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RFLP Report

TWO POLYMORPHIC *Ava*I AND *Hha*I SITES IN A DIFFERENTIALLY METHYLATED REGION OF THE HUMAN H19 GENE

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Summary The H19 gene is paternally imprinted both in the human and mouse (Bartolomei *et al.*, 1991; Zhang and Tycko, 1992), although its expression pattern seems somewhat different between the two species (Jinno *et al.*, 1995). DNA-methylation is a promising candidate for a parent-of-origin mark of the gene, and a paternal allele-specific methylation imprint was recently identified at the mouse H19 locus (Tremblay *et al.*, 1995). We found a 50% methylated region in the human H19 gene (Jinno, unpublished data). A search for polymorphisms in this region revealed two novel *Ava*I and *Hha*I RFLPs, which contribute to the detection of allele-specific methylation at the human H19 locus.

Key Words H19, methylation imprint, polymorphism, PCR-RFLP

PCR primers for the AvaI-site
PANL2: 5′-GAGCCTGCCAAGCAGAGCG-3′
PANR2: 5′-CACATAAGTAGGCGTGACTTGA-3′
PCR primers for the HhaI-site
ASMA: 5′-CAATGAGGTGTCCCAGTTCCA-3′
PANR2: 5′-CACATAAGTAGGCGTGACTTGA-3′

AvaI and HhaI polymorphisms. *Ava*I digestion produces a 342-bp fragment in A1 allele that lacks the *Ava*I site, while the digestion detects 270-bp and 72-bp fragments in A2 allele having the recognition site (Fig. 1a). A 64-bp fragment is constantly observed. *Hha*I digestion generates a 435-bp fragment and 245-bp+190-bp fragments in B1 allele without the *Hha*I site and in B2 allele with the site, respectively (Fig. 1b).

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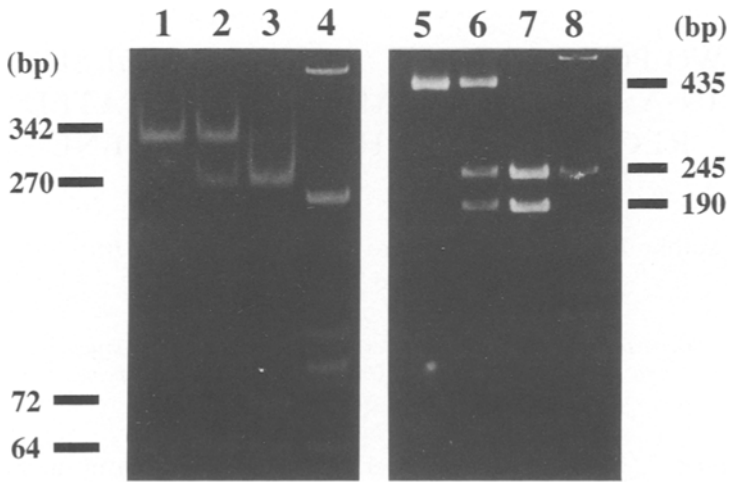

Fig. 1. *Ava*I RFLP (a) and *Hha*I RFLP (b). Lanes 1–3 indicate a homozygote for A1, heterozygote for A1/A2 and a homozygote for A2, and lanes 5–7 a homozygote for B1, heterozygote for B1/B2 and a homozygote for B2, respectively. Lanes 4 and 8 are size marker, *Alu*I-cut pUC19 DNA.

Table 1. Allele frequencies of *Ava*I- and *Hha*I-RFLPs among 30 Japanese.

Enzyme	Allele	Fragment size (bp)	Frequency	Heterozygosity
*Ava*I	A1	342	0.203	0.324
	A2	270+72	0.797	
*Hha*I	B1	435	0.667	0.444
	B2	245+190	0.333	

Frequencies. Estimated A1/A2 and B1/B2 allele frequencies among 30 normal Japanese are shown in Table 1.

Chromosomal localization. The human H19 gene has been assigned to chromosome 11p15.5.

Mendelian inheritance. Autosomal co-dominant inheritance for *Ava*I- and *Hha*I-RFLPs was confirmed in 2 families, respectively.

Other comments. For the *Ava*I RFLP, PCR was carried out in a total volume of 100 μl, containing 1 μg genomic DNA/1 μM of each primer/0.2 mM in each dNTP/10 μl 10×PCR-buffer/20 μl of 50% glycerol/3 U *Taq* polymerase (Takara Shuzo, Tokyo) for 32 cycles with an automated thermal cycler (GeneAmp PCR System 9600, Perkin Elmer, USA) as follows: denaturation at 94°C for 4 min for the first cycle and for 40 sec for further cycles, annealing at 59°C for 30 sec, and extension at 72°C for 110 sec. The PCR product (1.5 kb) was digested with *Bgl*I

and a 405-bp *Bgl*I-fragment was purified from a 4% polyacrylamide gel prior to *Ava*I digestion. The *Ava*I digests were subjected to electrophoresis on a 6% polyacrylamide gel.

Likewise, PCR was performed for the *Hha*I RFLP in a volume of 50 μl (300 ng genomic DNA/1 μM each primer/0.2 mM in each dNTP/5 μl 10×buffer/10 μl of 50% glycerol/1.5 U *Taq* polymerase) for 30 cycles with denaturation at 94°C for 105 sec, annealing at 59°C for 60 sec, and extension at 72°C for 100 sec (DNA Thermal Cycler PJ2000, Perkin Elmer). The PCR product (435 bp) was digested with *Hha*I and electrophoresed on a 4% polyacrylamide gel.

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REFERENCES

Bartolomei MS, Zemel S, Tilghman SM (1991): Parental imprinting of the mouse H19 gene. Nature **351**: 153–155

Jinno Y, Ikeda Y, Yun K, Maw M, Masuzaki H, Fukuda H, Inuzuka K, Fujishita A, Ohtani Y, Okimoto T, Ishimaru T, Niikawa N (1995): Establishment of functional imprinting of the H19 gene in human developing placentae. Nature Genet **10**: 318–324

Tremblay KD, Saam JR, Ingram RS, Tilghman SM, Bartolomei MS (1995): A paternal-specific methylation imprint marks the alleles of the mouse H19 gene. Nature Genet **9**: 407–413

Zhang Y, Tycko B (1992): Monoallelic expression of the human H19 gene. Nature Genet **1**: 40–44