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BRIEF REPORT — POLYMORPHISM REPORT

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A highly polymorphic CA repeat marker at the human tumor necrosis factor alpha (TNFAα) locus

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Abstract Tumor necrosis factor alpha (TNFα) in activated monocytes exerts cytotoxic activity and has a variety of other biological effects. We isolated a polymorphic dinucleotide (CA) repeat sequence from a genomic clone containing the gene located at 6p21.3. High heterozygosity (0.80) makes this polymorphism a useful marker in the genetic study of disorders affecting immunological response and cell differentiation.

Key words Tumor necrosis factor · Dinucleotide repeat rheumatoid arthritis · Obesity

Introduction

Tumor necrosis factor alpha (TNFα) selectively inhibits tumor cells, especially in combination with interferon, through the activation of JUN/AP-1 and other downstream genes. TNFα and its related peptide, TNFβ, have 30% amino acid homology with each other, have similar biologic activities, and bind to two common receptors on a variety of cells (Nedwin et al. 1985). To understand the relationship between genetic variations at the *TNF*α locus and disorders affecting the immunological system and cell differentiation, including rheumatoid arthritis and obesity (Mulcahy et al. 1996, Norman et al. 1995; Nakamura 1996; Yanase 1997), we isolated and characterized a highly informative dinucleotide repeat polymorphism (CA) at this locus.

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Source and isolation of CA repeat sequence

A human genomic clone containing the *TNF*α 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 the $(GT)_{20}$ probe, subcloned, and sequenced. An autoradiogram of the CA repeat sequence is shown in Fig. 1. PCR primers were designed to flank this new repeat sequence for polymorphism analysis.

PCR primers

The PCR priners used were:
Forward (TNFα, 3F) 5′ AGG AGC CAG GAT GGA GAC C 3′
Reverse (TNFα, 4R) 5′ CCA GCC TGG ATA ACA GAA CG 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 deoxynucleotide triphosphate (dNTP)s, 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, Foster City, USA CA) (Nakura et al. 1994). The 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. The 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.

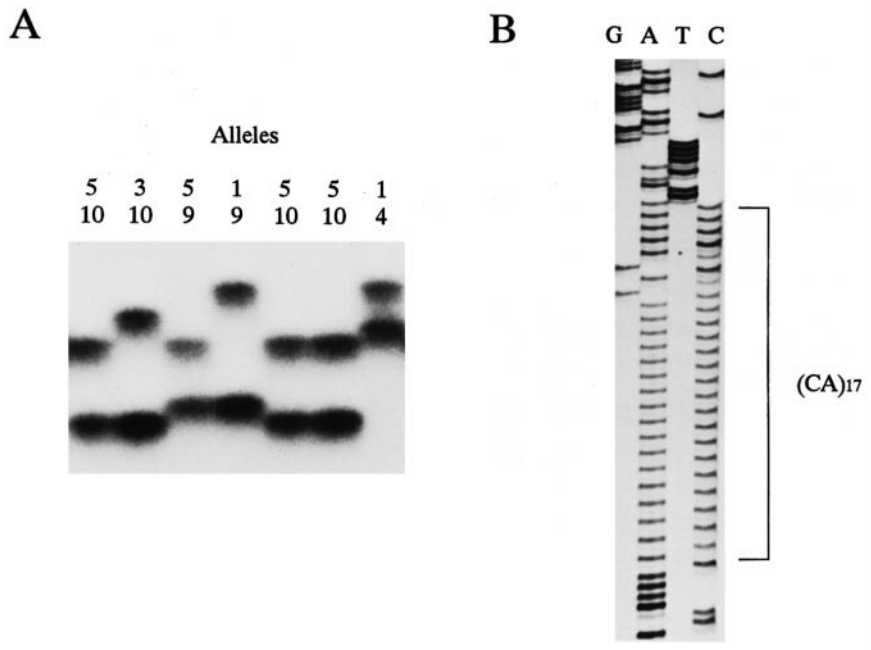

Fig. 1 **A** Autoradiogram showing a polymorphic CA repeat at the *tumor necrosis factor alpha* (*TNFα*) locus in seven unrelated individuals. **B** Nucleotide sequence of the CA repeat and flanking regions at the *TNFα* locus

Polymorphism and allele frequency

Ten alleles were detected in 192 chromosomes of unrelated Japanese individuals. (Table 1). A representative autoradiogram of the CA repeat polymorphism is shown in Fig. 1. Observed heterozygosity was 0.80.

Mendelian inheritance

Codominant inheritance was observed in two-generation families.

Chromosomal localization

The human *TNFα* gene was assigned to human chromosome 6p21.3 (Nedwin et al. 1985).

Radiation hybrid mapping

The newly isolated CA repeat at the *TNFα* locus was mapped to chromosome 6p21.3 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-7078 with a Logarithm of the ODds (LOD) score of more than 6.0.

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Table 1 Size and frequency of the alleles of the CA repeat polymorphism at the human *TNFα* locus

Allele	Size(bp)	Frequency
A1	131	0.13
A2	129	0.01
A3	127	0.07
A4	125	0.08
A5	123	0.28
A6	121	0.01
A7	119	0.01
A8	117	0.02
A9	115	0.09
A10	113	0.30

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