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Effects of the interaction between lean tissue mass and estrogen receptor α gene polymorphism on bone mineral density in middle-aged and elderly Japanese

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Abstract

Because both genetic and environmental factors influence bone mass, it is important to examine the effect of gene-environment interactions on bone mineral density (BMD) for the prevention of osteoporosis at an individual level. Estrogen receptor α (ERα) plays an important role in increasing BMD via mechanical strain and muscle mass is a reflection of the forces the muscle applies to the bone. The aim of this study is to investigate the effect of the interaction between lean tissue mass (LTM) and the ERα polymorphisms T→C (*PvuII*) [dbSNP: rs2234693] and A→G (*XbaI*) [dbSNP: rs9340799] on BMD in middle-aged and elderly individuals. Subjects were 2209 community-dwelling Japanese men and women, ages 40 to 79 years. ERα polymorphisms in the first intron, T→C and A→G were identified and lumbar spine and femoral neck BMD and LTM were measured by dual-energy X-ray absorptiometry. Both T→C and A→G polymorphisms were divided into two genotype groups (TT vs. TC/CC; AA vs. AG/GG). In postmenopausal women, the effect of LTM on femoral neck BMD was significantly larger for those with the TC/CC genotype than for those with the TT genotype for the T→C polymorphism, and larger for those with the AG/GG genotype than for those with the AA genotype for the A→G polymorphism. This gene–LTM interaction was observed at the femoral neck, but not at the lumbar spine. For men and premenopausal women, no gene–LTM interaction was found. In conclusion, there was an interaction between LTM and the ERα T→C and A→G polymorphisms with respect to their effect on femoral neck BMD in postmenopausal women and those with the TC/CC and AG/GG genotypes had larger effects of LTM than those with TT and AA genotypes.
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Keywords: Single nucleotide polymorphism; Estrogen receptor alpha; BMD; Lean tissue mass; Postmenopausal women

Introduction

It is generally accepted that dynamic loading acts as an osteogenic stimulus [1] and that the forces applied to bone are primarily the result of muscular contraction [2]. Therefore, muscular weakness is an important factor contributing to osteoporosis [3]. The importance of skeletal muscle in preserving bone [4] and the relation between low skeletal mass and poor structural parameters of bone in elderly men [5] have been reported. A previous study suggested that physical exercise maintains bone mineral density (BMD) in postmenopausal women [6]. Vainionpaa et al. showed that the intensity of exercise was significantly correlated with BMD changes [7] and Kerr et al. reported that postmenopausal bone mass can be significantly increased by strength training, but not by endurance training [8].

Animal studies have suggested that mechanical strain stimulates osteoblast proliferation through estrogen receptor α (ERα) [9], and osteoblast-like cells from ERα knockout mice have deficient responses to mechanical strain [10]. Thus, it is thought that ERα plays an important role in increasing BMD via mechanical strain [11,12]. Although the association between ERα genotype and the risk of osteoporosis in humans remains controversial [13], many studies have suggested a

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relation between ERα polymorphism and BMD [14–16]. A study previously carried out in our laboratory also showed that the ERα gene was a susceptibility locus for reduced bone mass, especially at the femoral neck, in elderly Japanese women [17].

Because the effects of environment on individuals might differ in accordance with individuals' different genetic make-ups, it is important to examine the effects of the gene-environment interaction on BMD, particularly for the prevention of osteoporosis at an individual level. Some studies have investigated the effect of ERα polymorphism on the relationship between exercise and BMD. These studies have shown an effect of the ERα gene (*PvuII*)–exercise interaction on BMD in middle-aged men [18] and prepubertal and early pubertal girls [19].

Because magnetic resonance imaging (MRI)-measured muscle area correlates with muscle strength [20], and the differences between MRI-measured and dual-energy X-ray absorptiometry (DXA)-predicted skeletal muscle mass are small [21], DXA-predicted total body lean mass can be legitimately used as an index of skeletal load. As mentioned above, a few studies have investigated the effects of the ERα gene–exercise interaction on BMD. However, the effects of the ERα gene–lean tissue mass (LTM) interaction were unknown. Furthermore, these previous studies involved single-sex populations within a limited age range. This study investigated for the first time the effects of the interaction between LTM and the typical ERα polymorphisms T→C (*PvuII*) and A→G (*XbaI*) on BMD in both men and women in a large population.

