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PRELIMINARY REPORT

Association of a Polymorphism of the Matrix Metalloproteinase–9 Gene With Bone Mineral Density in Japanese Men

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Matrix metalloproteinase–9 (MMP-9) is implicated in bone remodeling. A −1562C→T polymorphism in the promoter of the MMP-9 gene (*MMP9*) has been shown to influence gene transcription. The possible relation of this polymorphism to bone mineral density (BMD) was examined in 1,114 Japanese men and 1,087 women. BMD for the total body, lumbar spine, femoral neck, trochanter, or Ward's triangle was significantly lower in the combined group of men with the *CT* or *TT* genotypes or in men with the *CT* genotype than in those with the *CC* genotype. No significant differences in BMD among *MMP9* genotypes were observed in premenopausal or postmenopausal women. The −1562C→T polymorphism of *MMP9* was thus associated with BMD in Japanese men.

MATRIX metalloproteinase–9 (MMP-9) is produced by osteoclasts in human bone and is implicated both in bone resorption,[1-3] as well as in bone formation.[4] A C→T polymorphism at position −1562 in the promoter of the MMP-9 gene (*MMP9*) has been shown to affect transcriptional activity, with the T allele being associated with increased gene transcription.[5] We have now examined whether this polymorphism is associated with bone mineral density (BMD) in a population-based study.

MATERIALS AND METHODS

The National Institute for Longevity Sciences–Longitudinal Study of Aging is a population-based prospective cohort study of aging and age-related diseases.[6] We examined the possible association of BMD at various sites with the −1562C→T polymorphism of *MMP9* in 1,114 Japanese men and 1087 women. The study protocol was approved by the Committee on the Ethics of Human Research of the National Institute for Longevity Sciences, and written informed consent was obtained from each subject. BMD for the total body, lumbar spine (L2 to L4), right femoral neck, right trochanter, and right Ward's triangle was measured by dual-energy x-ray absorptiometry.

Genotypes were determined with a fluorescence-based allele-specific DNA primer assay system. The polymorphic region of *MMP9* was amplified by the polymerase chain reaction with allele-specific sense primers labeled at the 5′ end with either fluorescein isothiocyanate (5′-CCGAGTAGCTGGTATTATAGGXAT-3′) or Texas red (5′-CGAGTAGCTGGTATTATAGGXGT-3′) and with an antisense primer labeled at the 5′ end with biotin (5′-AAACCAGCCTGGT-CAACGTA-3′). The reaction mixtures (25 μL) contained 20 ng of DNA, 5 pmol of each primer, 0.2 mmol/L of each deoxynucleoside triphosphate, 4.5 mmol/L $MgCl_2$, and 1 U of Taq DNA polymerase in buffer. The amplification protocol comprised initial denaturation at 95°C for 5 minutes, 35 cycles of denaturation at 95°C for 30 seconds, annealing at 66.5°C for 30 seconds, extension at 68°C for 30 seconds, and a final extension at 68°C for 2 minutes. Amplified DNA was incubated in a solution containing streptavidin-conjugated magnetic beads in the wells of a 96-well plate at room temperature. The plate was placed on a magnetic stand, and the supernatants from each well were transferred to the wells of a 96-well plate containing 0.01 mol/L NaOH and then measured for fluorescence with a microplate reader.

Quantitative data were compared among 3 groups by 1-way analysis of variance and the Tukey-Kramer post hoc test, and between 2 groups by the unpaired Student's *t* test. BMD values were analyzed with

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Table 1. BMD and Other Characteristics of Men (n = 1,114) or of Premenopausal (n = 279) or Postmenopausal (n = 808) Women According to the −1562C→T Genotype of *MMP9*

