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Evaluation of Lewis genotyping by four PCR-based methods

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Abstract. We developed PCR-based methods, confronting two pair primers (PCR-CTPP) and sequence-specific primers with a PCR-positive control (PCR-SSPPC) to analyze SNPs at nucleotide position 59 to reflect α-1, 4-fucosyltransferase (Le transferase) activity, which were analyzed by ABI PRISM® 3100 genetic analyzer. We compared 4 kinds of PCR-based methods, PCR restriction fragment-length polymorphism (PCR-RFLP), PCR sequence-specific-primers (PCR-SSP), PCR-CTPP and PCR-SSPPC. We found that all of these methods could determine Lewis genotyping correctly. The frequencies of *Le* and *le* alleles were 67.2% and 32.8% respectively. Both PCR-CTPP and PCR-SSPPC for Lewis genotyping are simple, reliable and applicable for forensic and clinical investigation. © 2005 Published by Elsevier B.V.

Keywords: Lewis blood group; Genotype; Sialyl Lewis A antigen; PCR; Fragment analysis

1. Introduction

The antigenic epitope of CA19-9, i.e. sialyl Lewis A antigen, has been used clinically as a tumor marker for pancreatic cancer, colorectal cancer, and certain other malignancies. The synthesis of CA 19-9, however, is complex because there are three genes involved; Lewis gene encoding Le transferase, secretor gene encoding Se transferase, and gene encoding sialyltransferase. In the biosynthetic pathway, Le transferase is thought to be a key enzyme. The T59G missense mutation reflects Le transferase activity. The activity is genetically

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controlled by Lewis genotypes. Lewis phenotype Le(a−b+) or Le(a+b−) groups have the *Le*(*Le*/*Le*, *Le*/*le*) allele. The Le(a−b−) group is subdivided into two groups, genuine Le(a−b−) and non-genuine Le(a−b−) based on the Lewis genotype. Genuine Le(a−b−) groups have no *Le*(*le*/*le*) allele, while non-genuine Le(a−b−) groups have the *Le*(*Le*/*Le*, *Le*/*le*) allele [1]. *Le* is a functional allele, and *le* is a non-functional allele. Accordingly, there is a demand for a simple and reliable Lewis genotyping technique. For Lewis genotyping, PCR-RFLP has been reported [2]. PCR-RFLP involves three steps; PCR with a thermal cycler, incubation with a restriction enzyme for PCR product digestion, and electrophoresis for visualizing the genotypes. If the incubation step could be skipped, the result would be much simpler to obtain. PCR-SSP provides a powerful technique for the discrimination of alleles arising from single base substitutions without the incubation step. In a previous paper [3], we described PCR-based methods, PCR-SSP and PCR-CTPP to analyze SNPs at nucleotide position 59 to Le transferase activity. Here, we further developed a PCR-SSPPC with a PCR control to examine whether a target DNA can be amplified or not.

This study is developed simple and reliable Lewis genotyping methods to aid clinical and forensic investigation. Then 4 kinds of PCR-based methods, PCR-SSPPC, PCR-SSP, PCR-CTPP and PCR-RFLP were compared.

2. Materials and methods

Blood DNA was isolated from whole blood using QIAamp DNA Mini kit. Genotyping is our portion of the collaborative studies between our department and the urologic department. PCR-RFLP was performed as described by Nishihara et al. [2]. These three methods, PCR-SSP, PCR-SSPPC and PCR-CTPP, were developed to perform Lewis genotyping. Five kinds of primers were designed, including Le1-F(f5): ACC CAT GGA TCC CCT GGG TGC (AC: NML000149, from positions 629 to 649), Le2m-F: GCC GCT GTC TGG CCG CAt TG (AC: NML000149, from positions 673 to 692, small capital indicates mismatch sequence), Le3m-R: CCA CCA GCA GCT GAA ATA GaC G (AC: NML000149 from positions 710 to 690, small capital indicates mismatch sequence), Le4-R: GGC AGT GAT GTG GCA GTC GGC (AC: NML000149, from positions 917 to 897), Le2mm-F: GCC GCT GTC TGG CCG CAa GG (AC: D89325, from positions 41 to 60, small capital indicates mismatch sequence). For fragment analyses, Joe-Le-4R and Joe-Le3m-R were also prepared as dye-labeled primers. DNA amplification was performed in 25 ml reaction volumes. Template DNA (30 ng) were added to the reaction mixture containing PCR buffer (Invitorogen), 1.5 mM $MgCl_2$ (Invitorogen), 0.2 mM dNTPs (Promega), 0.625U platinum Taq DNA polymerase (Invitorogen) and primer sets. Thirty-five cycles were performed consisting