Materials and methods

Subjects

Study subjects were 1119 men and 1090 women, ages 40–79 years, who participated in the first wave (from April 1998 to March 2000) of the National Institute for Longevity Sciences-Longitudinal Study of Aging (NILS-LSA), which is a population-based prospective cohort study of aging and age-related diseases. Participants in the NILS-LSA were randomly selected age and sex stratified individuals selected from the pool of independent residents in the NILS neighborhood, Obu city and Higashiura town, Aichi Prefecture, central Japan. Details of the NILS-LSA have been given elsewhere [22]. The study protocol was approved by the Committee of Ethics of Human Research of the National Center for Geriatrics and Gerontology. Written informed consent was obtained from all subjects.

Anthropometric variables

Body weight was measured to the nearest 0.01 kg using digital scales, height was measured to the nearest 0.1 cm using a stadiometer, and body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2).

Menstrual status

Menopause was confirmed as the absence of menses by a questionnaire.

Dual-energy X-ray absorptiometry

Whole-body fat mass, LTM, bone mineral content (BMC), and BMD of the femoral neck and lumbar spine (L2–4) were assessed by DXA (QDR-4500; Hologic, Madison, OH, USA). Lean tissue mass is equal to the fat-free mass minus BMC, and is assumed to be an index of the amount of muscle mass.

ERα genotype analysis

DNA was extracted from peripheral blood lymphocytes by using a standard procedure. ERα genotypes were determined in accordance with a study by Yamada et al. [17] The ERα genotypes were analyzed by using an automated fluorescent allele-specific DNA primer assay system (Toyobo Gene Analysis, Tsuruga, Japan). To determine the T→C (*PvuII*) genotype, the polymorphic region of the gene was amplified by polymerase chain reaction (PCR) using allele-specific sense primers labeled at the 5′ end either with fluorescein isothiocyanate (5′-AGTTCCAAATGTCCCAGXTG-3′) or with Texas red (5′-AGTTCCAAATGTCCCAGXCG-3′) and an antisense primer labeled at the 5′ end with biotin (5′-TCTGGGAAACAGAGACAAAGC-3′). The reaction mixture (25 μl) contained 20 ng DNA, 5 pmol of each primer, 0.2 mmol/l of each deoxynucleoside triphosphate, 2.5 mmol/l $MgCl_2$, and 1U DNA polymerase (rTaq; Toyobo, Osaka, Japan) in rTaq buffer. The amplification protocol consisted of initial denaturation at 95 °C for 5 min; 35 cycles of denaturation at 95 °C for 30 s, annealing at 62.5 °C for 30 s, and extension at 72 °C for 30 s; and a final extension at 72 °C for 2 min. For determination of the A→G (*XbaI*) genotype, the polymorphic region of the gene was amplified by PCR using a sense primer labeled at the 5′ end with biotin (5′-CTGTTTCCCA-GAGACCCTGAG-3′) and allele-specific antisense primers labeled at the 5′ ends either with fluorescein isothiocyanate (5′-CCAATGCTCAT-CCCAACTXTA-3′) or with Texas red (5′-CCAATGCTCATCCCAACTXCA-3′). The reaction mixture (with the exception of the primers) and the amplification protocol (with the exception that the annealing temperature was 65 °C) were identical to those used for genotyping of the T→C polymorphism.

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 supernatant was discarded. After two washings, 0.01 M NaOH was added to the wells and mixed well. The plate was placed on a magnetic stand again and the supernatants were transferred to the wells of a new 96-well plate. The fluorescence was measured by using a microplate reader (Fluoroscan Ascent; Dainippon Pharmaceutical, Osaka, Japan) at excitation and emission wavelengths of 485 nm and 538 nm, respectively, for fluorescein isothiocyanate, and 584 nm and 612 nm, respectively, for Texas red.

