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BRIEF REPORT — POLYMORPHISM REPORT

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A highly polymorphic CA repeat marker at the human interleukin 6 receptor (IL6R) locus

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Abstract A polymorphic dinucleotide (CA) sequence was isolated from a genomic clone containing the human *interleukin 6 receptor* (*IL6R*) gene. High heterozygosity (0.81) makes this polymorphism a useful marker in the genetic study of disorders affecting the inflammation process and bone resorption.

Key words Interleukin 6 · Dinucleotide repeat · Inflammation · Osteoclast · Bone resorption

Introduction

Interleukin 6 is a multifunctional cytokine essential to the regulation of the immune response, hematopoiesis, and the bone resorption process. It exerts its actions through binding to its cell-surface receptor, IL6R. As interleukin 6 and its receptor stimulate osteoclast development and, thereby, the process of bone resorption, they are pathogenic factors in bone loss, especially that triggered by an estrogen deficiency state (Manolagas 1995). Yamasaki et al. (1988) isolated a cDNA encoding the IL6 receptor which consists of 468 amino acids. To understand the relationship between genetic variations at the *IL6R* locus and disorders affecting the inflammation process and bone resorption (Nakamura 1996; Yanase 1997), we isolated and characterized a dinucleotide repeat polymorphism at this locus.

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Source and isolation of CA repeat sequence

A human genomic clone containing the *IR6R* gene was identified from a P1-derived artificial chromosome (PAC) library by polymerase chain reaction (PCR) three-dimensional screening, using primer sequences derived from the 3′ portion of the gene. A fragment containing the CA repeat was identified by Southern blotting of PAC DNA digested by *Hae* III, *Sau* 3A, or *Rsa* I with $(GT)_{20}$ probe, subcloned, and sequenced. An autoradiogram of the CA repeat sequence is shown in Fig. 1B. PCR primers were designed to flank this new repeat sequence for polymorphism analysis.

PCR primers

The PCR primers used were: Forward (IL6R,123F) 5′ GGC AAC CGA GCA AGA CTC TC 3′, and reverse (IL6R,123R) 5′ GCG AGG ACA GAA GAT TTG TC 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 30 cycles of 94°C for 30 s, 65°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 PCR 9600 System (Perkin Elmer Cetus, Palo Alto, CA, USA) (Nakura et al. 1994). PCR products were electrophoresed in 0.3-mm-thick denaturing 6% polyacrylamide gels containing 36% formamide and 8 M urea, at 2000 volts for 2–4 h. The gels were transferred to filter papers, dried at 80°C, and autoradiographed. Sizes of alleles were determined by comparison with the sequencing ladder of a control plasmid.

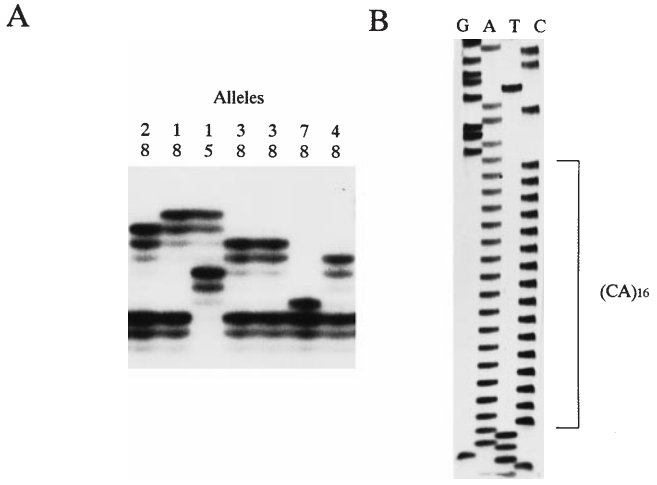

Fig. 1 **A** Autoradiogram showing a polymorphic CA repeat at the *IL6R* locus in seven unrelated individuals. **B** Nucleotide sequence of the CA repeat and the flanking regions at the *IL6R* locus

Polymorphism and allele frequency

Nine alleles were detected in 192 chromosomes of unrelated Japanese individuals. A representative autoradiogram of the CA repeat polymorphism is shown in Fig. 1A. Observed heterozygosity was 0.81. The size and frequency of the nine alleles are shown in Table 1.

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

Chromosomal localization. The human *IL6R* gene was assigned to human chromosome 1q21 (Kluck et al. 1993).

Table 1 Size and frequency of the nine alleles of the CA repeat polymorphism in the interleukin 6 receptor (IL6R) locus

Allele	Size (bp)	Frequency
A1	167	0.01
A2	165	0.10
A3	163	0.15
A4	161	0.16
A5	159	0.09
A6	157	0.04
A7	155	0.05
A8	153	0.39
A9	151	0.01

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