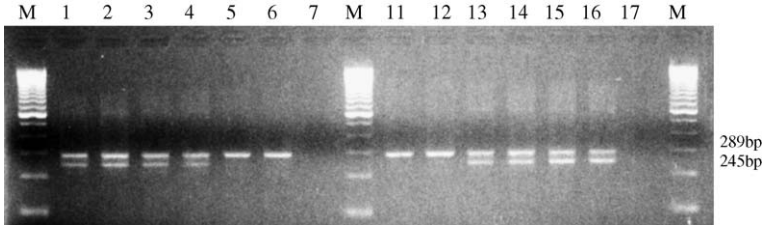

Fig. 1. Electrophoretic patterns for Lewis genotyping by PCR-SSPPC. From 1 to 7: Le-specific PCR, from 11 to 17: le-specific PCR, M: 100 bp marker; 1, 2, 11, 12: *Le/Le*; 3, 4, 13, 14: *Le/le*; 5, 6, 15, 16: *le/le*; 7, 17: negative control.

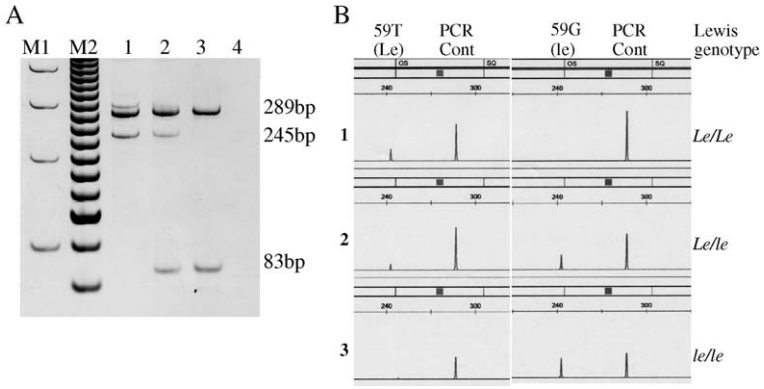

Fig. 2. (A) Electrophoretic patterns for Lewis genotyping by PCR-CTPP. M1: 100 bp marker, M2: 25 bp marker, 1: *Le/Le*, 2: *Le/le*, 3: *le/le*, 4: negative control. (B) Fragment analysis for Lewis genotyping by PCR-SSPPC 1: *Le/Le*, 2: *Le/le*, 3: *le/le*.

of 3 min at 94 °C, 2 min at 65 °C, and 2 min at 72 °C. For PCR-CTPP of Le and le typing, primer sets of 5 pmol primers of Le1-F(f5) and Le4-R, 10 pmol primer of Le2m-F, and 20 pmol primer of Le3m-R were used. For PCR-SSPPC of Le typing, primer sets of 2.5 pmol primers of Le1-F(f5), 10 pmol primers of Le2m-F, and 5 pmol primer of Le4-R were used. For PCR-SSPPC of le typing, primer sets of 2.5 pmol primers of Le1-F(f5), 10 pmol primers of Le2mm-F, and 5 pmol primer of Le4-R were used. Primer sets of PCR-SSP was similar to the PCR-SSPPC without addition of Le1-F(f5) primer. Amplified PCR product was analyzed by electrophoresis on 2% agarose gel stained with ethidium bromide or on 8% polyacrylamide gel stained with silver. Amplified PCR products using dye-labeled primers were also analyzed by an ABI 3100 PRISM® genetic analyzer.

3. Results and discussion

We developed PCR-based methods to analyze SNPs at nucleotide position 59 to reflect Le transferase activity. And we compared 4 kinds of PCR-based methods, PCR-SSPPC, PCR-SSP, PCR-CTPP and PCR-RFLP. All of the methods could determine Lewis genotyping correctly. The frequencies of *Le* and *le* alleles among 155 Japanese were 67.2% and 32.8% respectively. In Figs. 1 and 2A, illustrative examples of electrophoretic patterns of Lewis genotyping by PCR-SSPPC and PCR-CTPP are respectively shown. Both methods are very attractive in that they amplify a 289 bp product as a PCR control to examine whether a target DNA can be amplified or not. Fig. 2B shows fragment analyses of PCR-SSPPC products by an ABI 3100 genetic analyzer. PCR-SSPPC and PCR-CTPP for Lewis genotyping are simple, reliable and applicable for clinical and forensic investigation.

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