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Association of bone mineral density with polymorphism of the human matrix Gla protein locus in elderly women

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Abstract: The contribution of genetic factors has been implicated in the determination of bone mass in twin and family studies. Some of the genes involved would regulate bone metabolism, bone formation, and resorption, all processes that determine bone mass. One candidate gene, matrix Gla protein gene (MGP), has been implicated in the pathogenesis of bone loss through a repression of bone formation. To analyze the genetic background for osteoporosis in elderly women, we have investigated a possible association between the CA repeat polymorphism at the human MGP gene locus and bone mineral density (BMD) of radial bone in 460 elderly Japanese women. Genotypes were classified into six groups according to the number of CA repeats present, from 13 to 18 (alleles A1 through A6). BMD was expressed as the adjusted BMD (ADJBMD), which was the body mass index (BMI)- and age-adjusted average BMD. The 214 women who lacked an A2 allele (212 bp, containing 17 repeats of CA) had significantly lower adjusted BMD than the participants ($n = 246$) who possessed an allele of that size (mean ± SD; 0.303 ± 0.062 *vs* 0.315 ± 0.062 g/cm^2; $P = 0.0382$). This result suggests that genetic variation at the MGP locus is associated with some determinants for BMD in elderly women. Therefore, this locus should serve as one of the genetic markers for osteoporosis.

Key words: matrix Gla protein gene, bone mineral density, osteoporosis, microsatellite polymorphism, risk factors

Introduction

Osteoporosis is characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture. The most important predictor of fracture is bone mineral density (BMD), a measurement that reflects many genetic and lifestyle factors; its predictive value is well supported by results of twin [1–4] and family studies [5–8]. Knowing the genetic risk factors for an individual would assist diagnosis, prevention, and therapy of osteoporosis. Some genetic effects have been ascribed to polymorphisms of genes involved in bone metabolism.

Many endocrinological factors are known to play roles in bone maturation and in the process of bone loss that accompanies aging [9]. Because it is possible that the pathophysiology or key genetic background of each osteoporotic patient is heterogeneous, a rational approach to an understanding of the genetic background of osteoporosis would require expanding the panel of genes examined. One obvious candidate for such a study is matrix Gla protein gene (MGP), which is a 84-residue vitamin K-dependent protein expressed by chondrocyte and vascular smooth muscle cells in bone, cartilage, heart, kidney, and lung. It consists of a transmembrane signal peptide, a γ-carboxylase recognition site, and a Gla-containing domain. The human MGP gene spans 3.9 kb, consists of regulating exons, and is upregulated by vitamin D and retinoic acid [10]. It controls calcium deposition in cartilage, arterial walls, and other soft tissues, and is a potent inhibitor of inappropriate calcification of arteries and cartilage [11]. However, whether genetic variations of the MGP gene are correlated directly with bone metabolism is not clear, partly because no useful genetic markers for this gene have been recognized so far.

Dinucleotide-repeat polymorphic markers, known as microsatellites, are often invoked for analysis of multifactorial diseases [12–14]. Microsatellite polymorphisms can serve as markers for family-based studies, including sib-pair analysis, as well as for population-based association studies. To understand the relationship between genetic variation at the human MGP gene locus and osteoporosis, we determined the genotype in each elderly woman and analyzed the relationship

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between the genotype and radial mineral bone density (MBD).

Material and methods

Subjects

DNA samples were obtained from the peripheral blood of 460 elderly Japanese women living in the Akita area; their ages ranged from 66 to 92 (mean, 73.2 ± 5.8) years. All were nonrelated volunteers and gave their informed consent before the study. No participant had medical complications or was undergoing treatment for conditions known to affect bone metabolism, such as pituitary disease, hyperthyroidism, primary hyperparathyroidism, renal failure, adrenal disease, or collagen disease, and none was receiving estrogen replacement therapy.

