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BRIEF REPORT — POLYMORPHISM REPORT

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Isolation and mapping of a polymorphic CA repeat sequence at the human interleukin 6 locus

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Abstract A polymorphic dinucleotide (CA) sequence was isolated from a genomic clone containing the human interleukin 6 (interferon β-2) gene and was mapped to 7p21. This polymorphism will be useful in the genetic study of disorders affecting the inflammation process, calcium metabolism, and hematologic malignancies.

Key words Interleukin 6 · Dinucleotide repeat · Inflammation · Calcium metabolism · Interferon β-2

Introduction

Interleukin 6 (IL6) has been also called interferon β-2 (IFNB2), B-cell differentiation factor (BSF2), hepatocyte stimulatory factor (HSF), or hybridoma growth factor (HGF), according to the biological activities by which this peptide was identified by independent researchers (Zilberstein et al. 1986; Hirano et al. 1986; Sehgal et al. 1987). To understand the relationship between genetic variations at the *IL6* locus and disorders affecting the inflammation process, calcium metabolism, and hematologic malignancies, such as rheumatoid arthritis, Paget disease of bone, and multiple myeloma (Kawano et al. 1988; Roodman et al. 1992; 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 *IL6* gene was identified from a P1-derived artificial chromosome (PAC) library by polymerase chain reaction (PCR) 3-dimensional screening using primer sequences derived from the 3′ portion of the gene. A fragment containing a CA repeat was identified by Southern blotting of PAC DNA digested by *Hae*III, *Sau*3A, or *Rsa*I with a (GT) 20 probe and was 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

Forward (*IL6*, 6F) 5′ TTC TAC ATG ACA GCA GAA CAC 3′
Reverse (*IL6*, 7R) 5′ TCT GTG GGA AAG TAT ATG TGC 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 nonlabeled 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 PCR9600 System (Perkin Elmer Cetus, Norwalk, CT, 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 V for 2–4 h. Gels were transferred to filter papers, dried at 80°C, and autoradiographed. The sizes of alleles were determined by comparison with the sequencing ladder of a control plasmid.

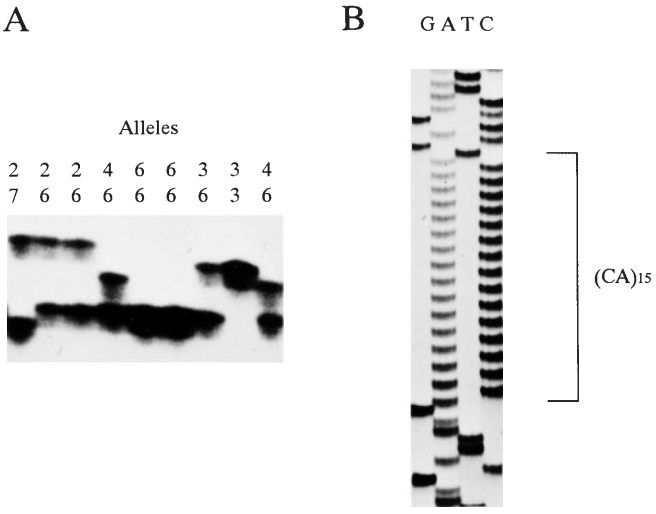

Fig. 1 **A** Autoradiogram showing a polymorphic CA repeat at the *IL6* locus in 9 unrelated individuals. **B** Nucleotide sequence of the CA repeat and the flanking regions at the *IL6* locus

Table 1 Size and frequency of the alleles of the CA repeat polymorphism

Allele	Size (bp)	Frequency
A1	134	0.05
A2	132	0.08
A3	130	0.07
A4	128	0.03
A5	126	0.62
A6	124	0.15

Polymorphism and allele frequency

Six alleles were detected in 192 chromosomes of unrelated Japanese individuals. A representative autoradiogram of the CA repeat polymorphism is shown in Fig. 1A. The observed heterozygosity was 0.57. The size and frequency of the six alleles are shown in Table 1.

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

Chromosomal localization. The human *IL6* gene was assigned to human chromosome 7p21 (Bowcock et al. 1988).

Radiation hybrid mapping. The newly isolated CA repeat at the *IL6* locus was mapped to 7p21 using 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-37360 with a logarithm of differences (LOD) score of 8.57.

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