J Hum Genet (1999) 44:421–422

BRIEF REPORT — POLYMORPHISM REPORT

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A common Ile796Val polymorphism of the human SREBP cleavage-activating protein (*SCAP*) gene

Received: August 17, 1999 / Accepted: August 19, 1999

Abstract We identified a new common amino acid polymorphism of isoleucine/valine at codon 796 in exon 16 of the gene for human sterol regulatory element binding protein (SREBP) cleavage-activating protein (SCAP), a central regulator of lipid synthesis and metabolism in animal cells. It can be detected as an *Msl*I restriction fragment length polymorphism. The allelic frequencies were: isoleucine (A) allele, 0.57 and valine (G) allele, 0.43. This polymorphism may be useful for genetic studies of disorders affecting intracellular lipid metabolism and hyperlipidemia.

Key words SREBP cleavage-activating protein (SCAP) · Single nucleotide polymorphism (SNP) · Restriction fragment length polymorphism (RFLP) · 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 et al. 1997). Point mutations in the sterol-sensing domain of SCAP causes resistance to sterol suppression (Hua et al. 1996; Nohturfft et al. 1998). Mutated cells continue to synthesize cholesterol and to take up low-density lipoprotein (LDL) even when they are massively overloaded with sterols. In transgenic mice that expressed mutant SCAP in liver there was resultant enlargement of the livers, which 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 the plasma lipoprotein levels and/or derangement of lipid metabolism within tissues. During screening for disease-associated mutations in hyperlipidemia, we identified a single nucleotide polymorphism in the human *SCAP* gene. Here we describe an *Msl*I polymorphism for Ile/Val amino acid substitution at codon 796 of the human *SCAP* gene.

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Source and isolation of polymorphisms

All 23 exons encoding the entire coding sequence of the *SCAP* gene were screened by polymerase chain reaction (PCR) single-strand conformational polymorphism (SSCP) analysis in 75 Caucasian individuals for nucleotide variations of the gene. PCR-SSCP was carried out following procedures described previously (Hirayama et al. 1998). Variant SSCP bands were identified in the PCR product of exon 16. These variants were subcloned and sequenced as described by Tsukamoto et al. (1998). Sequence analysis revealed an A-to-G transition at the first nucleotide of codon 796.

PCR primers

Forward 5′-TTGTGCTGCGCGGCCACCTCA-3′
Reverse 5′-AGGAGGAAAGGGCAGCCGCAC-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 mCi of [a-^{32}P] dCTP (3000 Ci/mmol; 10 mCi/ml), 2.5 pmol of a forward primer and a reverse primer, and 0.25 units of Taq polymerase. Cycle conditions were 94°C for 4 min, then 30 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, USA). PCR products were electrophoresed in a 5% polyacrylamide gel containing 5% glycerol in 0.5 × Tris-borate-EDTA (TBE) buffer at room temperature. Gels were transferred to filter papers, dried at 80°C, and autoradiographed.

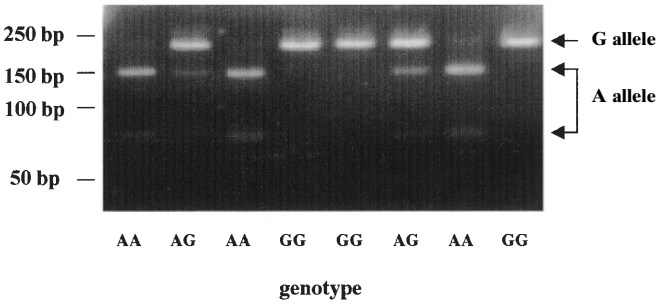

Fig. 1. *Msl*I restriction fragment length polymorphism (RFLP) showing an A-to-G substitution at codon 796 in the human *SCAP* gene. *Msl* I digestion produced a 235-bp fragment in the G (Val) allele and 158- and 77-bp fragments in the A (Ile) allele

Msl I polymorphism

An A (Ile)-to-G (Val) transition at the first nucleotide of codon 796 abolishes an *Msl* I restriction site. Thus, *Msl*I digestion produces a 235-bp fragment in the G (Val) allele that lacks the *Msl*I site, while the digestion detects 158- and 77-bp fragments in the A (Ile) allele having the recognition site, as demonstrated in Fig. 1.

Allele frequency

The estimated allele frequencies in 150 Caucasian individuals are shown in Table 1. The observed heterozygosity was 0.49.

Chromosomal localization. The human *SCAP* gene was assigned to chromosome band 3p21.3 by fluorescence in situ hybridization (Nakajima et al. 1999).

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

Table 1. Estimated allele frequencies of the *SCAP* gene in 150 caucasian individuals

Allele	Amino acid	*Msl*I fragments	Frequency
A allele	Ile	158 + 77 bp	0.57
G allele	Val	235 bp	0.43

Other comments. Two rare polymorphisms within the coding sequence of the *SCAP* gene were also identified during PCR-SSCP screening, as follows; a G-to-A silent substitution at the third nucleotide of codon 1190 in exon 22, and a 5-bp insertion in the 3′ untranslated region 48 bp downstream from the stop codon in exon 23.

Acknowledgments This work was supported by research grants for primary hyperlipidemia from the Ministry of Health and Welfare of Japan and the Novartis Foundation for Gerontological Research.

References

Brown MS, Goldstein JL (1997) The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. Cell 89:331–340

Hirayama T, Yamaki E, Hara A, Tsuji M, Hashimoto K, Yamamoto M, Emi M (1998) Five familial hypercholesterolemic kindreds in Japan with novel mutation of the LDL receptor gene. J Hum Genet 43:250–254

Hua X, Nohturfft A, Goldstein JL, Brown MS (1996) Sterol resistance in CHO cells traced to point mutation in SREBP cleavage-activating protein. Cell 87:415–426

Korn BS, Shimomura I, Bashmakov Y, Hammer RE, Horton JD, Goldstein JL, Brown MS (1998) Blunted feedback suppression of SREBP processing by dietary cholesterol in transgenic mice expressing sterol-resistant SCAP(D443N). J Clin Invest 102:2050–2060

Nakajima T, Hamakubo T, Kodama T, Inazawa J, Emi M (1999) Genomic structure and chromosomal mapping of the human SREBP cleavage-activating protein (SCAP) gene. J Hum Genet 44: (in press)

Nohturfft A, Brown MS, Goldstein JL (1998) Sterols regulate processing of carbohydrate chains of wild-type SREBP cleavage-activating protein (SCAP), but not sterol-resistant mutants Y298C or D443N. Proc Natl Acad Sci USA 95:12848–12853

Tsukamoto K, Haruta K, Shiba T, Emi M (1998) Isolation and mapping of a polymorphic CA repeat sequence at the human interleukin 6 locus. J Hum Genet 43:71–72