Measurement of bone mineral density (BMD)

We measured BMD at the distal radius consisting primarily of cortical bone, following 1995 Guideline for Osteoporosis Screening in a Health Check-up Program for Elderly conducted by the Ministry of Health and Welfare of Japan. This method was recommended for BMD measurement in elderly people who often have accompanying orthroarthritis of the spine. The BMD of radial bone (expressed in g/cm^2) of each participant was measured by dual-energy X-ray absorptiometry (DPX-L; Lunar, Madison, WI, USA). This parameter was recorded as adjusted BMD to correct for differences in age, height, and weight. The formulas were as follows: Body mass index (BMI) = (body weight) (kg) / (body height)^2 (m); adjusted BMD = BMD − 0.0052432908 × (73.1716102 − age) + 0.0088382998 × (23.2271299 − BMI). The formulation of adjusted BMD calculation was based on a previously published method [15]. The formula has been successfully applied to the Japanese population previously [16].

Determination of microsatellite polymorphism by polymerase chain reaction (PCR)

Each PCR amplification of the CA repeat polymorphism at the MGP locus was performed in a volume of 10 μl containing 20 ng of genomic DNA obtained from peripheral blood, 10 mM Tris HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 200 μM dNTP, 2.5 pmol [^{32}P]end-labeled primer MGP.2F and a nonlabeled primer MGP.2R, a microsatellite marker we described previously [16], 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). PCR products were electrophoresed for 2 h at 2000 V in 0.3-mm-thick denaturing 6% polyacrylamide gels containing 36% formamide and 8 M urea. Gels were transferred to filter papers, dried at −80°C, and autoradiographed. The size of each allele was determined by comparison with a sequencing ladder of control DNAs [17].

Statistical analysis

Adjusted BMD values between individuals who possessed one allele of a given genotype and individuals who did not were compared using a nonparametrical (Student-Newman-Keuls) analysis. Differences in means were considered statistically significant for $P < 0.05$.

Results

The 460 elderly Japanese women in our panel were genotyped for the CA repeat polymorphism at the MGP locus. The polymorphic PCR products had been classified into six alleles: A1 (214 bp, 18 CA repeats) to A6 (204 bp, 13 CA repeats). The distribution of the six alleles in this population is shown in Fig. 1. The distribution among women living in the rural Akita area was similar to that observed previously in the general Japanese population [17].

We sought to correlate genotype(s) at the MGP microsatellite locus with adjusted BMD of radial bone. The 214 women who lacked an A2 allele (212 bp, containing 17 repeats of CA) had significantly lower adjusted BMD than the participants (n = 246) who possessed an allele of that size (mean ± SD; 0.303 ± 0.062 *vs* 0.315 ± 0.062 g/cm^2; P = 0.0382) (Table 1). Figure 2 shows the effect of presence or absence of A2 allele at the MGP locus in adjusted BMD.

Background data of the groups with and without the A2 allele are summarized in Table 1. We found no significant differences between the two groups with respect to mean age, height, or weight. Of the 246 women possessing the A2 allele, 44 were homozygous at this locus. However, no significant differences in adjusted BMD were detected between women who were homozygous for the A2 allele and those who were heterozygous (data not shown).

Discussion

Matrix Gla protein (MGP) is a calcium-binding secreted protein isolated from bone matrix and is synthe-

Table 1. Comparison of age, height, and body weight between the groups with or without a A2 allele at the MGP locus

	A2 allele (+)	*n*	A2 allele (−)	*n*	Statistical significance
Age (years)	73.4 ± 5.7	246	72.8 ± 5.8	214	N.S.
Body height (cm)	144.6 ± 6.3	246	145.2 ± 5.6	214	N.S
Body weight (kg)	49.2 ± 8.3	246	49.9 ± 8.5	214	N.S.
ADJBMD (g/cm^2)	0.315 ± 0.062	246	0.303 ± 0.062	214	$P = 0.0382$

