International Congress Series 1261 (2004) 602–604

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Multiplex PCR with confronting two-pair primers for Se genotyping

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Abstract. Multiplex PCR with confronting two-pair primers (PCR-CTPP) for Se genotyping classified into Se, se1 (G428A), se2 (A385T), and se5 (fusion gene) alleles has been developed. Genotyping was performed using the electrophoretic patterns of the PCR products. The incidences of the Se, se1, se2 and se5 alleles were 55.75% (223/400), 0% (0/400), 39.75% (159/400) and 4.5% (18/400) in Japanese subjects, respectively. We were not able to find the se1 non-functional alleles among our Japanese samples. Se genotyping may be a useful marker for ethnic discrimination of biological stains obtained from Japanese, Caucasian, African and Iranian individuals. This method is simple, rapid and accurate.

Keywords: Se genotyping; DNA typing; Population genetics; Blood group

1. Introduction

The ethnic specificity of Secretor genotypes has been given special attention in recent years. The se1, G428A [1], is a unique non-functional allele in Caucasians, Africans and Iranians. Both the se2, A385T [2] and se5, fusion gene [3] are also unique non-functional alleles in Asians. In this paper, we developed a multiplex PCR with confronting two-pair primers (PCR-CTPP) [4,5] for Se genotyping classified into Se, se1, se2, and se5 alleles. This assay provides a rapid, accurate and simple method for Se genotyping that can provide useful information on ethnic specificity.

2. Materials and methods

All samples from Japanese subjects were obtained with informed consent and were collected at the Department of Urology, Juntendo University School of Medicine.

Lewis phenotypes were determined for fresh blood samples by standard hemagglutination methods. Typing was carried out with monoclonal Le^a and Le^b antibodies (Seraclone: Biotest, Germany).

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0531-5131/
doi:10.1016/S0531-5131(03)01535-8

DNA was isolated from blood using the QIAamp DNA Mini Kit (QIAGEN, Germany) or the standard phenol/chloroform extraction procedure. The extracted DNA was stored at 4 °C until the genotypes analysis.

Seven kinds of primers were designed, including T5-F: TCT CCC AGC TAA CGT GTC CCG (AC: U17894, the nucleotide sequences from position 24 to 44), T1-F: TGG GCA TAC TCA GCC CGT GT (AC: 82933, the nucleotide sequences from position 196 to 215), T2-R: CGG ACG TAC TCC CCC GGG AT (AC: U17894, the nucleotide sequences from position 500 to 481), NA1-F: GGA GGA GGA ATA CCG CCA CT (AC: U17894, the nucleotide sequences from position 462 to 481), T6-R: CAA TCC CTG TCC ACT CCG GCA (AC: U17894, the nucleotide sequences from position 1063 to 1043), T7-F: TGG GCA TAC TCA GCC CGT GT (AC: U17894, the nucleotide sequences from position 505 to 524), and T8-R: CGG ACG TAC TCC CCC GGG AT (AC: U17894, the nucleotide sequences from positions 544 to 524).

PCR-CTPP (confronting two-pair primers) was developed to perform secretor genotyping. DNA amplification was performed in 25 μl reaction volumes. PCR-CTPP for Se, se2 and se5 typing: 30 ng of template DNA was added to the reaction mixture containing PCR buffer (Invitorogen Life Technologies, Japan), 1.5 mM $MgCl_2$ (Invitorogen Life Technologies), 0.2 mM dNTPs (Promega Japan), 0.625 U platinum Taq DNA polymerase (Invitorogen life technologies). Primers (10 pmol) of T5-F and T1-F, 5 pmol primers of T2-R and NA1F, and 20 pmol primer of T6-R were used. PCR-CTPP for Se and se1 typing, 10 pmol primers of T5-F, T6-R, T7-F and T8-R was used as the primer pairs instead of the above pairs. After an initial denaturation step at 94 °C for 2 min, 35 cycles were performed consisting of 50 s at 94 °C, 1 min at 65 °C, and 1 min at 72 °C, followed by a 2-min extension at 72 °C in a TAKARA PCR Thermal Cycler MP (TP3000). Ten milliliters of each amplified PCR product was analysed by electrophoresis on 2% agarose gel stained with ethidium bromide.

3. Results and discussion

Genotyping was performed using the electrophoretic patterns of PCR products as follows, 1042 and 479 bp for Se/Se genotype, 1042, 602, and 479 bp for Se/se2 genotype,

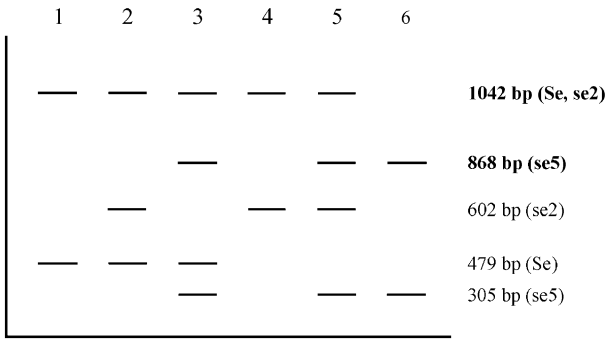

Fig. 1. Electrophoretic diagrams of PCR-CTPP products for Se, se2 and se5 genes. Genotypes of lanes; 1 = Se/Se, 2 = Se/se2, 3 = Se/se5, 4 = se2/se2, 5 = se2/se5, 6 = se5/se5.

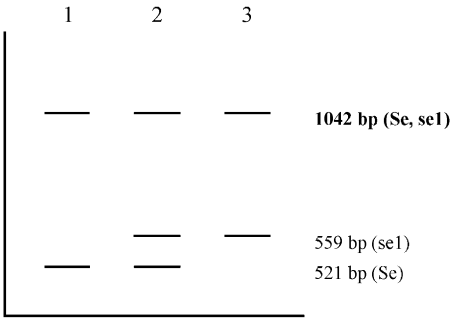

Fig. 2. Electrophoretic diagrams of PCR-CTPP products for Se and se1. Genotypes of lanes; 1 = Se/Se, 2 = Se/se1, 3 = se1/se1.

1042, 868, 479 and 305 bp for Se/se5 genotype, 1042 and 602 bp for se2/se2 genotype, 1042, 868, 602, and 305 bp for se2/se5A (Fig. 1). A 1042-bp positive control product is generated in the Se, se1 and se2 (Fig. 2). Similarly, a 868-bp positive control product was also generated in se5. The incidences of the Se, se1, se2 and se5 alleles were 55.75% (223/400), 0% (0/400), 39.75% (159/400) and 4.5% (18/400) in the Japanese subjects, respectively. Genotypes of Se/Se (54 cases), Se/se2 (104 cases), and Se/se5 (11 cases) belonged to phenotypes of Le(a − b+) or Le(a − b −). Those of se2/se2 (24 cases) and se2/se5 (7 cases) belonged to phenotypes of Le(a + b −) or Le(a − b −). No discrepancies were observed between the phenotypes and genotypes. We were not able to find the se1 non-functional alleles among our Japanese samples, consistent with the previous report [2]. Se genotyping may be a useful marker for ethnic discrimination of biological stains obtained from Japanese, Caucasian, African and Iranian individuals. This method is simple, rapid and accurate for forensic purposes.

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