Haplotype analysis

The haplotype distribution was calculated by using Haplotyper, a software program for haplotype inference, with the Bayesian algorithm [23,24].

Statistical analysis

Values are expressed as the mean±standard error (SE). The chi-squared test was used to identify significant departures from Hardy-Weinberg equilibrium. Both T→C and A→G polymorphisms were divided into two genotype groups (TT vs. TC/CC; AA vs. AG/GG). The differences between genotype groups were analyzed using one-way analysis of variance and the Tukey–Kramer post hoc test. A general linear model was employed to evaluate the effect of the LTM–genotype interaction on BMD (adjusted for age and BMI). When the effect of the interaction on BMD was significant for both T→C and A→G polymorphisms, further analysis (in accordance with haplotype groups) was

Table 1
Distribution of T→C and A→G genotypes of the ERα gene

	AA		AG		GG		Total	
	n	%	*n*	%	*n*	%	*n*	%
TT	787	35.6	1	0.1	0	0.0	788	35.7
TC	584	26.4	465	21.1	5	0.2	1054	47.7
CC	120	5.4	174	7.9	73	3.3	367	16.6
Total	1491	67.5	640	29.0	78	3.5	2209	100.0

Table 2
Physical characteristics of subjects with reference to the T→C and A→G genotypes of the ERα gene

	Men (n=1119)		Premenopausal women (n=278)		Postmenopausal women (n=812)	
	TT (n=398)	TC/CC (n=721)	TT (n=98)	TC/CC (n=180)	TT (n=292)	TC/CC (n=520)
Age (years)	58.9±0.6	59.3±0.4	46.2±0.5	46.2±0.3	62.8±0.5	64.6±0.4*
Weight (kg)	62.9±0.5	62.2±0.3	53.9±0.8	54.7±0.6	52.5±0.5	51.7±0.4
BMI (kg/m2)	23.2±0.1	22.9±0.1	22.5±0.3	22.9±0.2	23.1±0.2	23.0±0.2
LTM (kg)	47.2±0.3	46.6±0.2	36.3±0.4	36.5±0.3	33.9±0.2	33.7±0.2
L2–4 BMD (kg/cm^2)	0.99±0.01	0.98±0.01	1.03±0.01	1.02±0.01	0.82±0.01	0.80±0.01
Femoral neck BMD (g/cm^2)	0.76±0.01	0.75±0.004	0.78±0.01	0.77±0.01	0.66±0.01	0.64±0.004*
	AA (n=769)	AG/GG (n=350)	AA (n=192)	AG/GG (n=86)	AA (n=530)	AG/GG (n=282)
Age (years)	59.2±0.4	59.1±0.5	46.3±0.3	46.0±0.5	63.7±0.4	64.2±0.5
Weight (kg)	62.7±0.3	61.9±0.5	53.5±0.5	56.4±1.0	51.9±0.3	52.2±0.5
BMI (kg/m^2)	23.1±0.1	22.8±0.1	22.3±0.2	23.7±0.4**	22.9±0.1	23.3±0.2
LTM (kg)	47.0±0.2	46.5±0.3	36.1±0.3	37.0±0.5	33.8±0.2	33.7±0.2
L2–4 BMD (kg/cm^2)	0.99±0.01	0.97±0.01	1.03±0.01	1.02±0.01	0.81±0.01	0.81±0.01
Femoral neck BMD (g/cm^2)	0.75±0.004	0.75±0.01	0.77±0.01	0.78±0.01	0.65±0.004	0.64±0.01

Data are mean±SE. *p<0.05 vs. TT genotype, **p<0.01 vs. AA genotype.

carried out. Values of $p<0.05$ were considered to indicate statistical significance. Data were analyzed with the Statistical Analysis System (SAS) release 8.2 (SAS Institute Inc., Cary, NC, USA).

Results

Distribution of ERα genotypes

The distribution of genotype combinations was examined (Table 1). The distributions of ERα T→C and A→G genotypes were both in Hardy–Weinberg equilibrium. There were no subjects with the TT and GG genotypic combination and few with the TT/AG or TC/GG genotypic combination.

