment of HLA/HPA Gene Bank of Platelet Donors and Its Clinical Application

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Abstract The current study aimed at establishing a large-scale platelet donor database with known HPA and HLA genotypes in Ningbo, to provide matched platelets for the clinic and prevent the occurrence of ineffective immune platelet transfusion refractoriness (PTR). The platelet banks of clinical donor wtih HLA-A, HLA-B, HPA1, HPA2, HPA3, HPA4, HPA5, HPA6, HPA10, HPA15 and HPA21 genotyps were established. Meanwile, the platelet gene matching was performed on the donor. It was found that there were phenotype polymorphisms in 1000 donnors with HPA1, HPA2, HPA3, HPA4, HPA5, HPA6, HPA10, HPA15 and HPA21 genotyps, and allel polymorphism distribution in donnors with HPA1, HPA2, HPA3, HPA4, HPA5, HPA6, HPA15, HPA21 genotyps. The frequency of HPA10 was a gene, and not b gene, showing a single linear distribution. The HPA 2, HPA3, HPA15 system were the most polymorphic with three phenotypes: aa, ab, bb. In the HLA-A allele, the highest frequency is A*11:01 (24.25%). There were 13 alleles that were greater than 1%, such as A*24:02, A*02:01, A*33:03, and the accumulated frequency reached 96.20%. In the HLA-B allele, the highest gene frequency was B*40:01 (13.40%). There were 24 alleles that were greater than 1%, such as B*46: 01, B*58: 01, B*15: 01, and the accumulated frequency reached 91.60%. Platelet antibody cross matching was performed on 100 blood samples from patients with thrombocytopenia after multiple platelet transfusions. The number of consistent samples was 46 (46%). Twenty patients were transfused with platelet cross matching. Among them, 18 patients had obvious improvement in clinical symptoms and good hemostatic effect after transfusion, which was judged to be effective. Platelet donor HPA and HLA-A, B antigen genotyping database provided patients with individual appropriate platelets, and provided the effectiveness of immune platelet infusion ensuring effective platelet transfusion.

Keywords HPA · HLA · Platelet donor database

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Introduction

Platelet transfusion refractoriness (PTR) is one of the difficult problems in clinical blood transfusion. The ineffective platelet infusion caused by immune platelet antibodies (mainly HLA and/or HPA) accounted for about 18–25% [1]. HLA antigen, also known as tissue antigen, is widely found on the surface of nuclear cells with strong immunogenicity and easily causing alloimmune response. Patients with long-term repeated blood

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transfusion are prone to produce HLA antibodies and the antibody positive rate increases with the increase of blood transfusion times. HLA antibody is the main cause of ineffective platelet transfusion caused by immune reasons.

At present, there are two main ways to solve PTR: one way is to screen HLA/HPA specific platelets through serological platelet cross matching test. The second way is to establish a donor platelet bank with known HLA and HPA genotypes, and select blood donors with the HLA/HPA gene for platelet transfusion. Therefore, the establishment of HLA-A, HLA-B and HPA gene database of unpaid blood donors to provide gene matched donors platelet for patients is a feasible method to solve the ineffective platelet transfusion, improving the clinical efficacy and further improve the safety of blood transfusion.

Materials and Methods

Study Object

The platelet donor database was established by selecting 1000 blood donors who participated in the platelet donation for more than 3 times from January 2018 to December 2021 for HLA-A, HLA-B, HPA-1, HPA-2, HPA-3, HPA-4, HPA-5, HPA-6, HPA-10, HPA-15, and HPA-21 genotyping, including 550 cases men and 450 cases women, A (324 cases), B (237 cases), O (93 cases) and AB (346 cases), respectively, aged 18–55. All of them signed the informed consent form of platelet blood group genotyping of component blood donors, which voluntarily joined the platelet blood group genotyping database and was collected whole blood samples. 5 ml venous whole blood was collected, anticoagulated with EDTA and stored in 4 °C for 15 days or − 20 °C for 1 year.

Reagent and Instrument

DNA extraction kit (Tianlong Biotechnology Co., Ltd.), HLA-A, B genotyping detection kit (PCR-SBT method, Jiangsu Weihe Biotechnology Co., Ltd.), Human platelet-specific antigen HPA-1-6, 10,15,21 genotyping detection kit (quadruple fluorescent PCR method, Jiangsu Weihe Biotechnology Co., Ltd.), Taq DNA polyase and high fidelity enzymes (Promega Corporation), Big Dye Terminator (v3.1), ExoSAP-IT; Platelet antibody detection kit (Capture method, IMMUCOR Corporation), ABI 7500 fluorescent PCR instrument, ABI 3730 Sequencer, Frozen centrifuge (Sigma, Germany).

