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RAPID COMMUNICATION

DNA sequencing-based typing of HPA-1 to HPA-17w systems

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Abstract Although several DNA-based human platelet antigens (HPA) typing techniques, such as PCR-SSP and PCR-SSO, have been established, the typing errors and the lack of interlaboratory reproducibility are still the issues of concerns. In the present study, polymerase chain reaction primers were designed for identification of all the phenotypically different HPA-1 to HPA-17w types by sequencing-based typing (SBT) method using genomic DNA samples. No discrepancies were observed between PCR-SSP typing and SBT typing in typing a panel of HPA-typed platelet donors that included all common HPA types and the rare HPA-1b, 2b, 3b, and 6bw homozygous donors.

Keywords Human platelet antigens · HPA genotyping · Sequencing-based HPA typing

To date at least 24 human platelet antigens (HPA) have been defined by alloantibodies [1]. However, the usefulness of conventional serologic assays for HPA typing has been limited by the availability of specific typing sera. Following the determination of the molecular basis of 23 serologically defined antigens, several DNA-based HPA typing techniques have been developed. The polymerase chain reaction-sequence-specific priming (PCR-SSP), and PCR-sequence-specific oligo hybridization (PCR-SSO) are powerful methods for detecting genetic variants and have been widely used for HPA typing [2–5]. However, the DNA-based HPA typing errors and the lack of interlaboratory reproducibility are still the issues of concerns [6].

Here, we describe a sequencing-based typing method for HPA-1 to HPA-17w genotyping. This method is based on a single PCR amplification of a HPA gene fragment spanning a certain exon of interest. The amplification product is then used as cycle sequencing template. The sequencing reaction was performed using the same primers as used for PCR amplification. The sequence and the localization of the primers used in this study are indicated in the Table 1.

A panel of HPA-typed platelet donors (Table 2) selected from 1,831 individuals living in the Guangxi Zhuang Autonomous Region was typed for HPA-1 to HPA-17w using sequencing-based typing method. The panel of donors covered all HPA systems and included all available alleles detected in the lab. Informed consents were obtained from the participants in this study. The peripheral blood samples were collected using EDTA anti-coagulation tubes. Genomic DNAs for PCR amplification were prepared from whole blood using a QIAamp kit (Qiagen, Valencia, CA) and amplified by the Expand High Fidelity PCR System (Roche Diagnostics Corporation, Indianapolis, IN) using 0.5 μM of each forward and reverse primer according to the manufacture protocol. The PCR reaction was set up in a total volume of 100 μl. After denaturation for 5 min at 95°C, samples were subjected to 35 cycles of PCR in a DNA thermal cycler. Each cycle included 95°C for 30 s, 57°C for 30 s, and 72°C for 1.5 min followed by a final extension at 72°C for 5 min. This amplification generated a fragment encompassing the exon of interest. PCR products were analyzed by electrophoresis on a 2% agarose gel containing 0.5 μg/ml ethidium bromide and visualized

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Table 1 Primers used for sequencing-based HPA-1 to HPA-17w typing

