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ORIGINAL ARTICLE

A new method for ABO genotyping to avoid discrepancy between genetic and serological determinations

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Abstract In Japan and elsewhere, *ABO* genotyping is frequently used in forensic practice for identification of a decomposed body. However, the phenotype deduced from the genotyping data is occasionally inconsistent with the real phenotype. In this paper, we report a simple *ABO* genotyping method in which five single nucleotide polymorphism at nps 220, 261, 796, 802, and 803 are analyzed simultaneously to avoid discrepancies between genetic and serological determinations in *ABO*A204*, **O303*, **O207*, **cis-AB01* and **cis-AB02* alleles. This method can be used for the genotyping of badly decomposed remains or old bloodstains.

Keywords ABO blood group · Genotyping · Single nucleotide polymorphism · Single-base extension · Identification

Introduction

In Japan and elsewhere, *ABO* genotyping is frequently used in forensic practice for identification of a decomposed body. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis described by Lee and Chang [1] is the most popular *ABO* genotyping method; this method is based on the analysis of two single nucleotide polymorphisms (SNPs) at nps 261 and 703, which are characteristic of *O* and *B* alleles, respectively [2]. However, there are some serious problems to finally deduce a phenotype from the genotype determined by this method, because some of the *ABO* alleles have different SNPs from the characteristic *A*, *B*, or *O* allele [3]. For example, in the PCR-RFLP method, **O303* will be typed as an *A* allele, because the **O303* lacks the single-base deletion at np 261 characteristic of the *O* allele [4]. Similarly, **cis-AB01* [5] and **cis-AB02* [6] will be typed as *A* and *B* alleles, respectively. A discrepancy between postmortem genotyping and the antemortem agglutination test can be a serious problem in identification, and although approaches to address such mistyping have been proposed [7–11], none are free from a risk of mistyping.

In this paper, we report a novel *ABO* genotyping approach, which avoids the possible discrepancy between genetic and serological determinations.

Electronic supplementary material Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s00414-006-0148-0 and is accessible for authorized users.

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Materials and methods

The study protocol was approved by the ethical committees of the University of Tokyo and Osaka City University.

SNP analysis

PCR

PCR primers were designed to amplify the region including nps 220, 261, or 796–803 (See Supplementary table S1).

The size of each amplicon was restricted to under 200 base pairs to facilitate amplification of highly degraded DNA samples. PCR was performed in a reaction mixture containing 1× Ex buffer (TAKARA BIO, Otsu, Shiga, Japan), 200 μM of each dNTP, 0.75 U of Ex Taq polymerase (TAKARA BIO), each PCR primer pair (Supplementary table S1), and 50–200 ng of genomic DNA. The PCR conditions comprised 34 cycles of denaturation at 94°C for 40 s, annealing at 60°C (primer pair 1) or 56°C (primer pairs 2 and 3) for 40 s and extension at 72°C for 1 min, and final extension at 72°C for 5 min. Primers and unincorporated dNTPs were removed by incubating the PCR product with 2 U of Exonuclease I (*Exo*I, TAKARA BIO), and 5 U of Shrimp alkaline phosphatase (SAP, TAKARA BIO).

Single-base extension reaction using the SNaPshot™ system

The extension primer was designed so that the 3′-end of the primer hybridizes one base to the 5′-side of the SNP at np 220, 261, 796, 802, or 803 (See Supplementary table S1). Single-base extension was performed using an ABI PRISM® SNaPshot™ Multiplex Kit (Applied Biosystems, Foster City, CA). The extension reaction and the following minisequencing analysis were performed according to the kit protocol. Data were analyzed using ABI PRISM® GeneMapper™ Software version 3.0 (Applied Biosystems).

Direct sequencing

Direct DNA sequencing of exon 7 was performed with a BigDye® Terminator Cycle Sequencing Kit (Applied Biosystems) using dsDNA fragments.

Results and discussion

We focused on five relatively common alleles (**A204* [12], **cis-AB01* and *AB02* [13, 14], **O303* [4, 8], and **O207* [15]) that may cause discrepancies between genetic result from the PCR-RFLP method of Lee and Chang [1], and serological determinations. First, **A204* (also known as **R101*) is mistyped as a *B* allele due to a base substitution at np 703 characteristic of the *B* allele [16]. Second, **cis-AB01* with the G→C substitution at np 803 in the *A* background [5] and **cis-AB02* without the C→A substitution at np 796 in the *B* background [6] are mistyped as *A* and *B* alleles, respectively. For correct typing of these alleles, our method detects SNPs at nps 261, 796 and 803. Third, **O303* (**O^2* or **O03*) is mistyped as an *A* allele due to the lack of the deletion at np 261 characteristic of the *O* allele [17], whereas our method detects an SNP at np 802 that is characteristic of this allele only. Fourth, **O207* (**O^{1v}-B*) is a hybrid of *O* and *B* alleles with a deletion at np 261 and a base substitution at np 703 [18]. Since the genotypes **O207/A* and *B/O* have the same SNPs at all positions, no genotyping method can discriminate between them. However, our method decreases the risk of mistyping to about 20%, by distinguishing between *O* alleles with and without a substitution at np 220, similar to **O207* (GenBank database, accession no. AY268591).

We analyzed these five SNPs at nps 220, 261, 796, 802, and 803 by SNaPshot™ system, a commercially available mini-sequencing method. The SNaPshot system has been applied recently in forensic research [19–21] because the chemistry can be applied flexibly to several SNP sites [22, 23]. We classified all the *ABO* alleles into seven allele groups: *A*, *B*, *cisAB*, *O*, O^B, O^2, and O^{1v}; (Table 1). The zygote theoretically contains 28 pairs (See Supplementary table S2), and our method correctly determines all pairs except A/O^B and B/O^{1v}. In contrast, the PCR-RFLP method will mistype 12 of these 28 pairs.

The sensitivity of SNaPshot was evaluated using 32 specimens (nail, hair, muscle, or bone) from 19 decomposed bodies and was found to be equal to that of PCR-RFLP (data not shown). We also confirmed the reliability of the method based on correspondence of results with the correct serological phenotype in a blind test of 100 old bloodstains (5–10 years old), the phenotype of which had been determined by an agglutination test using liquid blood after each autopsy. The genotype of one sample determined using SNaPshot (A/B) differed from that determined by PCR-RFLP (*B*/*B*), and the correct phenotype was AB, consistent with the SNaPshot result. A sequence analysis showed the genotype of the sample to be a heterozygote of **A204* and **B101*, **B108*, **B301*, **Be102*, or **B(A)02*. **A204* has a base substitution at np 703 that is characteristic of the *B* allele, and this explains why the genotypes were determined as *A*/*B* and *B*/*B* by the SNaPshot and PCR-RFLP methods, respectively.

Table 1 Allele grouping at five SNP positions using SNaPshot method

Allele group	Classified allele	Nucleotide position				
		220	261	796	802	803
A	*A*	C	G	C	G	G
B	*B*			A		C
cisAB	**cisAB01*, **cis-AB02*					C
O	*O*		Δ			
O^B	**O207*	T	Δ	A		C
O^2	**O303*	T			A	
O^{1v}	**O204*, **O206*, **O201*	T	Δ			

In conclusion, we have established a new *ABO* genotyping method that avoids discrepancy between genetic and serological determinations caused by inappropriate detection of **A204*, **O303*, **O207*, and **cis-AB* alleles.

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