Physical characteristics

Physical characteristics of the subjects were compared with reference to the ERα T→C and A→G genotype groups (Table 2). For men and premenopausal women, age, weight, BMI, LTM, L2–4 BMD, and femoral neck BMD did not differ between subjects with the TT and TC/CC genotypes. In contrast, in postmenopausal women, age was significantly higher and femoral neck BMD was significantly lower in individuals with the TC/CC genotype than in those with the TT genotype. After adjusting for age, statistical significance was not achieved for the difference in femoral neck BMD in postmenopausal women (data not shown). In men and postmenopausal women, there were no differences in age and physical characteristics between subjects with the AA and AG/GG genotypes. In premenopausal women, age, weight, LTM, and BMD did not differ between subjects with the AA and AG/GG genotypes, whereas BMI was significantly greater in those with the AG/GG genotype than in those with the AA genotype. After adjusting for BMI, the relationship of L2–4 and femoral neck BMD between AA and AG/GG genotypes still did not show a significant difference in premenopausal women (data not shown).

ERα genotype and association between LTM and BMD

To investigate whether an interaction between ERα genotype and LTM had an effect on L2–4 and femoral neck BMDs, general linear models for BMD were analyzed using LTM, ERα

Table 3
General linear model for bone mineral density (BMD) with interaction between the ERα genotype and LTM

Dependent variables	Independent variables	Men		Premenopausal women		Postmenopausal women	
		F	p value	F	p value	F	p value
L2–4 BMD	LTM	45.65	<0.0001	24.73	<0.0001	25.53	<0.0001
	T→C genotype	0.91	ns	1.36	ns	2.41	ns
	LTM×(T→C genotype)	0.83	ns	1.29	ns	2.55	ns
Femoral neck BMD	LTM	63.90	<0.0001	15.07	<0.0001	25.35	<0.0001
	T→C genotype	0.03	ns	0.13	ns	8.15	0.004
	LTM×(T→C genotype)	0.03	ns	0.06	ns	7.48	0.007
L2–4 BMD	LTM	45.27	<0.0001	24.36	<0.0001	25.41	<0.0001
	A→G genotype	0.10	ns	0.16	ns	2.20	ns
	LTM×(A→G genotype)	0.05	ns	0.26	ns	2.14	ns
Femoral neck BMD	LTM	64.07	<0.0001	14.95	<0.0001	24.95	<0.0001
	A→G genotype	0.38	ns	0.07	ns	8.15	0.004
	LTM×(A→G genotype)	0.45	ns	0.05	ns	8.03	0.005

ns=not significant. Adjusted for age and BMI.

genotype, and the interaction between ERα genotype and LTM as independent variables, adjusting for age and BMI (Table 3). Lean tissue mass was significantly associated with L2–4 and femoral neck BMDs in both sexes and irrespective of menstrual status. In postmenopausal women, genotype and the interaction between genotype and LTM were significantly associated with femoral neck BMD for both the T→C and A→G genotypes, but not with L2–4 BMD. In men and premenopausal women, the effects of genotype and the interaction between genotype and LTM on BMD were not significant.

To clarify the influence of LTM on femoral neck BMD for T→C and A→G ERα genotypes in postmenopausal women, a general linear model for BMD was analyzed by each genotype, using LTM as an independent variable, adjusting for age and BMI. Fig. 1 shows the regression lines between femoral neck BMD and LTM for the ERα T→C and A→G genotype groups in postmenopausal women. For the T→C genotype (Fig. 1A), the slope was significantly larger ($p<0.01$) for TC/CC (slope = 0.0071, $p<0.0001$) than for TT individuals (slope = 0.0015, not significant). For the A→G genotype (Fig. 1B), the slope was significantly larger ($p<0.01$) for AG/GG (slope = 0.0081, $p<0.0001$) than for AA individuals (slope = 0.0033, $p=0.012$).

