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BRIEF REPORT — POLYMORPHSM REPORT

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A *Hha*I/*Bst*UI polymorphism in a novel gene at human chromosome 11p15.5

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Abstract We found a *Hha*I/*Bst*UI polymorphism in the 3′ untranslated region of a novel gene which was localized to 11p15.5. This region is one of prominent imprinting domains and contains multiple imprinted genes, such as *H19*, *IGF2* , *KVLQT1*, and $p57^{KIP2}$, which suggests that regional factors might contribute to the imprinting.

This polymorphism will be useful in the allelic analysis of expression and methylation of the novel gene.

Key words Imprinting · 11p15.5 · polymorphism · PCR-RFLP

Introduction

Imprinted genes are often found to cluster in particular chromosomal regions and form imprinted domains (Zemel et al. 1992), although the significance of their clustering in the imprinting mechanism remains to be revealed. In the mouse and the human, there are at least two major imprinted domains. One is the human chromosome 11p15.5 region, where the imprinted genes *H19*, *IGF2*, *KVLQT1*, $p57^{KIP2}$, and others are located (Zhang and Tycko 1992, Jinno et al. 1995, Giannoukakis et al. 1993, Lee et al. 1997, Matsuoka et al. 1996). We isolated a novel gene (*PEN11B*) which was localized to 11p15.5, using a repetitive sequence consisting of pentanucleotide elements found in the human *H19* enhancer region (GenBank Accession No. AF020089).

In order to analyze the allelic expression of the gene, we found a *Hha*I/*Bst*UI restriction fragment length polymorphism (RFLP) in its 3′ untranslated region (UTR).

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Polymerase chain reaction (PCR) primers

The PCR primers used were:
BSTA: 5′-GTCACCTGACCCCTCAGCAA-3′ and
BSTb: 5′-CACCAGCTCTGTCCTCAGAG-3′.

PCR conditions

For the *Hha*I/*Bst*UI RFLP, PCR was carried out in a total volume of 50 μl, containing 0.5 μg genomic DNA, 0.5 μM of each primer, 0.2 mM in each dNTP, 5 μl 10 × PCR-buffer, 10 μl of 50% glycerol, and 1.5 U *Taq* polymerase (Perkin Elmer, Norwalk, CT, USA) for 28 cycles with an automated thermal cycler (DNA Thermal Cycler PJ2000; Perkin Elmer) as follows: denaturation at 94°C for 3 min for the first cycle and for 90 s for further cycles, annealing at 59°C for 70 s, and extension at 72°C for 90 s. The PCR product was phenol/chloroform-extracted, ethanol-precipitated, and digested with *Hha*I/*Bst*UI. The restriction digests were separated in a 6% polyacrylamide gel.

Polymorphism and allele frequency

*Hha*I(*Bst*UI) digestion generated a 201 (263)-bp fragment in the A1 allele without the *Hha*I(*Bst*UI) and a 167 (208)-bp fragment in the A2 allele (Fig. 1). The estimated A1/A2 allele frequencies in 64 normal Japanese are shown in Table 1.

Table 1 Allele frequencies of *Hha*I restriction fragment length polymorphism in 64 normal Japanese individuals

Enzyme	Allele	Fragment size (bp)	Frequency	Heterozygosity
*Hha*I	A1	201	0.625	0.469
	A2	167	0.375	

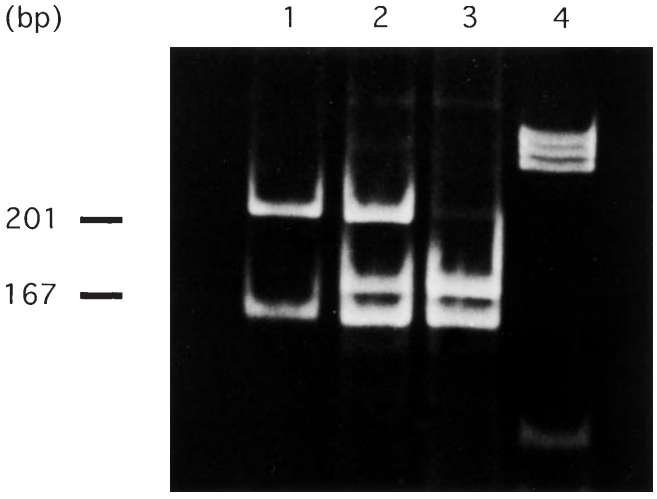

Fig. 1 *Hha*I restriction fragment length polymorphism. *Lanes 1–3* indicate a homozygote for A1, heterozygote for A1/A2, and a homozygote for A2, respectively. *Lane 4* is the size marker, *Alu*I-cut pUC19 DNA

Mendelian inheritance. Codominant segregation was observed in three two-generation families.

Chromosomal localization. The gene was assigned to 11p15.5 by fluorescence in situ hybridization.

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