HPA system	Glycoprotein location	Gene	Exon sequenced	Sequence specific primer (5′–3′)	Amplicon size (bp)	Nucleotide position	GenBank accession #
1, 10w	CD61	GPIIIa	3	F: CGGTCCTAAGGGATTATCCC	429	107232–107247	AC068234
				R: CCTCCTCAGACCTCCACC		106819–106836	AC068234
4, 16w	CD61	GPIIIa	4	F: GGGAGAAGAAGATAAAAACTAAC	306	106017–106039	AC068234
				R: AGGAGGGACTTACTCATAGCAG		105734–105755	AC068234
17w	CD61	GPIIIa	5	F: CTGTCTGGGTAACTGTGGTTGTATG	374	104295–104319	AC068234
				R: CAGAGTTCAGTCACTTCCTTTCCC		103946–103969	AC068234
6w, 7w	CD61	GPIIIa	10	F: TTGGCAGGGCAGGGAACAAC	523	98377–98396	AC068234
				R: CTGAGCACATCTCCCCCTTG		97874–97893	AC068234
14w	CD61	GPIIIa	11	F: TAGCCTGCTGCCATGGGAG	414	91182–91200	AC068234
				R: CTGGAGTGGACTCCTCTCCC		90787–90806	AC068234
8w, 11w	CD61	GPIIIa	12	F: GCATGGAGATCAGAGCTGGAC	342	90024–90044	AC068234
				R: CCAGCTCACATCAAGTGTC		89703–89721	AC068234
2	CD42B	GPIbα	1	F: CGAGCTCACCAAGCTCCAGG	464	148224–148243	AC109333
				R: GCCAGCGACGAAAATAGAGG		148668–148687	AC109333
3, 9w	CD41B	GPIIb	26	F: CCTCCGACCTGCTCTACATC	339	12671–12690	AC003043
				R: GAGAGCCTGCTCACTACGAG		12990–13009	AC003043
5	CD49B	GPIa	13	F: AATGAGCAAGTAAATGTTCAGTG	451	73278–73300	NC_000005
				R: CTCTCATGGAAAATGGCAGTAC		73707–73728	NC_000005
13w	CD49B	GPIa	20	F: TAATACAATAGCAACAAAGAACAATCC	354	83483–83509	NC_000005
				R: TACCGGCAGGGAGAATGATGC		83816–83836	NC_000005
12w	CD42C	GPIbβ	1	F: GCTTACTGCGGCGCTTCCCTT	368	20450–20470	AC000093
				R: GCAACGCAGGTCGCGGTA		20801–20817	AC000093
15	CD109	CD109	19	F: GTATTATGACCTTATGATGACCTATTC	387	133708–133734	AL590428
				R: GGAGCTGACGCTGTATTAGC		134075–134094	AL590428

The primers were used for both PCR amplification and sequencing analysis for both forward and reverse direction. Exon was numbered from ATG start codon. GenBank data were obtained from National Center for Biotechnology Information (NCBI) (http://www.ncbi.nih.gov)

F forward primer, *R* reverse primer

with UV transillummination. In most cases only one band was shown, suggesting that no nonspecific products were produced. PCR products were then purified with QIAquik PCR purification kit (Qiagen, Valencia, CA). If more bands were observed, a further gel purification was necessary as described below. The PCR products were purified either by ethanol precipitation or using MinElute PCR purification kit (Qiagen, Valencia, CA) and then further purified with QIAEX II gel extraction kit (Qiagen, Valencia, CA) after being separated on a 0.6% agarose gel. The purified PCR products were sequenced using BigDye Terminator Cycle Sequencing Kits on a 3700 ABI DNA Sequencer (Applied Biosystems, Foster City, CA, U.S.A.). The sequencing was performed from two directions using forward and reverse primers, respectively.

The samples listed in Table 2 have been typed for HPA-1, -2, -3, -4, -5, -6w, -9w, and -15 using two PCR-SSP HPA Typing Kits (INNO-TRAIN Diagnostik GmbH, Kronberg/Taunus, Germany, and G&T Biotech, Rockville, MD, USA). The types of HPA-7w, -8w, -10w, -11w, -12w, -13w, -14w, -16w and -17w were also determined using a PCR-SSP HPA Typing Kit (G&T Biotech, Rockville, MD, USA). However, only the common homozygous types aa were detected for these samples. Figure 1 shows the representative results for each available HPA types. No discrepancies were observed between PCR-SSP typing and SBT typing in typing these DNA samples.

DNA sequencing-based molecular typing technique is the most comprehensive method for characterizing genetic polymorphisms and is the only way to define new genetic variants. It could generate unambiguous and highly reproducible data. Recent advances in technology have created the ability to perform SBT at a high throughput level in a routine laboratory. SBT-HPA typing method could be used for verification of the HPA types determined by other techniques.