Method

Nucleic Acid Extraction

The ZTLYQ nucleic acid extraction kit and Tianlong NP968-S automatic nucleic acid extractor was performed, and the magnetic beads were adsorbed, transferred and released through a special magnetic rod based on the principle of magnetic bead adsorption, so as to realize the transfer of magnetic beads and nucleic acid and automatically complete the extraction of sample nucleic acid. The DNA concentration of the obtained sample should be between 15 and 40 ng/μL, the A260/A280 absorbance should be between 1.6 and 2.0, which can be tested immediately, stored at 4 °C for 10 days or − 20 °C for 6 months.

HLA-A, B Genotyping

The specific primers were designed according to the known human leukocyte antigen gene sequence and the specific primers for amplification reaction were selected according to the characteristic table of amplification primers. The amplification PCR reaction procedure was set as follows: 95 °C for 5 min, 93 °C for 30 s, 63 °C for 40 s, 72 °C for 2 min and 30 s, 36 cycles in total; 72 °C for 5 min; 4 °C until removed. Electrophores: prepare 2.0% agarose gel with grade DNA agarose and 0.5 × TBE buffer, running gel at 8–10 V/cm, 200 V, for about 10–20 min. Purification of PCR product: for the reaction wells that want to be sequenced, add 4 μL ExoSAP-IT to remove excess primers and DNA. Sequencing reaction: the sequencing reaction was carried out. The reaction system includes: 1.5 μl BDT sequencing reagent, 2.5 μl primers for sequencing, 1 μl purified PCR product. Sequencing PCR reaction program setting: 96 °C for 1 min, 98 °C for 25 s, 60 °C for 2 min 30 s, 25 cycles in total, 4 °C until removed. Purification of sequencing products: excess BDT was removed by ethanol precipitation. Preparation before sequencing: before sequencing, 15 μl HiDi formamide (HiDi formamide) was redissolved and treated with PCR at 95 °C for 2 min. Reading of sequencing results: HLA-A, B genotypes were analyzed by supporting intelligent analysis software.

HPA-1–6, 10, 15 and 21 Genotyping

The HPA-1, HPA-2, HPA-3, HPA-4, HPA-5, HPA-6, HPA-10, HPA-15 and HPA-21 genes were qualitatively genotyped by real-time fluorescent PCR and TaqMan probe technology. According to the HPA gene sequences published in the database [2], the allele-specific primers and probes were designed by the amplification refractory mutation system (ARMS). With FAM/VIC/ROX labeling, 18 alleles are distributed in 6 reaction tubes. ABI 7500 fluorescence quantitative PCR instrument was performed, and the PCR amplification cycle parameters are: 95 °C for 2 min 30 s, 1 cycle; 94 °C for 15 s, 65 °C for 55 s, 35 cycles, and set according to the operation of the instrument. The detection channels included FAM, VIC, ROX, CY5 and Cy5 was the internal standard gene reporting fluorescence. At the end of the reaction, the reference values of fluorescence threshold were adjusted to FAM 5000, VIC 5000, ROX 10,000, CY5 5000, and the baseline reference value 3–15. According to the test results, the HPA 1–6, 10, 15, 21 genotypes of the samples to be tested are directly analyzed by 7500 Software v2.0.6 intelligent software analysis.

The HPA Allele Frequency

The HPA allele frequency was calculated by direct counting method, The calculation formula of the HPA antigen mismatch rate was as follows: The a antigen mismatch rate = $b^2(1 - b^2)$, The b antigen mismatch rate = $a^2(1 - a^2)$; The HPA antigen random infusion mismatch rate Pab = 2ab (1 − ab).

Platelet Cross-Matching

Platelet cross-matching was performed by IMMUCOR platelet antibody detection kit (capture method). 50 µl donor platelet suspension was added to the microplate, centrifuged by 45–65 g for 5 min (platelets were fixed at the bottom of the microplate),.the liquid in the micropore was gently poured out, and washed with 0.9% NaCl for 6 times, and added 2 drops of low ionic strength solution (LISS), and added 50µL recipient serum or plasma, and added positive control and negative control into the control hole. The mixture was incubated at 37 °C for 45 min, washed the reaction plate with 0.9% NaCl for 6–8 times, and added 50 µL indicator red blood cells, and centrifuged at 700–900 g for 1 min. The experimental results were determined and observed.

