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BRIEF REPORT — POLYMORPHISM REPORT

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Isolation and radiation hybrid mapping of dinucleotide repeat polymorphism at the human matrix Gla protein *(MGP)* locus

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Abstract Matrix Gla protein (MGP) is an 84-residue, vitamin K-dependent protein expressed by chondrocytes and vascular smooth muscle cells, and is a potent regulator of calcium deposition in cartilage and arterial wall. We isolated a polymorphic dinucleotide CA repeat marker from a genomic clone containing the human *MGP* gene. This polymorphism will be useful in genetic studies of arteriosclerosis and osteoporosis.

Key words Matrix Gla protein · Polymorphism dinucleotide repeat · Arteriosclerosis · Osteoporosis

Introduction

Matrix Gla protein (MGP), a vitamin K-dependent protein expressed in bone, cartilage, heart, kidney, and lung (Cancela et al. 1990), controls calcium deposition in cartilage, arterial wall, and other soft tissues, and is a potent inhibitor of inappropriate calcification of arteries and cartilage (Luo et al. 1997). To understand the relationship between genetic variations at the *MGP* locus and the genetic backgrounds of arteriosclerosis and osteoporosis (Luo et al. 1997; 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 *MGP* 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 a CA repeat was identified by Southern blotting of a PAC library DNA digested by *Hae*III, *Sau*3A, or *Rsa*I with the $(GT)_{20}$ probe; subcloned; and sequenced. An autoradiogram of the CA repeat is shown in Fig. 1. PCR primers were designed to flank this new repeat sequence for polymorphism analysis.

PCR primers

Forward (MGP 2F) 5′ -GAA CTA GTG GAT CAC TAG GC- 3′
Reverse (MGP 2R) 5′ -CTT ACC AGA GTA TGA ATG CAC- 3′

PCR conditions

PCR was performed in a volume of 10 μl containing 20 ng genomic DNA, 10 mM TrisHCl (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 an unlabeled 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, 62°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 a sequencing ladder of a control plasmid.

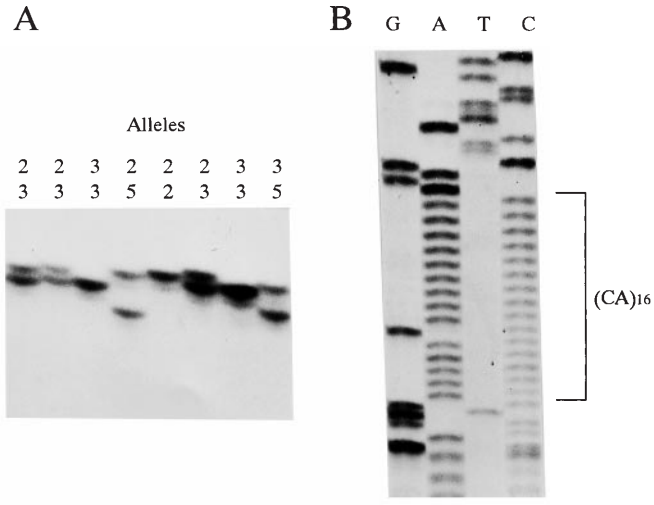

Fig. 1 **A** Autoradiogram showing a polymorphic CA repeat at the *MGP* locus in 8 unrelated individuals. **B** Nucleotide sequence of the CA repeat at the *MGP* locus and the flanking regions

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. 1. The observed heterozygosity was 0.59. 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 *MGP* gene was assigned to human chromosome 12p using a Chinese hamster/human hybrid cell panel (Cancela et al. 1990).

Radiation hybrid mapping. Radiation hybrid (RH) mapping of the newly isolated CA repeat was performed using the G3 RH mapping panel of 83 hybrid cell lines constructed by the Stanford Human Genome Center (SHGC) (Boehnke et al. 1991). The hybrid cell lines were typed by PCR for the presence of the new CA repeat. Two-point analysis of the RH data against SHGC RH framework markers indicated that the new CA repeat is linked to SHGC-9278 on 12p with a logarithm of differences (LOD) score of 6.23. This result is consistent with the previous mapping data on the *MGP* gene by Cancela et al. (1990).

Table 1 Size and frequency of the alleles of the CA repeat polymorphism at the human matrix Gla protein (*MGP*) locus

Allele	Size (bp)	Frequency
A1	214	0.01
A2	212	0.31
A3	210	0.54
A4	208	0.01
A5	206	0.11
A6	204	0.02

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