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Detection of T to G mutation at position 59 in the Lewis gene by mismatch polymerase chain reaction

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Abstract Recently, we discovered the missense mutations of T to G at position 59 and of G to A at position 508 in one of the Lewis-negative (*le*) genes (Koda et al. 1993). In the present study, we report a method to detect the mutation at position 59 using mismatch PCR amplification and endonuclease *Msp*I digestion. For this mutation, we found that 7 out of 12 Lewis-negative, and none of 15 Lewis-positive individuals were homozygous, while 4 out of 12 Lewis-negative, and 4 out of 15 Lewis-positive individuals were heterozygous. These results indicate that the mutation at position 59 is a common mutation in the *le* genes.

Key words Blood group · Lewis gene · Mismatch PCR

Introduction

Lewis gene-encoded α(1,3/1,4)fucosyltransferase (FucT III) cDNA has been isolated by Kukowska-Latallo et al. (1990), and we have isolated one of the Lewis-negative (*le*) genes from human gastric mucosa cDNA (Koda et al. 1993). This *le* gene showed missense mutations of T to G at position 59 (59G) and of G to A at position 508 (508A) from the adenine of the initiation codon. A failure in the expression.of Lewis antigens could occur due to the 508A mutation but not to the 59G mutation (Koda et al. 1993; Nishihara et al. 1993). Two other *le* genes have been isolated and one of these also contained the 59G mutation (Nishihara et al.1993; Elmgren et al. 1993). In the present study, we identified the 59G mutation from genomic DNA by PCR amplification using mismatch 5′-PCR-primer and *Msp*I digestion.

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Materials and methods

Blood and saliva samples were obtained from volunteer donors who were undergraduate students at our University. The Lewis blood group phenotype was determined by the hemagglutination method and by the immunoassay of Lewis substances in saliva samples using anti-Le^a and anti-Le^b monoclonal antibodies (Immucor, USA) (Wang et al. 1994). Genomic DNA was isolated from peripheral leukocytes by the organic solvent extraction method (Sambrook et al. 1989). PCR was performed on 100 µl of reaction mixture containing 500 ng genomic DNA, 2.5 U Taq DNA polymerase (Promega), 50 pmol of each primer (Fig. 1) and 20 nmol dNTPs, for 25 cycles (94° C–1 min, 65° C–0,5 min, 72° C–0,5 min). The resulting PCR products were digested by 12 U *Msp*I endonuclease for 2 hours followed by 12 U *Bam*HI for 2 hours and then separated on an 8% polyacrylamide gel. The gel was the stained with ethidium bromide. The 508A mutation of Lewis genes was examined as described previously (Koda et al. 1994).

Results and discussion

Since there are 3 different α(1,3)fucosyltransferase (FucT III, V, and VI) genes having a high degree (about 90%) of nucleotide sequence homology (Kukowska-Latallo et al. 1990; Weston et al. 1992 a, b), it is important to select a PCR primer for the specific and exclusive amplification of only the FucT III gene (the Lewis gene) of interest. As shown in Fig.1, the 5′-primer was designed to create the *Msp*I site (CCGG) in the PCR product from the Lewis gene containing the 59G mutation, while the 3′-primer was designed for the region in FucT V cDNA with an insert of 33 nucleotides and for the region in FucT VI cDNA with a deletion of 9 nucleotides compared to FucT III cDNA (Fig. 1).

PCR amplification produced a 120 bp product as expected (Fig. 2, lane 1). To examine the 59G mutation, the PCR products were digested by the endonuclease *Msp*I. As shown in Fig. 2, 3 patterns of *Msp*I-digested PCR products were subsequently observed; (1) not cleaved by *Msp*I digestion (lane 2), (2) completely cleaved into 97 bp and 23 bp (23 bp, not seen) fragments by *Msp*I digestion (lane 4), and (3) heterozygous for the *Msp*I site (lane 3). To rule out the possibility that the 120 bp and 97 bp DNA

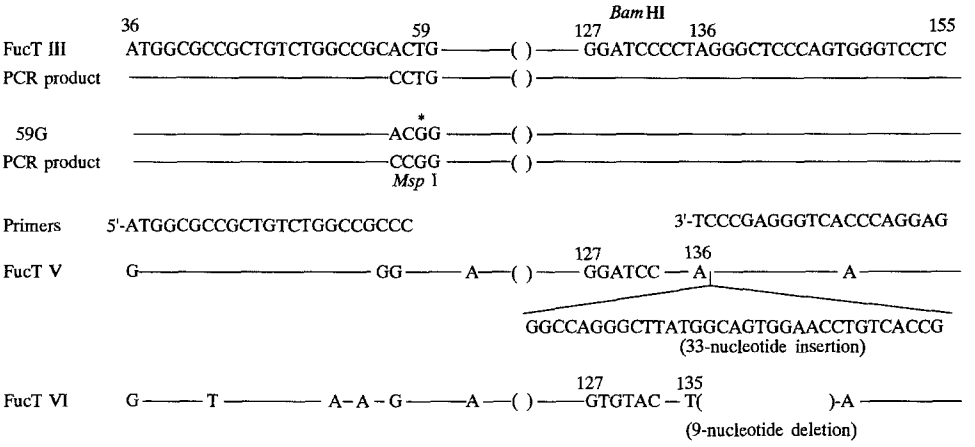

Fig. 1 DNA fragment and sites of digestion by endonuclease *Msp*I (CCGG) and *Bam*HI (GGATCC) of PCR product of the FucT III gene are indicated. The sequence of the primers used in the present study, and a part of the nucleotide sequence of the FucT V gene, and of the FucT VI gene, are also shown