Platelet Donor Selection

The donors were selected in the platelet donor database according to the cross reaction groups (CREGs) matching technology, and the matching classification was in accordance with the American AABB platelet HLA matching classification standard.

Statistical Analysis

Data were presented as mean ± SD. Corrected count increment, CCI $\geq 4.5 \times 10^9$/L indicated that the transfusion was effective and CCI $< 4.5 \times 10^9$/L indicated that the transfusion was invalid. CCI = ΔPLC × BSA/platelets transfused.

$$\Delta \mathrm{PLC} = 24 \text{ hours post} - \text{transfusion platelet count} - \text{pre-transfusion platelet count, Body surface area,}$$
$$\mathrm{BSA}\left(\mathrm{m}^2\right) = 0.0061 \times \text{height (cm)} + 0.0128 \times \text{weight(kg)} + 0.01529.$$

Statistical analyses were performed with SPSS 17.0. Statistical significance between groups was evaluated by Mann–Whitney U test. P value < 0.05 was considered statistically significant.

Result

Genotyping of HPA

The HPA-1, HPA-2, HPA-3, HPA-4, HPA-4, HPA-5, HPA-6, HPA-10, HPA-15 and HPA-21 genes were qualitatively genotyped by real-time fluorescent PCR method combined with TaqMan probe technology. Table 1 showed the genotypes and gene frequencies of the HPA genes in platelet donor. The HPA real-time fluorescent PCR diagram was shown in Fig. 1.

In the 1000 samples, there were polymorphisms in the HPA-1, HPA-2, HPA-3, HPA-4, HPA-5, HPA-6, HPA-10, HPA-15 and HPA-21 systems. The genotype aa frequency of HPA-1 system was 0.9870, the ab frequency was 0.0130; the genotype aa frequency of HPA-2 system was 0.8830, the ab frequency was 0.1140, the bb frequency was 0.0030; the genotype aa frequency of HPA-3 system is 0.3180, the ab frequency was 0.4840, the bb frequency was 0.1980; the genotype aa frequency of HPA-4 system was 0.9990, the ab frequency is 0.0010; the genotype aa frequency of HPA-5 system was 0.9690, the ab frequency was 0.0310; the genotype aa frequency of HPA-6 system was 0.9740, the ab frequency was 0.0260; the HPA-10 system a frequency was 1.0000, the b frequency was 0.0000; the genotype aa frequency of HPA-15 system was 0.3010, the ab frequency was 0.4830, the bb frequency was 0.2160; the HPA-21 system aa frequency was 0.9750, the ab frequency was 0.0250.

The frequencies of the HPA-1, HPA-2, HPA-3, HPA-4, HPA-5, HPA-6, HPA-10, HPA-15 and HPA-21 allele for platelet donors were shown in Table 1. All the alleles we detected revealing the polymorphic distribution apart from HPA 10. The frequency of HPA10 a gene was 1 and no b

Table 1 Genotypes and gene frequencies of HPA-1–6, 10, 15, 21 in platelet donor

Genotype	aa	aa (%)	ab	ab (%)	bb	bb (%)	a (%)	b (%)	Mismatch rate (%)
HPA-1	987	98.70	13	1.30	0	0.00	0.9935	0.0065	1.28
HPA-2	883	88.30	114	11.40	3	0.30	0.9400	0.0600	10.64
HPA-3	318	31.80	484	48.40	198	19.80	0.5600	0.4400	37.14
HPA-4	999	99.90	1	0.10	0	0.00	0.9995	0.0005	0.10
HPA-5	969	96.90	31	3.10	0	0.00	0.9845	0.0155	3.01
HPA-6	974	97.40	26	2.60	0	0.00	0.9870	0.0130	2.53
HPA-10	1000	100.00	0	0.00	0	0.00	1.0000	0.0000	0
HPA-15	301	30.10	483	48.30	216	21.60	0.5425	0.4575	37.32
HPA-21	975	97.50	25	2.50	0	0.00	0.9875	0.0125	2.44

