J Hum Genet (2000) 45:58–59

BRIEF REPORT — POLYMORPHISM REPORT

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Identification of a novel single nucleotide polymorphism (SNP) in the human organic cation transporter-like 2-antisense (*ORCTL2S*) gene

Received: September 13, 1999 / Accepted: September 27, 1999

Abstract We found a single nucleotide polymorphism (SNP) in exon 3 of the human organic cation transporter-like 2-antisense (*ORCTL2S*) gene: a base substitution A266G which was confirmed by direct sequencing. Heterozygosity of the polymorphic alleles was 0.45 in a Japanese population. This polymorphism will be useful in the allelic expression analysis of the *ORCTL2S* gene.

Key words Imprinting · 11p15.5 · Single nucleotide polymorphism (SNP) · PCR-SSCP · *ORCTL2S*

Introduction

Imprinted genes are frequently found to cluster on particular chromosomes (Zemel et al. 1992). In the human, there are at least two common imprinted domains. One is the human chromosomal band 11p15.5, 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). The human *ORCTL2S* is localized near the three imprinted genes, *IPL*, *ORCTL2*, and $p57^{KIP2}$ (Qian et al. 1997; Cooper et al. 1998). Thus, one would expect *ORCTL2S* to show imprinted status. In order to analyze its allelic expression, we searched and found a single nucleotide polymorphism (SNP) in exon 3 of *ORCTL2S*.

Polymerase chain reaction (PCR) primers

The PCR primers used were:
OR3F3: 5′-CAGGCTGACTGGAGGAAGGA-3′ and
OR3R: 5′-GCCGTTATCTATGCTATTCTCT-3′

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Primer labeling and PCR conditions

Before the PCR reaction, the primers were labeled by [γ-^{32}P]dATP, using a Kination kit (Toyobo, Tokyo, Japan), following the manufacturer's protocol. PCR was carried out in a total volume of 10 μl, containing 100 ng genomic DNA, 0.1 μM of each primer, 0.25 mM of each dNTP, 1 × Expand PCR buffer with $MgCl_2$, and 0.35 U Expand Taq polymerase (Boehringer-Mannheim, Mannheim, Germany), and performed as follows: an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 20 s, annealing at 53°C for 20 s, and extension at 72°C for 30 s, with a final extension at 72°C for 5 min.

Single-strand conformation polymorphism (SSCP)

After the PCR reaction, 5 μl each of the radiolabeled PCR product was added to 45 μl of stop solution, which consisted of 95% formamide, 10 mM NaOH, 0.25% bromophenol blue, and 0.25% xylene cyanol. These mixtures were incubated at 94°C for 3 min, then 1.5-μl aliquots of each were loaded and electorophoresed on 0.5 × Super Detection Solution (Toyobo) containing 5% glycerol and 0.6 × TBE (53.4 mM Tris, 53.4 mM boric acid, 1.32 mM ethylene diamine tetraacetic acid [EDTA], at 4°C (7–8 W, 10 h). After electrophoresis, the gel was dried, then exposed to the imaging plate for 30 min, and analyzed with a BAS-2000 bio imaging analyzer (Fuji Film, Tokyo, Japan).

Polymorphism and allele frequency

The lower band of SSCP was generated from the A allele and the upper one from the G allele (Fig. 1). The estimated allele frequency of the A allele and the G allele in 50 normal volunteer Japanese is shown in Table 1.

Chromosomal localization. The human *ORCTL2S* gene was assigned to human chromosome 11p15.5 (Cooper et al. 1998).

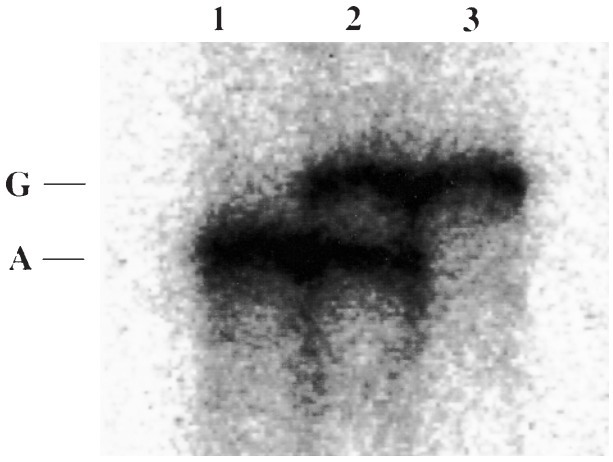

Fig. 1. Single nucleotide polymorphism of *ORCTL2S*. *Lanes 1-3* indicate a homozygote for the A allele (*A*), heterozygote (A/G), and a homozygote for the G allele (*G*), respectively

Table 1. Allelic frequencies of single-strand conformation polymorphism in 50 normal Japanese individuals

Allele	Frequency	Heterozygosity
A	0.34	0.45
G	0.66	

Acknowledgments This work was supported, in part, by a Health Science Research Grants (Research on Human Genome and Gene Therapy) from the Ministry of Health and Welfare of Japan.

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