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BRIEF REPORT — POLYMORPHISM REPORT

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Human cathepsin S gene (*CTSS*) promoter −25G/A polymorphism

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Abstract We found a novel G → A change at nucleotide −25 within the promoter of the *CTSS* gene encoding the elastase cathepsin S. The *CTSS* −25G/A polymorphism could be detected by digestion with endonuclease *Bfm*I. The frequency of the *CTSS* −25A allele was 0.457 in Caucasians and 0.431 in Canadian Inuit. Because of the importance of the *CTSS* gene product in vascular matrix remodeling, this polymorphism may be useful in the study of associations with atherosclerosis and related phenotypes.

Key words Atherosclerosis · Arterial wall matrix · Lipodystrophy

Introduction

Human cathepsin S (CTSS, EC3.4.22.27) is a cysteine protease that belongs to the papain superfamily (Shi et al. 1994). CTSS and related elastases are involved in physiological protein degradation and are considered to play a role in pathological tissue destruction and invasion (Shi et al. 1994). An important role for CTSS in vascular biology and atherosclerosis has been suggested by the presence of CTSS, and cathepsin K, at sites of arterial wall matrix remodeling (Sukhova et al. 1998). Extracts of atheromatous tissues have higher elastase activity, due mainly to higher cysteine protease mass and activity (Sukhova et al. 1998). Cultured smooth muscle cells, when stimulated with atheroma-specific cytokines, secrete active CTSS (Sukhova et al. 1998). Thus, the gene encoding CTSS is an important candidate gene for atherosclerosis and possibly for its related phenotypes.

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The *CTSS* gene has been mapped to chromosome 1q21 (Shi et al. 1994) and is contained within a region that has shown linkage to interesting metabolic disorders, such as Dunnigan-type partial lipodystrophy (Anderson et al. 1999) and familial combined hyperlipidemia (Pajukanta et al. 1998). The availability of a marker for the *CTSS* gene would be useful for the study of its possible association not only with atherosclerosis but also with distinctive metabolic phenotypes related to insulin resistance and lipid metabolism. In the course of DNA sequencing all of the coding regions and the 5′- and 3′-untranslated regions of the *CTSS* gene, we identified a novel G → A change at nucleotide −25 within the promoter region.

Polymorphism and allele frequency

Primers for the polymerase chain reaction (PCR). For PCR, we used the following primers:
CTSS-25F 5′-ACCTCATGTGACAAGTTCCAAT-3′
CTSS-25R 5′-ACCAAATGGGAGAAAAAGAACA-3′

*Bfm*I *polymorphism.* The PCR fragment size was 164 bp. Digestion of the less common −25A allele produced two smaller fragments, with sizes 104 and 60 bp. Digestion of the more common −25G allele produced a single 164-bp fragment.

Chromosomal localization. The human *CTSS* gene has been localized to chromosome 1q21 (Shi et al. 1994).

Mendelian inheritance. Mendelian inheritance was confirmed in three large families.

Other comments. The polymorphism was detected through direct sequencing of all coding regions of the *CTSS* gene in Caucasian subjects with partial lipodystrophy. Nucleotide −25 is within exon 1. Target DNA was amplified using an initial melting temperature of 94°C for 5 min, followed by 30 cycles of 94°C for 20 s, 55°C for 20 s, and 72°C for 20 s. A

Table 1. Allele frequencies of *CTSS* −25G/A polymorphism as detected by *Bsm*I

Allele	Nucleotide	Fragments (bp)	Frequency	
			Caucasian (n = 186)	Inuit (n = 281)
−25G	G	164	0.543	0.569
−25A	A	104 + 60	0.457	0.431

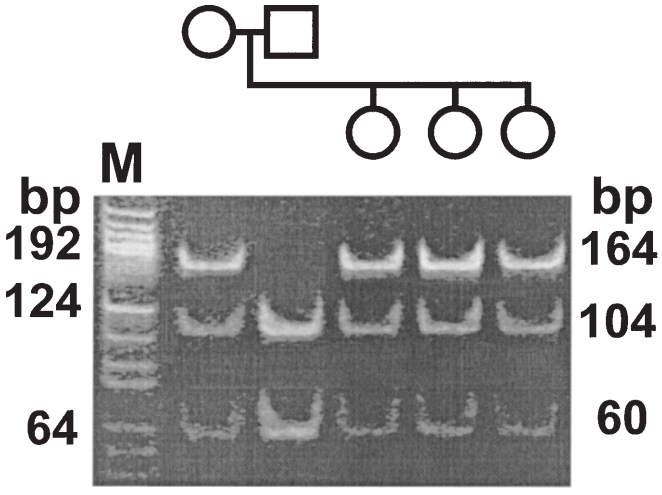

Fig. 1. *Bsm*I restriction fragment length polymorphism (RFLP) detecting *CTSS* −25G/A polymorphism. *Bsm*I digests were electrophoresed in 8% polyacrylamide gels. *Pedigree structure* indicates familial relationships between samples. *M* indicates molecular weight marker. *Numerals on left side* correspond to molecular weight marker band sizes. *Numerals on right side* correspond to fragment sizes

final 72°C extension step for 10 min terminated the process. The fragments were visualized in 8% polyacrylamide gels.

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