HPA mismatch rate Pab = 2ab (1 − ab)

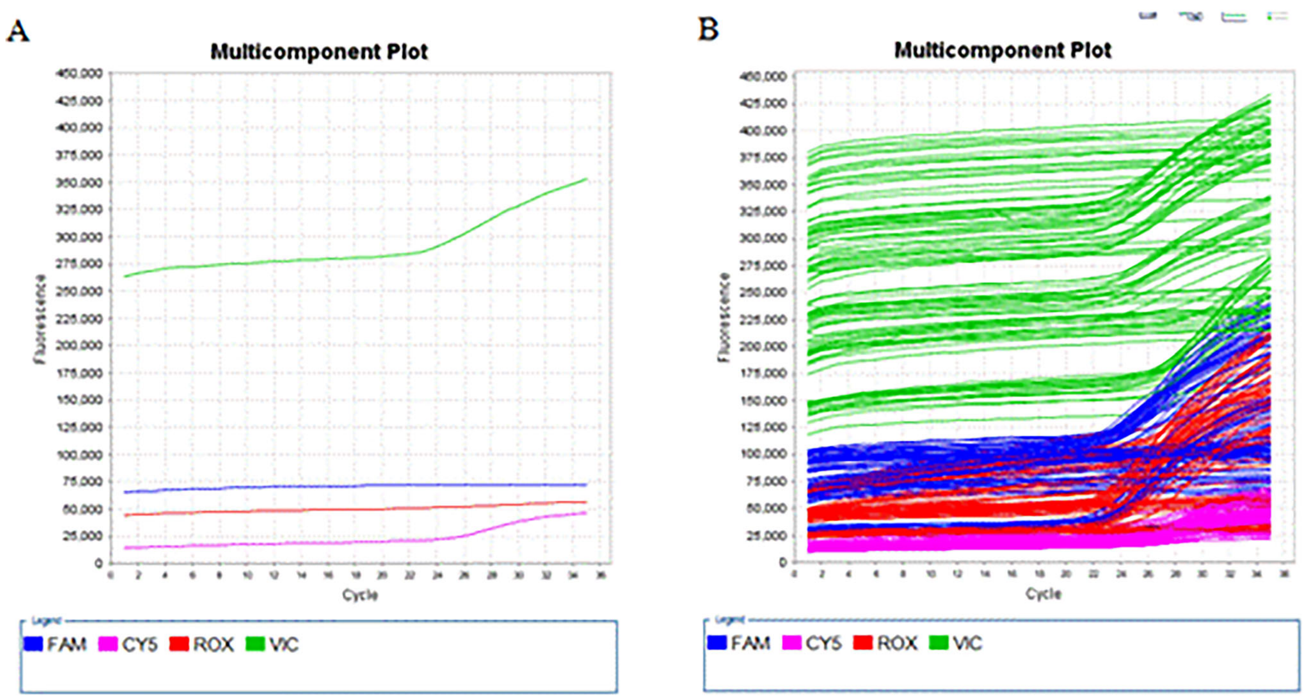

Fig. 1 **A** and **B** show some HPA real-time fluorescent PCR diagram

gene was found, showed a single linear distribution. The HPA-2, HPA-3 and HPA-15 systems had the most nucleotide polymorphim with three phenotypes: aa, ab, bb.

The HLA-A, B Genes and Frequencies

Detection of HLA-A and HLA-B Alleles

The direct sequencing method was adopted for gene detection, and the reading results of HLA-A and B genotypes were analyzed by using the intelligent analysis software matched with the instrument. The frequencies of HLA-A and HLA-B allele in platelet donors were shown in Tables 2 and 3, respectively. The genotyping and sequencing diagram was shown in Figs. 2 and 3.

Frequencies of HLA-A, HLA-B Antigens in Platelet Donors

The frequencies of HLA-A, HLA-B antigens of platelet donors were shown in Table 4. The highest frequency of HLA-A antigen was A11 (26.7%), and HLA-A antigens with gene frequency were greater than 1% included A2, A24, A33, A30, A31, A203, A26, A1, A3 and A29, with a cumulative frequency of 97.75%. Meanwhile, the highest frequency of HLA-B antigen was B60 (12.7%), and 19 kinds of HLA-B antigens with gene frequency were greater than 1%, with a cumulative frequency of 95.20%, including B46, B13, B58, B62, B61, B75, B51, B35, B55, B27, B54, B38, B3901, B7, B48, B52, B44, B71 and B5102.

