

Selective detection of parental alleles in imprinted gene, *H19*

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Abstract. An autosomal polymorphism, designated H19FR which located upstream of imprinted *H19* gene, was investigated. Three allelic bands of H19FR system were detected by PCR amplification followed by constant denaturing gel electrophoresis (CDGE). In digested genomic DNA by methylation-sensitive endonuclease or *Mcr*BC, paternal or maternal allele of H19FR was selectively detectable, respectively. This parental typing was applicable to the DNA isolated from oral swab and hair with sheath in one individual with heterozygous genotype, but failed in the fingernail DNA. The DNA from bloodstain showed a tendency to obscure the parental discrimination. © 2003 Elsevier B.V. All rights reserved.

Keywords: Polymorphism; *H19* gene; Parent-of-origin; Genomic imprinting; Methylation

1. Introduction

Genomic imprinting is defined as an epigenetic modification that leads to parent-of-origin-specific expression of a gene. It is assumed that main causative mechanism of genomic imprinting would be related to methylation of cytosine residue in CpG dinucleotide of the imprinted gene. A number of imprinted genes have been found and investigated so far. We describe here the H19FR polymorphism in paternally imprinted *H19* gene and the selective detection of parental allele in the H19FR system.

2. Materials and methods

Genomic DNA was extracted from peripheral blood, oral swab, hair with sheath, fingernail and bloodstain by routine phenol/chloroform method.

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2.1. *H19FR* polymorphism detected by constant denaturing gel electrophoresis (CDGE)

The PCR cocktail (25 μ l) was composed of following reagents: 1 \times PCR buffer (QIAGEN), 2.5 mM MgCl₂, 200 μ M of each dNTP, 10 pM of each primer (*H19F*, 5' - ggctcttgca tagcacatgt-3' and *H19R-GC*, 5' -cgcccgcgcgcccgcgcc cgtcccgcgcgccccgcgcg tcccacatccatccatggaact-3'), 0.5 U of Taq DNA polymerase (QIAGEN) and 10–20 ng of genomic DNA. After pre-heating the cocktail at 94 °C for 15 min, PCR by 32 cycles of 94 °C for 30 s, 61 °C for 30 s and 72 °C for 1 min, and post-extension at 72 °C for 4 min was carried out. In CDGE analysis [1,2], the PCR product was mixed with the same volume of 2 \times loading dye (70% glycerol, 0.05% bromophenol blue and 0.05% xylene cyanol) and separated in a polyacrylamide gel (6% T and 2.6% C, 160 \times 100 \times 1 mm in size), including 45% denaturant (final concentration of 3.15 M urea and 18% formamide) and 1 \times TAE buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA, pH 8.0), in the 1 \times TAE buffer at 60 °C, at 120 V for 3 h. After run, the band was visualized by silver staining.

2.2. Selective detection of parental allele by enzymatic pre-treatment of genomic DNA

One of two methylation-sensitive restriction enzymes (msRE), *Hpa*II (recognition site, C^mCGG) and *Hha*I (G^mCGC) was used to cleave a non-methylated allele, while *Mcr*BC [5' . . .Pu^mC(N_{40–3000})Pu^mC. . .3'] was used to digest a methylated allele [2,3]. 10–20 ng of genomic DNA was treated with 5 U of each enzyme at 37 °C for 2 h, and subjected to the PCR–CDGE analysis.

3. Results and discussion

From the SNP and genome databases, the region including three SNPs (nucleotide positions 7523, 7547 and 7591, accession no. AF125183) was amplified and designated *H19FR*, which located 2.5 kb upstream of paternally imprinted *H19* gene. The *H19FR* polymorphism was analyzed by CDGE technique, and three alleles were observed in the Japanese population (Fig. 1A) [2]. The frequencies of *H19FR**1, *H19FR**2 and *H19FR**3 were 0.5093, 0.3426 and 0.1481, respectively, accompanying the useful statistical values of heterozygosity (0.6013), polymorphic information content (0.5238) and probability for paternity exclusion (0.3161) [2].

Selective detection of parental allele was referred to the reports that upstream region of the human *H19* gene was widely and highly methylated in paternal allele [4–6]. The strategy of genomic DNA digested by either msRE or *Mcr*BC was subjected to *H19FR* analysis [3]. As a result, paternal allele or maternal allele was exclusively amplified from msRE- or *Mcr*BC-treated DNA samples, respectively [2] (Fig. 1B). No inconsistency was observed in 17 family studies (data not shown). Moreover, we investigated the *H19FR* typing and selective parental detection for forensic DNA samples isolated from oral swab, hair with sheath, fingernail and bloodstains (stored at 4 °C, room temperature and 37 °C for 1 day, 1 week, 1 month and 3 months). Though the correct genotype was detected from all of the samples, the parental typing was not succeeded in the fingernail DNA showing both alleles and in bloodstain DNA with faint appearance of another allele (data not shown). In the nail DNA, relatively slow growing would affect the methylated

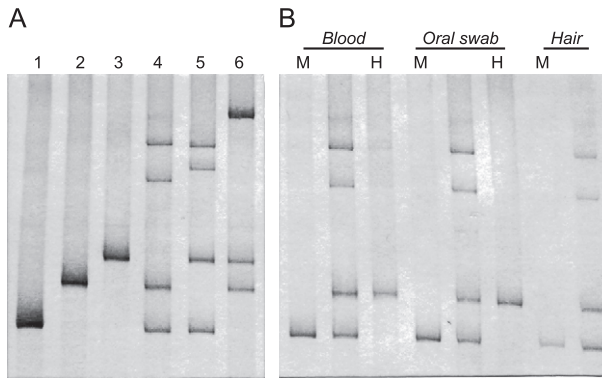


Fig. 1. Electrophoretic band pattern of H19FR genotypes detected by CDGE analysis. (A) Lanes 1–6 are six common genotypes 1–1, 2–2, 3–3, 2–1, 3–1 and 3–2, respectively. (B) Selective detection of parental allele from one individual with type 2–1. Genomic DNA was pre-digested by *McrBC* (M) or *HpaII* (H) and subjected to the CDGE analysis. Single band amplified from *McrBC*-digested DNA and that from *HpaII*-digested DNA were corresponding to the maternal and paternal alleles, respectively.

situation, or a possibility of different modification mode might be remained, because most of imprinted genes exhibit a manner of organ- or tissue-specific methylation status. On the other hand, the bloodstain DNA showed unstable results that the other parental band was becoming visible even in the bloodstain stored at 4 °C for 1 day, although the discrimination was still possible in bloodstain stored over 1 month. The reason was not clear, however, the methylated status in bloodstain DNA might be denaturated in early period after preparation.

As described above, the H19FR polymorphism will be a practical genetic marker with a possibility of selective detection of parental allele. By choosing suitable DNA sources, this typing method would supply effective information of parental origin not only to paternity test and pedigree investigation but also to the study on the genomic imprinting.

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