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ABO genotyping by TaqMan assay and allele frequencies in a Japanese population

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ABSTRACT

ABO genotyping have become common tools for forensic casework. We developed a new rapid ABO genotyping method using a fast real-time PCR system with the TaqMan® Sample-to-SNP™ Kit. Eight single nucleotide polymorphism (SNP) sites in the ABO gene (nt 261, 297, 467, 657, 703, 829, 930 and 1061) were selected to determine the ABO genotypes. ABO genotypes were easily determined by examining allelic discrimination patterns. This method enabled analyses to be completed in about 1 h per plate with no postmortem change influences. The detection limit in each SNP site was examined as 100 pg per reaction. ABO genotyping from 1000 Japanese individuals was also examined to determine the distribution of ABO genotypes and allele frequencies. Thus, 31 genotypes were clearly identified, and these were controlled by four common and seven rare alleles. The power of discrimination, heterozygosity and polymorphism information contents were 0.913, 0.775 and 0.812, respectively. Therefore, selecting these eight SNP sites could be useful for high specific ABO genotyping. This rapid, sensitive and accurate genotyping method is useful for forensic casework.

1. Introduction

The ABO blood group is one of the most important systems in DNA profiling as well as transfusion medicine. Yamamoto et al. elucidated the molecular genetic basis of the ABO blood system and identified seven nucleotide substitutions (nt 297, 526, 657, 703, 796, 803 and 930) resulting in differences in only four amino acids (residues 176, 235, 266 and 268) between the coding regions of the cDNA for A and B transferases [1,2]. Most O alleles are characterized by a nucleotide deletion at position 261, which caused a frame shift mutation and stopped the activity of A transferases. A101, B101 and O01 are the most common alleles and numerous rare alleles have recently been described. Many types of techniques have been reported for the ABO genotyping such as PCR-restriction fragment length polymorphism (RFLP) [3], PCR-SSCP (single strand confirmation polymorphism) [4,5], PCR-APLP (amplification product length polymorphism) [6], SNaP-shot [7,8]. However, these methods require a considerable length of time for PCR amplification with many manipulations.

In the present study, we developed a rapid and high-specific ABO genotyping method based on the TaqMan assay with the TaqMan® Sample-to-SNP™ Kit (Applied Biosystems, USA). The TaqMan assay is one of the high-sensitivity technique that is used widely for single nucleotide polymorphism (SNP) genotyping and gene expression. The Sample-to-SNP™ Kit can be used to perform direct DNA extraction from forensic samples such as blood, old bloodstain, tissue, hair follicle and buccal swab and also enables fast PCR cycling. Allele frequencies of the ABO blood group in a Japanese population were also examined using this technique.

2. Materials and methods

2.1. Samples

One thousand blood samples were collected from unrelated Japanese individuals living in Kanagawa prefecture whose ABO phenotypes were determined by serological methods. Forensic samples such as blood, muscle, nail, hair follicle, tooth and bone were obtained from decomposed human bodies. Bloodstains were made by spotting the blood and stored at room temperature. Buccal swab was prepared from a healthy volunteer.

2.2. DNA extraction

The DNA Extract All Lysis Reagents, which were included in the TaqMan® Sample-to-SNP™ Kit, was used for the direct the extraction of DNA from bloods, bloodstains, muscles, hair follicles and buccal swabs. Each sample required: blood, 2 μl; bloodstain, 2 mm^2; muscle 2 mm^3; 2 hair follicles and 1 buccal swab. The lysis solution was directly added to these samples and incubated for 3 min at room temperature or 95 °C. Finally, adding the DNA

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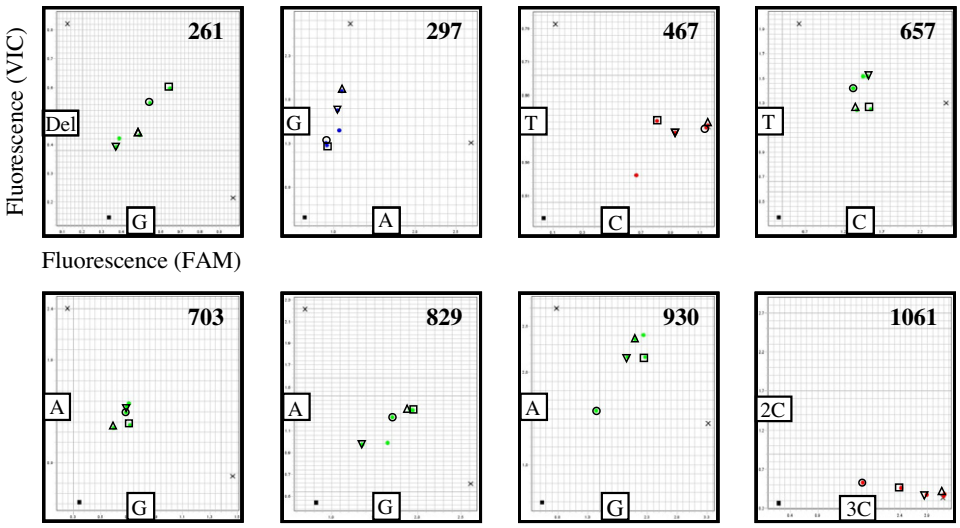

Fig. 1. ABO genotyping using the TaqMan assay with a Sample-to-SNP Kit. The ABO genotypes of blood, bloodstain, muscle, hair follicles and buccal swab were successfully detected. All of the sample genotypes were known as B101O02. Blood (None), bloodstain (○), muscle (□), hair (△), buccal swab (▽).

stabilizing solution completed the sample lysate. Quick Gene-800 (FUJIFILM, Tokyo, Japan) was used to extract DNA from hard tissues, such as nail, tooth and bone.