Table 2 HLA-A allele frequencies in platelet donors

HLA-A	n	Frequency (%)	HLA-A	n	Frequency (%)
A*11:01	485	24.25	A*24:07	4	0.20
A*24:02	320	16.00	A*69:01	4	0.20
A*02:01	237	11.85	A*30:02	3	0.15
A*33:03	197	9.85	A*23:01	2	0.10
A*02:07	193	9.65	A*33:01	2	0.10
A*30:01	96	4.80	A*74:01	2	0.10
A*02:06	85	4.25	A*02:02	1	0.05
A*31:01	67	3.35	A*11:03	1	0.05
A*02:03	60	3.00	A*02:28	1	0.05
A*11:02	57	2.85	A*02:53	1	0.05
A*26:01	47	2.35	A*03:02	1	0.05
A*01:01	31	1.55	A*24:03	1	0.05
A*03:01	28	1.40	A*24:08	1	0.05
A*29:01	21	1.05	A*24:10	1	0.05
A*32:01	15	0.75	A*26:03	1	0.05
A*68:01	12	0.60	A*30:04	1	0.05
A*24:20	7	0.35	A*30:18	1	0.05
A*02:05	6	0.30	A*31:32	1	0.05
A*02:10	6	0.30	A*74:03	1	0.05

Platelet Cross-Match

Platelet antibody cross matching was performed on 100 blood samples from patients with thrombocytopenia after multiple transfusions. The number of compatible samples was 46 (46%). The number of platelets before and after platelet transfusion was counted among 100 patients. The number of platelets before platelet transfusion was $(8.13 \pm 3.52) \times 10^9$/L, and the number of platelets after transfusion was $(35.28 \pm 12.52) \times 10^9$/L, and the average platelet count increased by 27.15×10^9/L, and CCI value was 20.16, $P < 0.005$, which were significant differences between pre-transfusion and post-transfusion.

Clinical Application of HLA/HPA Gene Database

HLA database was used to search for platelet donors matched with gene "digital" data, and platelets were collected and infused. Twenty clinical patients were required HLA/HPA genotyping.

In twenty cases of HLA genotyping patients, one case from Grade A donors of HLA genotyping, the number of pre-transfusion platelet was 7.15×10^9/L, and the number of platelet at 24 h post-transfusion was 38.26×10^9/L. Platelet count increased by 31.11×10^9/L and CCI value

Table 3 Allele frequencies of HLA-B in platelet donors

HLA-B	n	Frequency (%)	HLA-B	n	Frequency (%)	HLA-B	N	Frequency (%)
B*40:01	268	13.40	B*15:18	23	1.15	B*18:02	2	0.10
B*46:01	240	12.00	B*15:27	22	1.10	B*35:08	2	0.10
B*58:01	176	8.80	B *27:05	19	0.95	B*38:01	2	0.10
B*15:01	122	6.10	B*07:05	15	0.75	B*39:05	2	0.10
B*13:02	109	5.45	B*67:01	15	0.75	B*40:40	2	0.10
B*50:01	103	5.15	B*35:03	11	0.55	B*49:01	2	0.10
B*13:01	79	3.95	B*08:01	9	0.45	B*51:01	2	0.10
B*15:02	74	3.70	B*37:01	9	0.45	B*15:10	1	0.05
B*55:02	61	3.05	B*56:01	9	0.45	B*15:15	1	0.05
B*54:01	58	2.90	B*35:05	6	0.30	B*15:17	1	0.05
B*38:02	53	2.65	B*40:03	6	0.30	B*15:25	1	0.05
B*35:01	51	2.55	B*44:02	6	0.30	B*15:35	1	0.05
B*39:01	51	2.55	B*48:03	6	0.30	B*15:38	1	0.05
B*40:06	50	2.50	B*15:07	5	0.25	B*18:01	1	0.05
B*40:02	48	2.40	B*35:02	3	0.15	B*27:06	1	0.05
B*27:04	39	1.95	B*53:01	3	0.15	B*39:15	1	0.05
B*52:01	36	1.80	B*55:04	3	0.15	B*39:24	1	0.05
B*15:11	31	1.55	B*59:01	3	0.15	B*41:01	1	0.05
B*48:01	31	1.55	B*14:02	2	0.10	B*45:01	1	0.05
B*57:01	31	1.55	B*15:05	2	0.10	B*55:01	1	0.05
B*07:02	29	1.45	B*15:12	2	0.10	B*55:07	1	0.05
B*44:03	24	1.20	B*15:29	2	0.10	B*81:01	1	0.05
B*51:02	24	1.20	B*15:32	2	0.10			