Characteristic	*CC*	*CT*	*TT*	*CT* + *TT*
Men				
No. (%)	794 (71.3)	280 (25.1)	40 (3.6)	320 (28.7)
Age (yr)	59.0 ± 0.4	59.9 ± 0.7	58.7 ± 1.7	59.7 ± 0.6
BMI (kg/m^2)	22.9 ± 0.1	22.8 ± 0.2	23.1 ± 0.4	22.9 ± 0.2
Fracture (%)	201 (25.3)	76 (27.1)	11 (27.5)	87 (27.2)
BMD values (g/cm^2)				
Total body	1.090 ± 0.003	1.076 ± 0.006	1.081 ± 0.015	1.077 ± 0.005*
L2-L4	0.988 ± 0.006	0.965 ± 0.010	0.981 ± 0.026	0.967 ± 0.009*
Femoral neck	0.758 ± 0.004	0.739 ± 0.006*	0.736 ± 0.017	0.739 ± 0.006†
Trochanter	0.673 ± 0.004	0.655 ± 0.006*	0.659 ± 0.017	0.655 ± 0.006*
Ward's triangle	0.559 ± 0.004	0.534 ± 0.007*	0.532 ± 0.020	0.534 ± 0.007‡
Premenopausal women				
No. (%)	200 (71.7)	70 (25.1)	9 (3.2)	79 (28.3)
Age (yr)	46.2 ± 0.3	45.6 ± 0.5	49.9 ± 1.5§	46.1 ± 0.5
BMI (kg/m^2)	22.8 ± 0.2	22.8 ± 0.4	22.7 ± 1.1	22.8 ± 0.4
Fracture (%)	23 (11.5)	8 (11.4)	3 (33.3)	11 (13.9)
BMD values (g/cm^2)				
Total body	1.091 ± 0.006	1.102 ± 0.010	1.088 ± 0.028	1.100 ± 0.009
L2-L4	1.019 ± 0.009	1.035 ± 0.014	1.031 ± 0.041	1.035 ± 0.014
Femoral neck	0.770 ± 0.007	0.775 ± 0.016	0.793 ± 0.033	0.777 ± 0.011
Trochanter	0.654 ± 0.006	0.664 ± 0.011	0.689 ± 0.030	0.667 ± 0.010
Ward's triangle	0.659 ± 0.009	0.654 ± 0.015	0.693 ± 0.042	0.658 ± 0.014
Postmenopausal women				
No. (%)	563 (69.7)	214 (26.5)	31 (3.8)	245 (30.3)
Age (yr)	63.9 ± 0.4	64.1 ± 0.6	65.0 ± 1.5	64.2 ± 0.5
BMI (kg/m^2)	23.0 ± 0.1	22.8 ± 0.2	23.3 ± 0.6	22.9 ± 0.2
Fracture (%)	114 (20.2)	45 (21.0)	8 (25.8)	53 (21.6)
BMD values (g/cm^2)				
Total body	0.920 ± 0.004	0.915 ± 0.006	0.914 ± 0.016	0.915 ± 0.006
L2-L4	0.808 ± 0.006	0.806 ± 0.009	0.841 ± 0.025	0.810 ± 0.009
Femoral neck	0.645 ± 0.004	0.642 ± 0.006	0.637 ± 0.016	0.641 ± 0.006
Trochanter	0.540 ± 0.004	0.538 ± 0.006	0.533 ± 0.016	0.537 ± 0.006
Ward's triangle	0.452 ± 0.005	0.451 ± 0.008	0.461 ± 0.022	0.452 ± 0.008

NOTE. Data are means ± SE. BMD values are adjusted for age.
*$P < .05$, †$P < .01$, ‡$P < .005$ *v CC.*
§$P < .05$ *v CC* or *CT.*

adjustment for age by the least squares method in a general linear model. A P value $< .05$ was considered statistically significant.

RESULTS

Age, body mass index (BMI), and the prevalence of nontraumatic fractures did not differ among −1562C→T genotypes in men or in premenopausal or postmenopausal women (Table 1). We compared BMD values among the 3 genotypes (*CC, CT,* and *TT*), as well as between 2 groups of genotypes in dominant (*CC* and *CT* + *TT*) and recessive (*CC* + *CT* and *TT*) genetic models to examine the effect of the *T* allele on BMD. BMD for the total body, lumbar spine, femoral neck, trochanter, or Ward's triangle was significantly lower in the combined group of men with the *CT* or *TT* genotypes or in men with the *CT* genotype than in those with the *CC* genotype (Table 1). The differences in BMD between men with the *CC* genotype and those with either the *CT* or *TT* genotypes (expressed as a percentage of the corresponding larger value) were 1.5% for the total body, 2.2% for the lumbar spine, 2.8% for the femoral neck, 2.7% for the trochanter, and 5.2% for Ward's triangle. For premenopausal or postmenopausal women, BMD did not differ among −1562C→T genotypes (Table 1).

DISCUSSION

We previously showed that the −1607G→GG polymorphism of *MMP1* was associated with BMD at the radius in postmenopausal women,[6] with the *GG* genotype, which exhibits an increased transcriptional activity,[7] representing a risk factor for reduced BMD. The T allele of the −1562C→T polymorphism in the promoter of *MMP9* also exhibits higher transcriptional activity than does the *C* allele.[5] A 9-bp sequence (−1567 to −1559) containing the −1562C→T site has been suggested to function as an important regulatory element by serving as a binding site for a transcriptional repressor protein. In addition, the serum concentration of MMP-9 was shown to

be higher in individuals with the *TT* genotype than in those with the *CC* or *CT* genotypes.[5] We have now shown that the −1562C→T polymorphism of *MMP9* was associated with BMD at various sites in Japanese men, with the *T* allele being related to reduced bone mass. Given that MMP-9 degrades collagen in the bone matrix, an increased activity of this protease might be expected to result in reduced bone mass. Our results are thus consistent with the previous observations that the *T* allele of *MMP9* exhibits higher transcriptional activity and is associated with a higher serum concentration of the encoded protein.[5]

Given that BMD values for the total body, lumbar spine, femoral neck, trochanter, and Ward's triangle in men with the *TT* genotype were similar to those in men with the *CT* genotype, the *T* allele may exert a dominant effect on BMD. The lack of statistical significance for differences in BMD between the *CC* and *TT* genotypes may be attributable to the small number of subjects with the *TT* genotype (n = 40), compared with the number of those with the *CT* genotype (n = 280). This polymorphism was associated with BMD in men but not in women. The reason for this gender difference remains unclear, but differences in the concentrations of estrogen and other sex hormones between men and women might be contributing factors. Although it is possible that the −1562C→T polymorphism of *MMP9* is in linkage disequilibrium with polymorphisms of other nearby genes that are actually responsible for reduced BMD, our present results suggest that this polymorphism of *MMP9* is associated with BMD in Japanese men.

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