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RFLP Report

A NOVEL *H19*/*Hha*I RFLP AND ITS ALLELE FREQUENCY IN THE JAPANESE

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A novel *Hha*I RFLP was found in the H19 gene (*H19*) located to 11p15.5 and its allele frequency was estimated in the Japanese to be 0.64 for allele a and 0.36 for allele b.

Key Words Novel RFLP, H19 gene, genomic imprinting

Genomic DNAs from 50 Japanese individuals (25 males and 25 females) were amplified by polymerase chain reaction (PCR) followed by *Hha*I digestion and electrophoresis.

Primers for PCR. A primer set designed by Rainer *et al.* (1991) as H195, 5′-TACAACCACTGCACTACCTG-3′, and H196, 5′-TGGAATGCTTGAAGGCTG-CT-3′.

PCR condition. 100 ng of genomic DNA was amplified with 50 pmol of each primer in 50 μl reaction mixture (10 mM Tris-Cl, pH 8.4/1.5 mM $MgCl_2$/50 mM KCl/250 μM of each dNTPs/3 units of Taq polymerase). Denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extention at 72°C for 2 min for 30 cycles. Digested PCR products were electrophoresed on 6% polyacrylamide gel (PAG) in 1 × TBE buffer, then stained with ethidium bromide.

Polymorphic and constant DNA fragments. A two allele RFLP with 386 bp fragment (allele a) and 346 bp+40 bp fragments (allele b), and 3 constant fragments (129 bp, 84 bp, and 56 bp).

Allele frequency. 0.64 for allele "a" and 0.36 for allele "b". PIC=0.35. Expected frequency of genotypes, a/a, a/b, b/b, was 0.41, 0.46, and 0.13, respectively. Observed frequency of each genotype was 0.60, 0.32, and 0.08, respectively. This RFLP segregated in Mendelian inheritance.

Comments. H19 is paternally imprinted (the paternally-derived allele is inactive), and the imprinting is inverse to that of *IGF2* located near *H19*. As two RFLPs, an *Alu*I RFLP and an *Rsa*I RFLP (Zhang and Tycko, 1992) have been known, the present RFLP is the third one in *H19*. Since these RFLPs sites are

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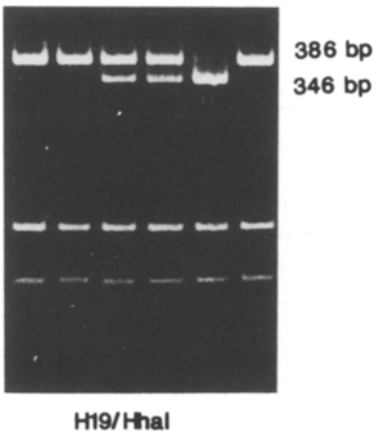

Fig. 1. *H19*/*Hha*I RFLP. Band intensity of polymorphic 40 bp fragments was so weak not to be seen in this figure.

localized within exon 5 of *H19* and they segregate independently, they are useful for imprinting studies of the gene.

References. Rainer S, Johnson LA, Dorby CJ, Ping AJ, Grundy PE, Feinberg AP (1993): Nature **362**: 747–749; Zhang Y, Tycko B (1992): Nature Genet **1**: 40–44.