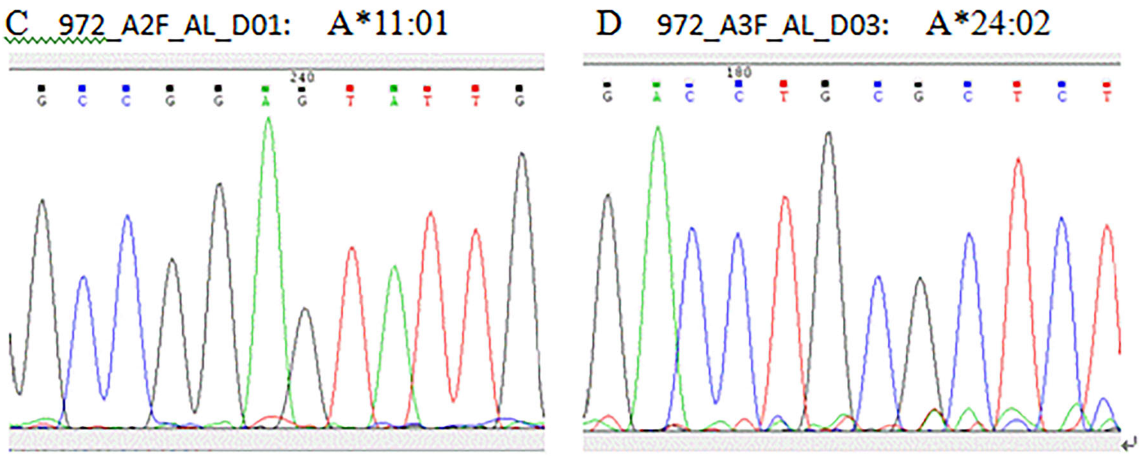

Fig. 2 **C** and **D** show the genotyping and sequencing map of A*11:01 and A*24:02

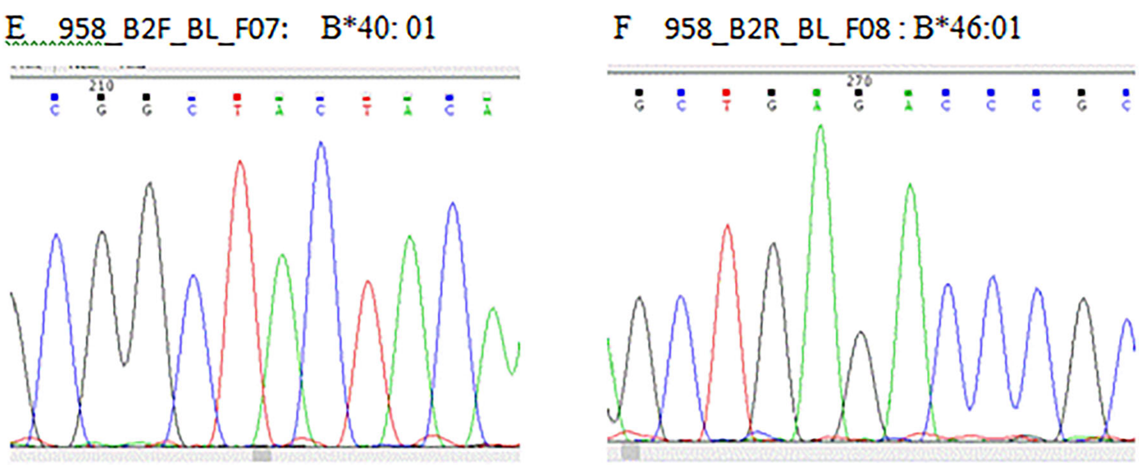

Fig. 3 **E** and **F** show the genotyping and sequencing map of B*40:01 and B*46:01

was 59.73 $\times$ 10^9/L, P value $<$ 0.05. In addition, Grade B1U four cases, grade BU four cases, grade B1X five -cases, BUX grade three cases, grade B2UX one case had been screened, there were a significant improvement in the mean CCI. However, in two patients (one grade B1U and one grade B2UX), there was no noticeable increase in CCI values, as the values were 3.72 $\times$ 10^9/L and 2.13 $\times$ 10^9/L, respectively. Comparison of 24 h CCI in HLA genotyping patients was shown in Table 5.

