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BRIEF REPORT — POLYMORPHISM REPORT

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An *Nsi*I RFLP in the human long QT intronic transcript 1 (*LIT1*)

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Abstract An *Nsi*I polymorphic site has been found in the human long QT intronic transcript 1 (*LIT1*). In this transcript, we found a C-to-T transition, which was located between exons 10 and 11 of *KVLQT1*, and was confirmed by sequencing analysis. The allelic frequency of this polymorphism, was 0.82: 0.18 in Japanese individuals. Our novel polymorphism, combined with other polymorphisms, could be very useful in helping to determine whether the imprinting of *LIT1* is disrupted in Beckwith-Wiedemann syndrome (BWS) or in human cancers.

Key words Imprinting · 11p15.5 · *Nsi*I RFLP · *LIT1*

Introduction

Chromosomal region 11p15.5 is one of the common imprinting clusters. This region is associated with Beckwith-Wiedemann syndrome (BWS) and a variety of human cancers (Brown et al. 1996; Hatada et al. 1996; Besnard-Guerin et al. 1996; Kondo et al. 1996). Multiple cases of BWS patients with chromosomal rearrangements involve the disruption of the *KVLQT1* gene (Lee et al. 1997). This suggests that the *KVLQT1* locus may harbor some imprinting control elements (Reid et al. 1997). Recently, the human long QT intronic transcript 1 (*LIT1*) was identified as an imprinted antisense RNA which was expressed preferentially from the paternal allele in the *KVLQT1* locus, and it was suggested that disrupted imprinting of *LIT1* could contribute to BWS pathophysiology in many patients (Mitsuya et al. 1999; Smilinich et al. 1999; Lee et al. 1999). Genomic analysis of the *LIT1* identified an *Nsi*I restriction fragment length polymorphism (RFLP) which was a novel single nucleotide polymorphism (SNP) and was located in the intronic sequence between exons 10 and 11 of the *KVLQT1* gene. Several different SNPs have been reported previously (Mitsuya et al. 1999; Lee et al. 1999). Our novel SNP, combined with the reported SNPs could lead to further investigation of the pathogenesis of BWS and human cancers, and the correlation between the disruption of *LIT1* imprinting and these diseases could be elucidated.

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Polymerase chain reaction (PCR) primers

The PCR primers used were:
SMS2-B: 5′-AAGAAAGTGTTGAGTGGTAA-3′ and
SMS2-C: 5′-CAAGCAGTACTGTTTCAGAA-3′

The primers were designed from the EST and the genomic sequence data of Genebank (Genebank accession No. H88273 and U90095).

PCR conditions and *Nsi*I digestion

PCR was carried out in a total volume of 10 μl, containing 100 ng genomic DNA, 0.6 μM of each primer, 0.25 mM of each dNTP, 1 × Expand PCR buffer (Boehringer-Mannheim, Mannheim, Germany), 2.0 mM $MgCl_2$, and 0.35 U Expand Taq polymerase (Boehringer-Mannheim), and was performed as follows: initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 20 s, annealing at 50°C for 20 s, extension at 72°C for 50 s, and a final extension at 72°C for 5 min. The PCR products were digested with *Nsi*I and electrophoresed on 1% agarose gel. To confirm complete digestion, the PCR products generated from genomic DNAs with known sequences were simultaneously digested with *Nsi*I as the controls.

Polymorphism and allele frequency

The fragment size of the PCR product was 801 bp. *Nsi*I digestion produced an 801-bp undigested fragment from the

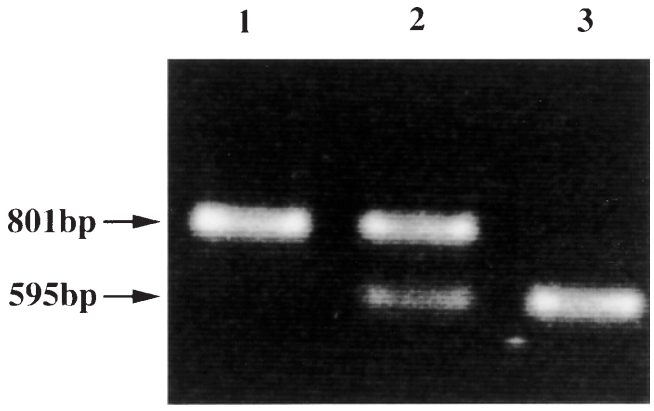

Fig. 1. *Nsi*I restriction fragment length polymorphism (RFLP). *Nsi*I digests were electrophoresed on 1% agarose gel. *Lane 1* indicates a homozygote for the C allele; *lane 2* is a heterozygote (C/T); *lane 3* indicates a homozygote for the T allele

Table 1. Allele frequencies of the polymorphisms in 56 Japanese individuals

Allele	Frequency	Heterozygosity
C	0.82	0.30
T	0.18	

C allele that lacked the *Nsi*I site, while from the T allele having the *Nsi*I restriction site, two fragments, with lengths of 595 and 206 bp, were generated. Figure 1 shows a representative result of the analysis. The allele frequencies of the polymorphisms in 56 volunteer Japanese individuals are shown in Table 1.

Chromosomal localization. The human *LIT1* was assigned to human chromosome 11p15.5 (Mitsuya et al. 1999; Smilinich et al. 1999; Lee et al. 1999).

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