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BRIEF REPORT — POLYMORPHISM REPORT

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A *Sac*II polymorphism in the human *ASCL2 (HASH2)* gene region

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Abstract The mouse achaete-scute homolog-2 gene (*Ascl2* or *Mash2*) encodes a transcription factor playing a role in the development of the trophoblast. The *Ascl2* gene is paternally imprinted in the mouse, but whether this applies to its human homolog is unknown. In the present study, we found a *Sac*II polymorphism in the possible 3′ untranslated region (UTR) of the gene. It would be very useful to determine definitively whether the gene is imprinted, as well as to analyze the allelic methylation status of the gene.

Key words *Ascl2* · 11p15.5 · Polymorphism · Imprinting

Introduction

The mouse achaete-scute homolog-2 gene (*Ascl2* or *Mash2*) encodes a transcription factor playing a role in the development of the trophoblast (Guillemot et al. 1994). The *Ascl2* gene is paternally imprinted in the mouse (Guillemot et al. 1995), but whether this applies to its human homolog is unknown. Previously, we isolated a phage clone covering the human *ASCL2* gene and mapped it to 11p15.5, an imprinted cluster region (Miyamoto et al. 1996). Paternal imprinting of the *ASCL2* gene was suggested by comparing its expression in a hydatidiform mole and a normal placenta (Alders et al. 1997). In this study, we found a *Sac*II polymorphism in the possible 3′ untranslated region (UTR) of the gene. It would be very useful to determine definitively whether the gene is imprinted, as well as to analyze the allelic methylation status of the gene.

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Primers for the polymerase chain reaction (PCR)

TM-F:5′-CGGCCCAGCCTGACCAATG-3′
TM-R:5′-GAAGCCGCCAGCCCTTATG-3′

SacII polymorphism. *Sac*II digestion produces a 68-bp fragment in the A1 allele that lacks the *Sac*II site, while the digestion detects 45-bp and 23-bp fragments in the A2 allele having the recognition site (Fig. 1).

Allele frequencies. The estimated A1/A2 allele frequencies among 36 normal Japanese women are shown in Table 1.

Chromosomal localization. The human *ASCL2* gene has been assigned to chromosome 11p15.5.

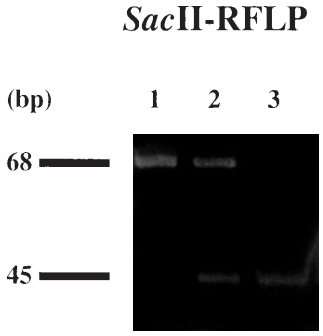
Mendelian inheritance. Mendelian inheritance was confirmed in four families.

Other comments. For the *Sac*II restriction fragment length polymorphism (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, and 3 U *Taq* polymerase (Takara Shuzo, Tokyo, Japan) for 31 cycles with a DNA Thermal Cycler PJ2000 (Perkin-Elmer, Norwalk, CT, USA) as follows: denaturation at 94°C for 5 min for the first cycle and for 90 s for further cycles, annealing at 59°C for 75 s, and extension at 72°C for 90 s. The PCR products (68 bp) were digested with *Sac*II and the digests were subjected to electrophoresis on an 8% polyacrylamide gel. The polymorphic *Sac*II site was localized to a nucleotide position 788 bp downstream from the *ASCL2* stop codon (Alders et al. 1997) and 108 bp down-

Table 1 Allele frequencies of *Sac*II-RFLPs among 36 Japanese women

Enzyme	Allele	Fragment size (bp)	Frequency	Heterozygosity
*Sac*II	A1	68	0.542	0.639
*Sac*II	A2	45 + 23	0.458	

Fig. 1 *Sac*II restriction fragment length polymorphism (*RFLP*). *Lanes 1–3* indicate a homozygote for A1, heterozygote for A1/A2, and a homozygote for A2



stream from the 5′ end of a partial cDNA isolated from a human placental cDNA library (Miyamoto, in preparation). This cDNA is identical to a part of the *ASCL2* genomic sequence. It is very likely that the partial cDNA represents a part of the *ASCL2* 3′UTR, from comparisons of its sequence to those of the rat cDNA and the mouse *Ascl2* gene.

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