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BRIEF REPORT — POLYMORPHISM REPORT

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Isolation and radiation hybrid mapping of a highly polymorphic CA repeat sequence at the SREBP cleavage-activating protein (SCAP) locus

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Abstract Sterol regulatory element binding protein (SREBP) cleavage-activating protein (SCAP) is a central regulator of lipid synthesis and uptake in animal cells. A polymorphic dinucleotide (CA) repeat sequence was isolated from a genomic clone containing the *SCAP* gene and was mapped to chromosome 3p21.3. High heterozygosity (0.89) makes this polymorphism a useful marker in the genetic study of disorders affecting lipid metabolism.

Key words SREBP cleavage-activating protein (SCAP) · Dinucleotide repeat · Lipid metabolism

Introduction

Sterol regulatory element binding protein (SREBP) cleavage-activating protein (SCAP) is a central regulator of lipid synthesis and uptake in animal cells (Hua et al. 1996; Brown and Goldstein 1997). Located in the endoplasmic reticulum, SCAP stimulates the proteolytic cleavage of SREBPs that activate the synthesis of cholesterol and unsaturated fatty acids and their uptake from plasma via the low-density lipoprotein (LDL) receptor. SCAP-stimulated proteolysis releases active fragments of SREBPs from membranes of the endoplasmic reticulum and allows them to enter the nucleus, where they activate transcription. The activity of SCAP is abolished by sterols, a regulatory mechanism that maintains the constant lipid composition of cell membranes.

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Point mutations in the sterol-sensing domain of SCAP cause resistance to sterol suppression (Hua et al. 1996; Nohturfft et al. 1998). Cells with a point mutation continue to synthesize cholesterol and to take up LDL even when they are massively overloaded with sterols. In transgenic mice that expressed mutant SCAP in liver, the result was enlarged livers that were engorged with cholesterol and triglycerides (Korn et al. 1998).

Given the central role of SCAP in the regulation of lipid metabolism, molecular variants in the human *SCAP* gene would likely result in alterations in plasma lipoprotein levels and/or derangement of lipid metabolism within tissues. As a first step in determining whether molecular variants in the *SCAP* gene contribute to human disease, we isolated a highly polymorphic dinucleotide repeat at this locus.

Source/Isolation of CA repeat sequence

A bacteriophage P1 library (Genome Systems, St. Louis, MO, USA) was screened by the polymerase chain reaction (PCR), using two oppositely oriented oligonucleotides (forward: 5′-TGGGGATGTCACCTCCCTTAC-3′ and reverse: 5′-TGCTGAATGGAGTAGAACTTGATGC-3′) that correspond to nucleotide positions 3476–3495 and 3564–3588 of human SCAP cDNA (KIAA0199; GenBank Accession no. D83782) (Nagase et al. 1996). A fragment containing the CA repeat was identified by Southern blotting of PAC DNA digested by *Hae*III, *Sau*3A, or *Rsa*I with the $(GT)_{20}$ probe, subcloned, and sequenced (Tsukamoto et al. 1998); Sequences were determined by Dye Terminator cycle sequencing, using the 377 ABI DNA sequencer Perkin-Elmer; Norwalk, CT (DNA sequences are available through GenBank Acc no. AF141945). PCR primers were designed to flank this new repeat sequence for polymorphism analysis.

Table 1. Size and frequencies of the 13 alleles of the CA repeat polymorphism

Allele	Size (bp)	Frequency
A1	170	0.03
A2	168	0.03
A3	166	0.10
A4	164	0.01
A5	162	0.03
A6	160	0.09
A7	158	0.12
A8	156	0.19
A9	154	0.19
A10	152	0.14
A11	150	0.06
A12	148	0.01
A13	144	0.01

PCR primers

The PCR priners used were:
Forward (SCAP-F) 5′-CAGGCTGTATAAGCTAAGTATG-3′
Reverse (SCAP-R) 5′-CTGGATGTAGTGGCAGGCAC-3′

PCR conditions

PCR was performed in a volume of 10 μl containing 20 ng genomic DNA, 10 mM Tris HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% of gelatin, 200 μM dNTPs, 2.5 pmol of a [^{32}P] end-labeled forward primer and a non-labeled reverse primer, and 0.25 units of Taq polymerase. Cycle conditions were 94°C for 4 min, then 35 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s, with a final extension step of 5 min at 72°C in a Gene Amp PCR9600 System (Perkin Elmer Cetus, Norwalk, CT, CA, USA). PCR products were electrophoresed in 0.3-mm-thick denaturing 6% polyacrylamide gels containing 36% formamide and 8 M urea, at 2000 V for 2–4 h. The gels were transferred to filter papers, dried at 80°C, and autoradiographed. Sizes of the alleles were determined by comparison with the sequencing ladder of a control plasmid (Watanabe et al. 1998).

Polymorphism and allele frequency. Thirteen alleles were detected in 154 chromosomes of unrelated Japanese individuals. Observed heterozygosity was 0.89. The sizes and frequencies of the 13 alleles are shown in Table 1.

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

Radiation hybrid mapping. The newly isolated CA repeat at the *SCAP* locus was mapped to chromosome 3p21.3, using described procedures on the G3 RH mapping panel of 83 hybrid cell lines of the Stanford Human Genome Center (Boehnke et al. 1991), by linkage to a marker SHGC-500 (D3S1322E) with a logarithm of differences (LOD) score of >7.85.

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