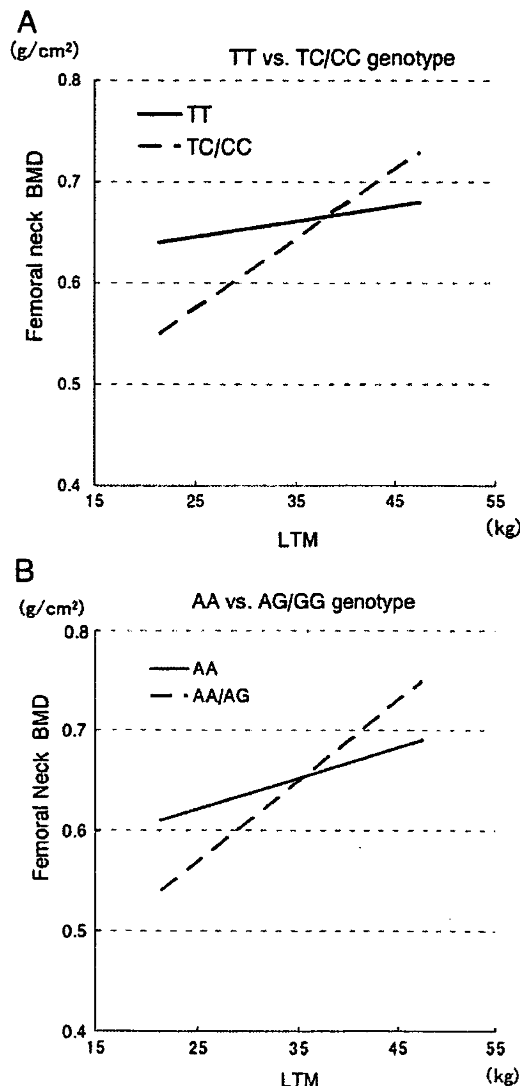

Fig. 1. Relationship between femoral neck BMD and LTM with reference to ERα T→C and A→G genotypes in postmenopausal women, adjusted for age and BMI. (A) T→C genotype. Solid line, TT genotype; Dotted line, TC/CC genotype. (B) A→G genotype. Solid line, AA genotype; Dotted line, AG/GG genotype.

Table 4
Physical characteristics of postmenopausal women with reference to ERα T→C and A→G haplotype

	Haplotype		
	TA	CA	CG
N	965	349	307
Age (years)	63.4±0.3	64.8±0.5*	64.3±0.5
Weight (kg)	52.2±0.3	51.6±0.4	51.8±0.5
BMI (kg/m^2)	23.0±0.1	22.9±0.2	23.2±0.2
LTM (kg)	33.8±0.1	33.7±0.2	33.6±0.2
BMD L2–4 (g/cm^2)	0.81±0.004	0.80±0.01	0.80±0.01
BMD femoral neck (g/cm^2)	0.65±0.003	0.64±0.01	0.64±0.01

Data are mean±SE. *$p<0.05$ vs. TA.

ERα haplotype and association between LTM and BMD in postmenopausal women

Because there were significant genotype-LTM interactions on femoral neck BMD for both T→C and A→G polymorphisms in postmenopausal women, further analysis was carried out to evaluate the effect of the haplotype–LTM interaction. The distribution of haplotypes is shown in Table 4. The possible haplotype combinations for the ERα T→C and A→G polymorphisms were TA, CA, TG, and CG, but very few subjects had the TG haplotype. For postmenopausal women, the number of TA haplotype was 965; CA was 349; and CG was 307. Physical characteristics and BMD were compared with reference to these three haplotypes (Table 4). Age was significantly higher for those with the CA haplotype than for those with the TA haplotype. Weight, BMI, LTM, and L2–4 and femoral neck BMDs did not differ among haplotypes.