Discussion and Conclusion

Platelet transfusion is a supportive therapy for blood diseases, tumors and other diseases. In China, platelet transfusion strategy is usually only based on ABO isotype, and patients are transfused with random platelets multiple times, generating various of alloimmune antibodies in blood. The genaration of alloimmune antibodies caused by the HPA and/or HLA is one of the reasons for the ineffective blood transfusion. It is a feasible method to detect the HPA and/or HLA genes, antigens and antibodies preventing PTR in clinic [3].

In the HPA system, we found that the HPA-2, HPA-3 and HPA-15 systems had the most nucleotide polymorphim and HPA-2b, HPA-3b and HPA-15b antigens had the highest proportion and mismatch rate, which were of important immunological significance in blood transfusion. In addition,the proportion of HPA-3b and HPA-15b antigen frequency was close to that reported by Tomoya H et al. (HPA-3b, 41.3%; HPA-15b, 48.8%) [4].

At present, the main HLA genotyping methods include PCR-SSP, PCR-SSO, PCR-SBT, flow cytometry, gene chip and so on. Among them, direct sequencing typing is the most accurate, complete and reliable genotyping method. In this study, we had established a platelet HLA gene database of 1000 donors by using direct sequencing technology.

Table 4 Frequencies of HLA-A and B antigens in platelet donors

Antigen	n	Frequency (%)	Antigen	n	Frequency (%)
HLA-A			HLA-B		
A11	534	26.7	B60	254	12.70
A2	522	26.1	B46	236	11.80
A24	334	16.7	B13	194	9.70
A33	198	9.9	B58	190	9.50
A30	102	5.1	B62	161	8.05
A31	72	3.6	B61	109	5.45
A203	64	3.2	B75	105	5.25
A26	48	2.4	B51	98	4.90
A1	31	1.55	B35	73	3.65
A3	29	1.45	B55	65	3.25
A29	21	1.05	B27	59	2.95
A32	16	0.8	B54	59	2.95
A68	12	0.6	B38	56	2.80
A210	6	0.3	B3901	51	2.55
A69	4	0.2	B7	44	2.20
A74	3	0.15	B48	37	1.85
A23	2	0.1	B52	36	1.80
A2403	2	0.1	B44	30	1.50
			B71	24	1.20
			B5102	23	1.15
			B57	18	0.90
			B67	15	0.75
			B50	10	0.50
			B8	9	0.45
			B37	9	0.45
			B56	9	0.45
			B39	3	0.15
			B59	3	0.15
			B53	3	0.15
			B18	3	0.15
			B81	3	0.15
			B49	2	0.10
			B15	2	0.10
			B76	2	0.10
			B65	2	0.10
			B45	1	0.05
			B41	1	0.05
			B63	1	0.05
Total	2000	100	2000	100	

It can be seen from Table 2 that the highest frequency of HLA-A alleles was A*11:01, accounting for 24.25%. Alleles greater than 1% included A*24:02, A*02:01, A*33:03, A*02:07, A*30:01, A*02:06, A*31:01, A*02:03, A*11:02, A*26:01, A*01:01, A*03:01 and A*29:01, with a cumulative frequency of 96.20%. The common HLA allele HLA-A*24:02 accounts for 16.0%, which was close to South Korea (19.5%), Chinese in Hong Kong (14.7%) and Syria (12.9%), Other countries such as Japan (37.9%) and the Philippines (31.3%) are higher than our data, while the frequencies of American whites (7.5%), British whites (6.9%) and Saudis (8.5%) were lower than ours[5, 6].

It can be seen from Table 3 that the highest frequency of HLA-B alleles was B*40:01, accounting for 13.4%. Alleles were greater than 1% included B*46:01, B*58:01, B*15:01, B*13:02, B*50:01, B*13:01, B*15:02, B*55:02, B*54:01, B*38:02, B*35:01, B*39:01, B*40:06, B*40:02, B*27:04, B*52:01, B*15:11,, B*48:01, B*57:01, B*07:02, B*44:03, B*51:02, B*15:18 and B*15:27. The cumulative frequency reached 91.60%. Among them, the gene frequencies of B*40:01, B*46:01, B*58:01 were the highest, closed to those reported by F Wang et al., which were 14.0%, 11.7%, and 8.9% respectively[7].