2.3. Real-time PCR assay

Primers and TaqMan® MGB probes were designed to detect eight SNP sites (nt 261, 297, 467, 657, 703, 829, 930 and 1061) of exon 6 and 7 in ABO genes. These SNP sites were selected in order to identify common and rare ABO alleles (Table S1). The sequences of these primers and probes are shown in Table S2. PCR was performed in a reaction mix containing 3.13 μl of 2× TaqMan® GTXpress™ Master Mix consisting of 0.56 μl each of forward and reverse primers with a final concentration of 0.9 μM, and 0.25 μl each of the probes with a final concentration of 0.2 μM. After addition of a 0.75 μl genomic DNA template, the final reaction volume was brought to 6.0 μl by adding distilled water. Thermal cycling was undertaken in a StepOne™ Real-time PCR System (Applied Biosystems, USA) using the following conditions: initial denaturation at 95 °C for 20 s followed by 40 cycles of 95 °C for 3 s and 60 °C for 20 s. PCR products were analyzed by StepOne™ Software Ver2.01 (Applied Biosystems, USA). The study protocol was approved by the Kitasato University Medical Ethics Committee (B 03–17).

3. Results and discussion

The ABO alleles were defined according to the nomenclature described in the Blood Group Antigen Gene Mutation Database (http://www.ncbi.nlm.nih.gov/projects/gv/rbc/xslcgi.fcgi?cmd=bgmut/home).

3.1. ABO genotyping using the TaqMan assay

ABO genotyping was examined using the TaqMan assay with the Sample-to-SNP™ Kit. The DNA extraction procedure using DNA Extract All Lysis Reagents only required a small amount of the samples and only took 3 min. Moreover, utilizing a fast PCR cycling enabled the clear classification of eight SNP sites within 1 h (Fig. 1).

Because personal identification in forensic cases is frequently required, forensic samples obtained from decomposed bodies were also examined. Bloods and tissues were collected from decomposed bodies 1 day to 4 months and 1 day to 2 months after death, respectively. From 1- to 15-year-old bloodstains were also collected. As shown in Fig. S1–3, genotypes of these samples were successfully detected. These results suggested that the TaqMan® Sample-to-SNP™ Kit could be useful for degraded forensic samples.

Although DNA Extract All Lysis Reagents cannot be used for hard tissues, such as nail, tooth and bone, fast PCR could be performed using the TaqMan® GTXpress™ Master Mix with the ordinary extraction method such as Quick Gene-800 (Fig. 2). The amount of DNA obtained from decomposed bodies and bones is often limited in forensic cases. The detection limit of this method was investigated at each SNP site using the B101O02 samples that quantified the intact template DNA in the range of 1.0×10^{-3}–30 ng/μl. As indicated in Fig. S4, at least 100 pg of genomic DNA could be detected at all eight SNP sites. In addition, using the short amplicon (no longer than 119 bp) was small enough to allow partially degraded DNA to be used for genotyping. This advantage, consequently, made our method one of high sensitivity.

The ABO blood system is one of the most widely used identification tools in forensic casework. The PCR-RFLP was the first ABO genotyping method for forensic samples and became the most common method [3]. However, this method requires many restriction enzymes to simultaneously detect multiple SNP sites. The SNaP-shot system is also commonly used, but this assay consists of multistep reactions and requires many attempts before the detection can be made [6,7]. Recently, newly introduced SNP genotyping tools, such as the MALDI/TOF-MS (matrix assisted laser desorption ionization/time of flight mass spectrometry) [9] and DNA array [10], can be used for forensic casework; however, they require expensive equipment and are inconvenient to use on a routine basis. There are some reports of ABO genotyping using real-time PCR, but they could only discriminate a few alleles [11,12]. In contrast, our method could classify alleles in detail and carry out fast PCR by the total volume of 6 μl. This was a sufficient volume for the detection of ABO genotyping and the cost estimation of one sample could be kept minimal. In conclusion, our high sensitivity, rapid, cost-efficient and accurate ABO genotyping will be helpful in forensic casework.

3.2. Allele frequencies of the ABO blood group in a Japanese population

In DNA samples extracted directly by the DNA Extract All Lysis Reagents from 1000 Japanese subjects, ABO genotyping was examined by the new method of TaqMan assay developed in this study.