To clarify the influence of the interaction between ERα haplotype and LTM on femoral neck BMD in postmenopausal women, general linear models for BMD were analyzed using LTM, ERα haplotype, and the interaction between ERα haplotype and LTM as independent variables, adjusting for age and BMI (Table 5). Lean tissue mass, haplotype, and the interaction between haplotype and LTM were significantly

Table 5
General linear model for femoral neck BMD with interactions between ERα haplotype and LTM in postmenopausal women

Dependent variables	Independent variables	Postmenopausal women	
		F	*p*
Femoral neck BMD	LTM	49.80	<0.0001
	haplotype	6.63	0.001
	LTM×haplotype	6.23	0.002

Adjusted for age and BMI.

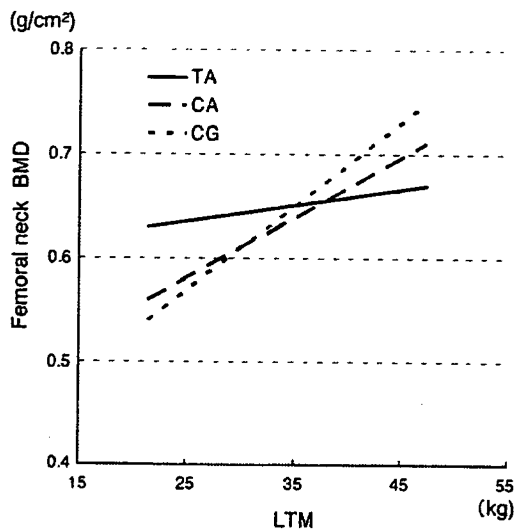

Fig. 2. Relationship between femoral neck BMD and LTM with reference to ERα haplotype in postmenopausal women, adjusted for age and BMI. Solid line, TA haplotype; Dotted line, CA haplotype; Fine dotted line, CG haplotype.

associated with femoral neck BMD. To evaluate the extent of the influence of LTM on BMD with respect to different haplotypes, general linear models were analyzed by each haplotype, using LTM as an independent variable, adjusting for age and BMI. Fig. 2 presents the relationship between femoral neck BMD and LTM with respect to the ERα haplotypes in postmenopausal women. The slope was significantly larger ($p<0.01$) for subjects with the CG (slope=0.0081, $p<0.0001$) and CA (slope=0.0063, $p<0.0001$) haplotypes than for those with the TA haplotype (slope=0.0035, $p=0.0003$), but there was no difference between the CG and CA haplotypes.

Discussion

We found that ERα polymorphisms influence the relationship between LTM and femoral neck BMD in postmenopausal women and that the effect of LTM on BMD was significantly larger in individuals with the TC/CC genotype of the T→C polymorphism than in those with the TT genotype, and larger in those with the AG/GG genotype of the A→G polymorphism than in those with the AA genotype. Haplotype analysis revealed that the effect was significantly larger for those with the CG and CA haplotypes than for those with the TA haplotype. This is the first study to investigate the effect of ERα gene–LTM interaction on BMD and to reveal the significant interaction in postmenopausal women.

In this study, a significant gene–LTM interaction in postmenopausal women was found at the femoral neck, but not at the lumber spine. It has been reported that hip joint compression forces reach 2.5 to 3 times body weight during walking [25,26]. The significant results found only at the femoral neck could be explained by the fact that high loading occurs at this site even in ordinary daily life.

We also analyzed data for different combinations of genotypes (TC/TT vs. CC; AG/AA vs. GG) (data not shown). In premenopausal women, no significant gene–LTM interaction was found. In postmenopausal women, significant interaction was found at femoral neck between TT/TC and CC genotype groups; however, no significant genotype–LTM interaction was found between GG and AA/AG genotype groups. This might be due to the small number in the GG genotype group ($n=28$). In men, when divided into TT/TC and CC genotypes, significant interaction was found at L2–4 and the femoral neck (L2–4, $p=0.04$; femoral neck, $p=0.02$) and the effect of LTM on BMD was larger for those with CC genotype than for those with TC/TT genotype. However, these significant interactions in men were weak in spite of the large number and the coefficients of determination (R^2) in the analysis model were low in men compared with postmenopausal women (men at the femoral neck, 0.28; men at L2–4, 0.18; postmenopausal women at the femoral neck, 0.38). Therefore, there might be other related factors in men and we considered that these results in men are insufficient to draw a clear conclusion about the effect of ERα gene–LTM interaction between TT/TC and CC genotype groups. We will examine this problem by adding other factors and analyzing the data longitudinally.