fragments were from nonspecific PCR products, *Msp*I-digested PCR products were further digested by *Bam*HI. The 120 bp PCR products not cleaved by *Msp*I (Fig. 2, lanes 2 and 3) were cleaved into 94 bp and 26 bp fragments by *Bam*HI (lanes 5 and 6) and the *Msp*I-digested 97 bp fragments (lanes 3 and 4) were cleaved into 71 bp and 26 bp fragments by *Bam*HI digestion (lanes 6 and 7). The results suggested that the PCR product did not contain either the corresponding FucT V gene product (an expected size was 153 bp because of the 33-nucleotide insertion) or FucT VI gene product (without a *Bam*HI site). Recent studies have indicated that the 3 FucT genes (FucT III, FucT V and FucT VI) can be detected in human genomic DNA by Southern blot analysis using FucT III catalytic domain (Nishihara et al. 1993; Weston et al. 1992b), suggesting that only the FucT V and FucT VI genes showed a high degree of nucleotide sequence homology with the FucT III gene. Although we cannot completely exclude the possibility that our PCR product contained other gene(s) similar to the FucT III gene, it was likely that only the FucT III was specifically amplified by PCR in the present study.

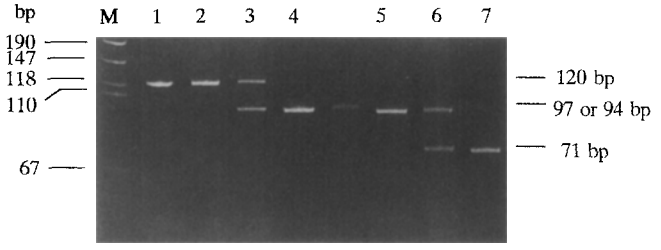

Fig. 2 Restriction endonuclease digestion of Lewis gene PCR products amplified between the 36th and 155th nucleotides of the Lewis gene were digested by *Msp*I (12U) for 2 hours followed by *Bam*HI for 2 hours, and then applied onto an 8% polyacrylamide gel. Lane M: *Msp*I-digested pBluescript (molecular weight marker), lane 1: PCR product of genomic DNA, lanes 2–4: *Msp*I-digested PCR products, lanes 5–7: *Msp*I- and *Bam*HI-digested PCR products of lanes 2–4. The expected DNA fragments (120, 97, 94 and 71 bp) are indicated

Table 1 The incidence of the 59G and 508A mutations in a Lewis gene in Lewis-positive and -negative individuals

Mutations	Le(+)	Le(–)
59G (–/–), 508A (–/–)	11	1
59G (+/–), 508A (+/–)	4	4
59G (+/+), 508A (–/–)	0	1
59G (+/+), 508A (+/–)	0	4
59G (+/+), 508A (+/+)	0	2
Total	15	12

We have examined the 59G and 508A mutation in genomic DNA from 15 Lewis-positive and 12 Lewis-negative individuals (Table 1). We found that 7 out of 12 Le(–) and none out of 15 Le(+) individuals were homozygus, while 4 out of 12 Le(–) and 4 out of 15 Le(+) individuals were heterozygous for the 59G mutation. We also detected the 508A mutation in some Le(+) and in some Le(–) individuals. However, 2 Lewis-negative individuals showed no 508A mutation, suggesting another mutation in the *le* genes. Although expression studies of FucT III cDNA into COS cells previously suggested that the 59G mutation was not responsible for the failure of the Lewis antigen expression (Koda et al. 1993) and the α(1,3/1,4) fucosyltransferase activity (Nishihara et al. 1993), the incidence of the 59G mutation was higher than that of the 508A mutation in Lewis-negative individuals (Table 1), suggesting that the 59G mutation plays at least some role in the failure of the expression of Lewis antigens on red blood cells.

Our results indicated that the 59G mutation was a common mutation in the *le* gene, and that PCR was useful to detect the 59G mutation in the Lewis gene.

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References

Elmgren A, Rydberg L, Larson G (1993) Genotypic heterogeneity among Lewis negative individuals. Biochem Biophys Res Commun 196:515–520

Koda Y, Kimura H, Mekada E (1993) Analysis of Lewis fucosyltransferase genes from the human gastric mucosa of Lewis-positive and -negative individuals. Blood 82:2915–2919

Koda Y, Soejima K, Kimura H (1994) Detection of G to A missense mutation of Lewis-negative gene by PCR on genomic DNA. Vox Sang 67:327–328

Kukowska-Latallo JF, Larson RD, Nair RP, Lowe JB (1990) A cloned human cDNA determines expression of a mouse stage-specific embryonic antigen and Lewis blood group α(1,3/1,4) fucosyltransferase. Genes Dev 4:1288–1303

Nishihara S, Yazawa S, Iwasaki H, Nakazato M, Kudo T, Ando T, Narimatsu H (1993) α(1,3) Fucosyltransferase (FucT-III) gene is inactivated by a single amino acid substitution in Lewis histo blood type negative individuals. Biochem Biophys Res Commun 193:624–631

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor, NY, Cold Spring Harbor Laboratory

Wang B, Akiyama K, Kimura H (1994) Quantitative analysis of Le^a and Le^b antigens in human saliva. Vox Sang 66:280–286

Weston BW, Nair RP, Larson RD, Lowe JB (1992a) Isolation of a novel human α(1,3) fucosyltransferase gene and molecular comparison to the human Lewis blood group α(1,3/1,4) fucosyltransferase gene. J Biol Chem 267:4152–4160

Weston BW, Smith PL, Kelly RJ, Lowe JB (1992b) Molecular cloning of a fourth member of a human α(1,3) fucosyltransferase gene family. J Biol Chem 267:24575–24584