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BRIEF REPORT — POLYMORPHISM REPORT

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Dinucleotide repeat polymorphism in the first intron of the CSR gene

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Abstract The CSR (cellular stress response) gene encodes a protein that structurally resembles the macrophage scavenger receptor, and is a potent regulator of intracellular reactive oxygen intermediates. We found a polymorphic dinucleotide repeat in the first intron of the CSR gene. This polymorphism will be a useful genetic marker to study diseases associated with oxidative stress.

Key words CSR (cellular stress response) gene · Oxidative stress

Introduction

We isolated the CSR (cellular stress response) gene, whose expression was significantly induced by cellular oxidative stress (GenBank accession numbers, AB007829 and AB007830) (Han et al. 1998). The CSR protein was suggested to have a crucial role in protecting cells from the consequences of oxidative stress, as a scavenger of reactive oxygen species and their by-products. The oxidative stress exerted by reactive oxygen intermediates (ROI) such as H_2O_2, superoxide (O_2^-), and hydroxyl radicals (OH^-) can cause severe cellular damage and plays important roles in aging, and cancer and various other diseases (Schwartz et al. 1993). The brain, in particular, is highly sensitive to oxidative damage generated by ROI following ischemia and reperfusion (Sussman and Bulkley 1990), or as a consequence of neuronal degenerative diseases (Schubert et al. 1995). Therefore a better understanding of genes responding to the presence of oxidative agents is important from both the biological and the clinical points of view. To investigate the susceptibility of the CSR gene to pathogenesis due to cellular oxidative stress, we characterized a dinucleotide repeat polymorphism within the CSR gene.

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Source and description

A sequence containing the dinucleotide (CA) repeat was identified in the first intron of the CSR gene through genome sequence analysis. Polymerase chain reaction (PCR) primers were designed to amplify a fragment containing the dinucleotide repeat region.

Primer sequence

GT strand 5′-GCATGGCTCTCATACATGCA-3′
CA strand 5′-ACTTCATCCCAGTGATAAAGC-3′

PCR conditions

PCR was performed in 25 μl volumes of a mixture containing 20 ng of genomic DNA, 0.5 units of Ex-Taq DNA polymerase (Takara, Tokyo, Japan), 1 × PCR buffer [67 mM Tris (pH 8.8), 16.6 mM NH_2SO_4, 6.7 μM ethylene-diamine tetraacetic acid (EDTA), 10 mM β-mercaptoethanol], 10 pmol of cold (GT strand) and hot (CA strand) primer labeled with [γ-^{32}P] adenine triphosphate (ATP), 250 μM of each dideoxynucleotide, and 5 mM $MgCl_2$ for 35 cycles, using a thermocycler (Perkin Elmer Cetus 9600; Norwalk, CT, USA]). Each cycle consisted of 94°C for 30 s, 57°C for 30 s, and 72°C for 1 min. The final polymerization step was at 72°C for 5 min. The PCR products were electrophoresed on 6% polyacrylamide gels containing 7.7 M urea and 32% formamide.

Polymorphism and allele frequency

Frequency

Allele frequencies were estimated from the genomic DNA of 74 unrelated Japanese individuals (Table 1). The observed frequency of heterozygotes was 0.66.

Table 1 Allele frequencies estimated from genomic DNA of 74 unrelated Japanese individuals

Allele	Size (bp)	Frequency
a1	143	0.176
a2	149	0.277
a3	153	0.155
a4	155	0.216
a5	157	0.128
a6	159	0.021
a7	161	0.027

Chromosomal localization

The CSR gene was localized to chromosome 8p21 (Han et al. 1998).

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