Table 2 HPA types of a panel of platelet donors

Donor	HPA-1	HPA-2	HPA-3	HPA-4	HPA-5	HPA-6w	HPA-15
NNITM-1	1aa	2bb	3bb	4aa	5aa	6aa	15ab
NNITM-2	1aa	2aa	3ab	4aa	5aa	6bb	15bb
NNITM-3	1ab	2aa	3ab	4aa	5aa	6aa	15ab
NNITM-4	1bb	2aa	3ab	4aa	5aa	6aa	15aa
NNITM-5	1aa	2ab	3aa	4aa	5ab	6aa	15ab
NNITM-6	1aa	2bb	3aa	4aa	5aa	6aa	15bb
NNITM-7	1aa	2aa	3ab	4aa	5ab	6aa	13ab
NNITM-8	1aa	2ab	3ab	4aa	5aa	6bb	15bb
NNITM-9	1aa	2ab	3aa	4aa	5aa	6aa	15ab
NNITM-10	1aa	2aa	3ab	4ab	5aa	6aa	15ab
NNITM-11	1ab	2aa	3ab	4aa	5aa	6aa	15ab
NNITM-12	1aa	2aa	3bb	4aa	5aa	6aa	15ab
NNITM-13	1aa	2aa	3aa	4aa	5aa	6ab	15ab

All donors are "aa" homozygous for HPA-7w, -8w, -9w, -10w, -11w, -12w, -13w, -14w,-16w and -17w

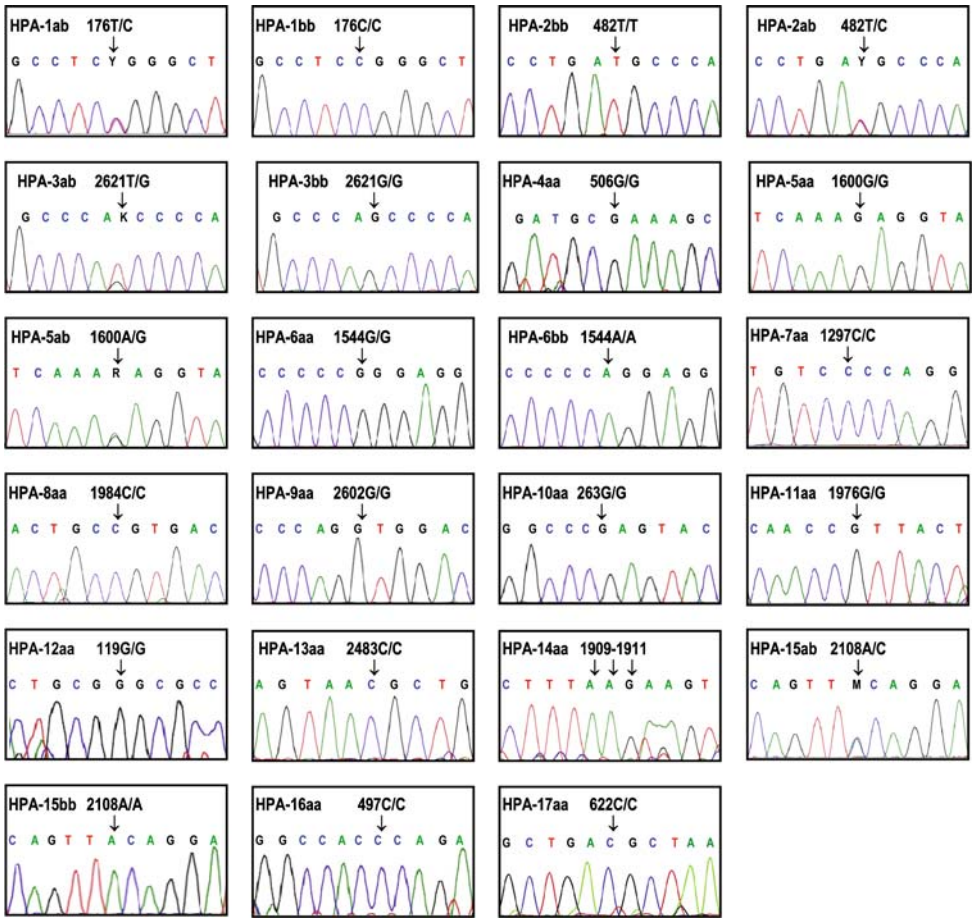

Fig. 1 DNA sequencing chromatograms. The *arrows* indicate the SNP position or deletion in a heterozygous or homozygous state. The number of nucleotide position according to the nomenclature of human platelet antigens [1]

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