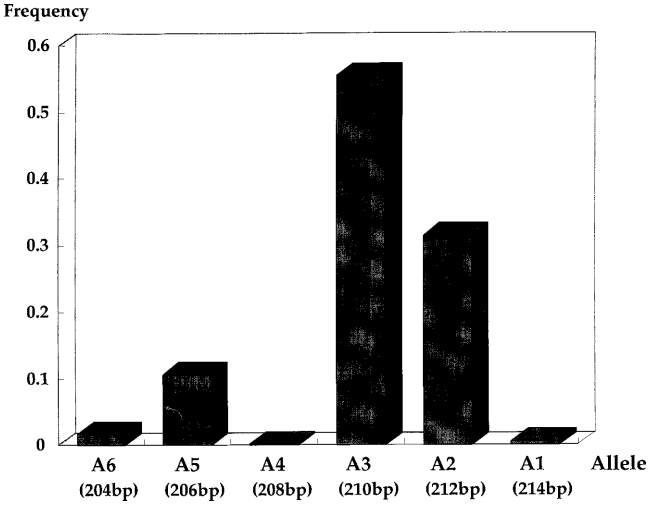

Fig. 1. Frequency distribution of alleles containing the CA repeat polymorphism at the matrix Gla protein (MGP) locus among 460 elderly Japanese women

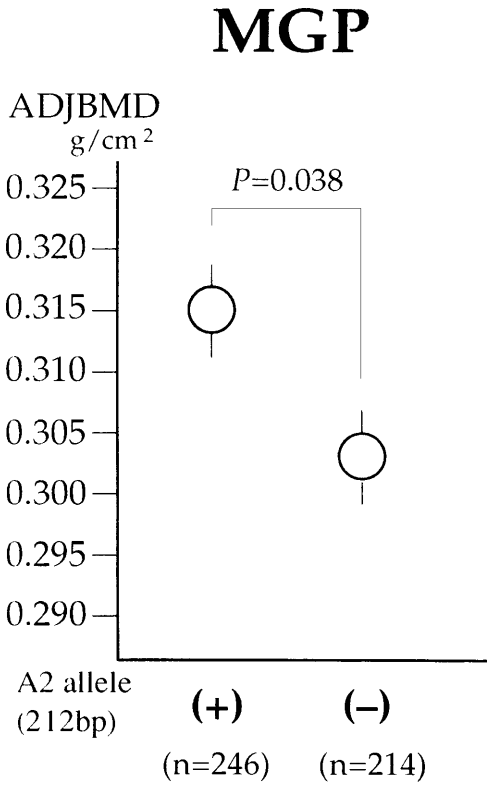

Fig. 2. Comparison of mean adjusted bone mineral density (BMD) of radial bone between the group of individuals that carried an A2 allele and the group that did not

sized in a wide variety of human tissues as well as bone [18,19]. Its function is unknown but it is suggested that it inhibit bone formation [19]. Moreover, MGP gene expression is regulated by retinoic acid receptor-beta on skin, bone, and cartilage [20]. The retinoic acid-responsive element in the retinoic acid receptor b gene is detected in the human MGP promoter [21]. This receptor gene is a co-transcription factor for vitamin D receptor and steroid hormone receptor. Thus, MGP would be implicated in the pathogenesis of bone loss through repression of bone formation and regulation by the retinoic acid receptor.

For the work reported here, we genotyped a large panel of elderly Japanese women at a newly isolated microsatellite at the MGP locus. Presence or absence of one genotype, A2 (212 bp, 17 CA repeats), affected the levels of BMD in these women. The data presented here might suggest that variation or some mutation in or adjacent to the MGP gene may affect bone metabolism and eventually cause variation in BMD. The elderly Japanese women in our panel who lacked an A2 allele at the human MGP locus showed lower adjusted BMD than those who carried the alleles of this size. Lowered BMD in elderly women could be a result of abnormally rapid bone loss and/or lower peak bone mass that had occurred when they were young adults. To clarify these issues it is necessary to carry out a further genetic study or to investigate the genomic structure of the entire region containing the MGP gene and its control elements in affected individuals.

Investigation of genetic variations in humans have been attempted at the vitamin D receptor locus [22], estrogen receptor-alpha loci [23,24], interleukin-6 locus [25], or the collagen type I alpha-1 locus [26] as to their role in bone metabolism. We measured BMD at the radius following the 1995 Guideline for Osteoporosis

Screening in a Health Check-up Program for Elderly conducted by the Ministry of Health and Welfare of Japan. This method was recommended for BMD measurement in elderly people who often have accompaning orthroarthritis of the spine. The present study showed a novel association between radial BMD and a microsatellite polymorphism that lies within 110 kb of the MGP gene. It would be important to confirm the association between the polymorphism and BMD measured at other anatomical sites including spine and femur in the future.

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