There have been some human studies investigating the effects of ERα gene polymorphism on exercise-induced effects on BMD. In a 4-year exercise intervention study, Remes et al. [18] reported that middle-aged Finnish men with the Pp (TC) or PP (CC) ERα genotype had increased lumbar spine BMD values. In the study of Remes et al., the subjects were middle-aged men, and the exercise intervention group spent 45–60 min on prescribed aerobic exercise five times a week for 4 years. Because we did not intervene as in the research of Remes et al., there might not have been a significant interaction between TT and TC/CC genotype groups in men.

Suuriniemi et al. [19] found that prepubertal and early pubertal Finnish girls with the Pp (TC) ERα genotype and high levels of physical activity had significantly higher bone mass and BMD at loaded bone sites. The subjects were 10- to 13-year-old prepubertal and early pubertal girls, whose estrogen concentrations were low, like those of postmenopausal women. A previous report indicated that estrogen can affect bone strength and mass by lowering the remodeling threshold, and that loss of estrogen would raise the threshold and help cause postmenopausal bone loss [27]. ERα expression in osteoblasts and osteocytes depends on estrogen concentration [28]. The increase in the potential of mechanical loading to stimulate bone gain in the peripubertal period is associated with marked increases in serum estrogen [11]. Thus, in the peripubertal period, when estrogen concentrations are high, the response to mechanical loading might be greater than in the prepubertal and postmenopausal periods, when estrogen concentrations are low. However, in the present study, significant interactions were found for postmenopausal women and in the study of Suuriniemi et al. [19] significant interactions were found for prepubertal and early pubertal girls. Accordingly, the effect of the gene–LTM interaction on BMD, that is, differences between individuals with different single nucleotide polymorphisms, might more readily appear in groups with low estrogen concentrations than in those with high concentrations.

In the study of Suuriniemi et al. [19], the interaction was found only for individuals with the Pp (TC) genotype (heterozygotes). This was not in agreement with our results. In this previous study, girls with low levels of physical activity bearing the Pp genotype had lower values for bone parameters compared with other groups. Because there are differences in the subject characteristics, age, sex, lifestyle, and study design, it is difficult to simply compare the results of the present study with those of the study by Suuriniemi et al. Further investigations are necessary to clarify these differences.

The mechanisms by which the ERα T→C and A→G polymorphisms might affect the femoral neck BMD are not clear, because the affected regions lie in an intronic and non-functional area of the gene. However, single nucleotide polymorphisms are usually linked to each other, so the two polymorphisms in intron 1 may be in linkage disequilibrium with causal polymorphisms elsewhere in the ERα gene or in genes nearby. In this regard, it is known that the T→C and A→G polymorphisms are in linkage disequilibrium with an upstream TA repeat polymorphism in the promoter region of the ERα gene [29]. An association between the TA repeat polymorphism and BMD has been shown in postmenopausal Japanese [30] and Italian women [29]. The number of TA repeats could be important in ERα gene transcription [31].

There are conflicting results regarding the association between ERα genotype and BMD in previous studies. Gene–LTM interactions might be one of the reasons for these differing results. That is, differences in the amount of muscle mass might change the association between ER genotype and BMD. For example, as shown in Fig. 1A, it is considered that in the group with low LTM, postmenopausal women with the TT genotype for T→C polymorphism have higher femoral neck BMD than those with the CC genotype. Conversely, in the group with high LTM, postmenopausal women with the CC genotype have higher BMD than those with the TT genotype.

In the present study, we evaluated the relationship between LTM and BMD with reference to ERα genotype, but we did not evaluate cause and effect directly. A previous exercise intervention study [18] showed an interaction between ERα genotype and exercise (i.e. the effect of mechanical loading) on BMD in men. Because LTM correlates with muscle strength [20] and it can be used as an index of skeletal load, the result of this previous exercise intervention study supports our present results regarding the gene–LTM interaction.

