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BRIEF REPORT—POLYMORPHISM REPORT

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Isolation and radiation hybrid mapping of a highly polymorphic CA repeat sequence at the human nuclear factor kappa-beta subunit 1 (NFKB1) locus

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Abstract The transcriptional factor nuclear factor kappa-beta (NFKB) consists of a multicomponent protein complex that plays a major role in the regulation of many viral and cellular genes. The NFKB complex has two alternative DNA binding subunits. We isolated a polymorphic dinucleotide (CA) repeat sequence from a genomic clone containing the *NFKB subunit 1 (NFKB1)* gene located at 4q23–24. High heterozygosity (0.813) makes this polymorphism a useful marker in the genetic study of disorders affecting the immune response and cell differentiation.

Key words nuclear factor kappa-beta subunit 1 · dinucleotide repeat · immune response · cell differentiation

Introduction

The transcriptional factor nuclear factor kappa-beta (NFKB) consists of a multicomponent protein complex that plays a major role in the regulation of many viral and cellular genes. The NFKB complex has two alternative DNA binding subunits, p105 and p49/p100. The generation of nfkb1 null mice resulted in altered immune responses, but had no effect on development (Sha et al. 1995). Similarly, nfkb2 knockout mice did not show a developmental defect. However, mice lacking nfkb1 and nfkb2 exhibited osteopetrosis (Iotsova et al. 1997).To understand the relationship between genetic variations at the *NFKB1* locus and disorders affecting the immunological system and cell differentiation (Nakamura, 1996; Yanase 1997), we isolated and characterized a highly informative dinucleotide repeat polymorphism at this locus.

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Source/Isolation of CA repeat sequence

A human genomic clone containing the *NFKB1* 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 (Tsukamoto et al. 1998). The nucleotide sequence of the CA repeat is shown in Fig. 1A. PCR primers were designed to flank this new repeat sequence for polymorphism analysis.

PCR primers

The PCR primers used were: forward (NFKB1,1F) 5′ CTTCAGTATCTAAGAGTATCCT 3′, and reverse (NFKB1,1R) 5′ CAAGTAAGACTCTACGGAGTC 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% 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 PCR9600 System (Perkin Elmer Cetus, Foster, CA, USA) (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 volts 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 (Watanabe et al. 1998).

Polymorphism and allele frequency. Thirteen alleles were detected in 192 chromosomes of unrelated Japanese indi-

A

ACAGACAATC CAGTCTTCTT CAGTATCTAA GAGTATCCTC
ATTTTATATG TA**CACACACA CACACACACA CACACACACA**
CACACACACA CACACACACA GCCTTATCGA GGGGATAAGG
GGACTTAGGG CTGGGACTCC GTAGAGTCTT ACTTGGTAAA

B

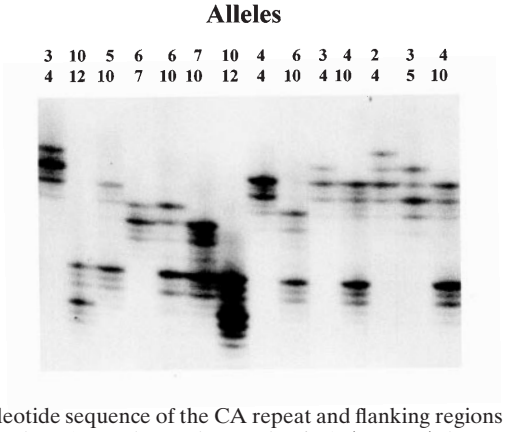

Fig. 1 **A** Nucleotide sequence of the CA repeat and flanking regions at the human nuclear factor kappa-beta subunit 1 (*NFKB1*) locus. **B** Autoradiogram showing a polymorphic CA repeat at the *NFKB1* locus in 12 unrelated individuals

viduals. A representative autoradiogram of the CA repeat polymorphism is shown in Fig. 1B. The observed heterozygosity was 0.813.

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

Chromosomal localization. The human *NFKB1* gene was assigned to human chromosome 4q23–q24 (Mathew et al. 1993).

Radiation hybrid mapping. The newly isolated CA repeat at the *NFKB1* locus was mapped to clomosome 4q, 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 WI-4262 with a logarithm of differences (LOD) score of 6.30.

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