More and more researches were reported on the correlation research between alleles of HLA-B locus and disease. We found the ratio of susceptible genes B*15:27 and B*39:01 of coronavirus disease-2019 (COVID-19) was 1.10% and 2.55% respectively, which was close to the results of 1.071–1.471% and 1.289–1.945% reported by Wei W, Wang BX [8, 9]. The mechanism of association between HLA-B alleles and diseases might be that HLA-B allele polymorphism lead to the diversity of intracellular folding and assembly characteristics and affected the diversity of CTL and NK cell-mediated immune responses [10].

It can be seen that the highest proportion of HLA-A antigens was A11, accounting for 26.7%, HLA-A antigens with gene frequency were greater than 1% included A2, A24, A33, A30, A31, A203, A26, A1, A3 and A29, with a cumulative frequency of 97.75%. Among them, the highest HLA-B antigen was B60, accounting for 12.7%, HLA-B antigens with gene frequency were greater more than 1% included B46, B13, B58, B62, B61, B75, B51, B35, B55, B27, B54, B38, B3901, B7, B48, B52, B44, B71 and B5102, with a cumulative frequency of 95.20%.

Platelet cross matching is aimed to detect compatibility between platelet recipients and donors. If there is no antibody corresponding to the donor platelet in the recipient's serum, the two platelets are compatible. Platelet cross matching can solve some problems of PTR. The CCI value of platelet count is one of the indicators of the effectiveness of platelet transfusion, which has extremely important clinical significance.

The platelet count was significantly increased in 18 of 20 post-transfusion clinical patients (24 h CCI $\geq 4.5 \times 10^9$/L), with obvious improvement of clinical symptoms and good hemostatic effect, which was judged that the transfusion was effective. In addition, the

Table 5 Comparison of 24-h CCI in HLA genotyping patients ($\bar{\chi} \pm s$, $\times 10^9$/L)

Group	n	Pre-transfusion	Post-transfusion	24 h CCI	P value
Grade A	1	7. 1 5	38.26	59.73	< 0.05
Grade B1U	4	9.13 ± 3.45	36.18 ± 10.52	51.93 ± 6.99	< 0.05
Grade BU	4	8.79 ± 3.12	32.28 ± 9.48	45.10 ± 6.30	< 0.05
Grade B1X	5	7.95 ± 3.04	24.27 ± 8.63	31.33 ± 584	< 0.05
Grade BUX	3	7.85 ± 2.98	23.19 ± 8.67	29.45 ± 5.83	< 0.05
Grade B2UX	1	7.69	20.22	24.06	< 0.05
Grade B1U	1	9. 11	11.05	3.72	> 0.05
Grade B2UX	1	7.15	8.26	2.13	> 0.05

platelet counts of grade B1U 1 case (CCI = 3.72 $\times$ 10^9/L) and grade B2UX 1 case (CCI = 2.13 $\times$ 10^9/L) about HLA genotyping patients did not reach the expected target, so it was judged as invalid transfusion due to that the HPA typing was mismatch.

In order to avoid ineffective platelet transfusion, most patients require platelet matching services to carry out platelet matching quickly and efficiently. We used serological platelet cross matching allowing most patients to obtain matched platelets, which could to solve the problem of ineffective transfusion in most patients. Data from 100 patients are provided in this paper. In clinical practice, these patients will still have ineffective platelet transfusion after platelet cross matching. At this time, we need to determine genotype and use the established gene bank to find donors to achieve the effect of accurate transfusion.

PTR is a big difficult problem in clinical transfusion. The main cause of alloimmune response caused by repeated transfusion of incompatible platelets is HLA antibody, followed by platelet specific antibody (HPA). Platelet transfusion with serological matching and HLA /HPA gene matching can significantly improve the efficacy of platelet transfusion and ensure the safety and effectiveness of blood.

The establishment of a database can solve the problem of ineffective platelet transfusion in some clinical patients, and understand the polymorphism characteristics of the HLA/HPA in the Chinese population, and predict the risk of ineffective platelet transfusion of the HLA/HPA related alloimmunity and provide basic data for human hematoimmunology and genetics.

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Declarations

Conflict of interest The authors declare no potential conflict of interest.

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