The strengths of the present study are the large sample size, the inclusion of both sexes, and the wide range of ages. Previous research has evaluated only one sex and a limited age range [18,19]. So far, the ERα gene–environment interaction concerning mechanical loading has not been investigated in postmenopausal women, who are particularly susceptible to osteoporosis. In the present study, both T→C and A→G ERα polymorphisms were examined and haplotype analysis was carried out.

As already described, previous studies have investigated the effects of ERα gene polymorphism on exercise-induced effects on BMD, but there has been no study that has evaluated the effect of the ERα gene–LTM interaction on BMD. The well-known phenomenon of reduction in muscle mass with aging is known as sarcopenia. Recent studies have reported a high prevalence of sarcopenia in postmenopausal woman with osteoporosis [32]. It would be very useful to identify individuals in this group who would experience a marked effect from increasing muscle mass, and the results of this study might assist in developing this process.

ERα plays an important role in the increase of BMD via mechanical strain [9–11]. A recent study has suggested that the effect of chronic immobility might be more marked on bone formation than bone resorption [33]. On the basis of our results, we can speculate that the influence of the mechanical loading increase on BMD via ER is different according to the ERα T→C and A→G polymorphisms in postmenopausal women.

In conclusion, there was an interaction between LTM and the T→C and A→G ERα polymorphisms with respect to their effect on femoral neck BMD in postmenopausal women and those with the TC/CC and AG/GG genotypes had larger effects of LTM than those with TT and AA genotypes.

Acknowledgments

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第48回日本老年医学会学術集会記録
〈市民公開シンポジウム：高齢者の健康と食〉

1. 食生活と長寿

下方　浩史

要　約　肥満はさまざまな生活習慣病の原因であり，最近はメタボリックシンドロームとしてその病態が注目されている．健康長寿には健全な食生活によって肥満を防止することが重要である．しかしやせているほど健康というわけではなく，人間には理想的な肥満度がある．この理想的な肥満度は年齢によって異なり，高齢者では生命予後を考えた場合，肥満の予防よりもむしろやせの予防の方が重要である．栄養摂取の不足は高齢者では寿命を短くすることが多い．高齢者の栄養のかかえるさまざまな栄養問題，栄養評価に関する考え方を述べるとともに，健康な長寿を目指すための理想的な肥満度，内臓脂肪や体脂肪分布と健康，そして急激な体重変動が健康障害をもたらす等の知見を示し，長寿と食生活，栄養との関連について幅広く紹介する．

Key words：長寿，肥満，食事，老化，栄養

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はじめに

厚生労働省による平成16年度の簡易生命表では日本人の平均寿命は，女性が85.59歳，男性が78.64歳であった[1]．男女とも5年連続で過去最高を更新したことになる．女性は20年連続の世界一であり，男性は前年の3位から香港を抜いて2位となり，世界最速のペースで長寿化が進んでいる．

日本人の長寿には食生活が重要な要因となっていると考えられる．日本には独特の食習慣がある．先進諸国中で脂肪摂取量が飛び抜けて少なく，米飯を中心として炭水化物の摂取が多い．また魚の摂取が多いことも特徴である．豆腐や納豆，味噌などの大豆製品の摂取が多く，これらは動脈硬化の進行を防ぐには理想に近い食習慣である．またカテキンやビタミンCなどの抗酸化物質が多く含まれる緑茶の摂取は，動脈硬化や癌を防いでいる可能性がある．ここでは長寿や高齢者の健康と栄養との関わりについて述べてみる．

理想的肥満度

自由無制限の食餌を与えたラットより食餌を制限したラットの方が長生きするという結果は基礎老化の研究者の間ではよく知られている[2]．しかし他の動物においても食餌制限が有効かどうかについては議論のあるところで，サルを使ったプロジェクトがアメリカ国立老化研究所で行われつつあるが，サルの寿命は長く最終的な結論がでるのはまだまだ先である[3]．

人間ではやせていればいるほど健康にいいのか．もしそうでないなら，どの程度の体重であるのが医学的には理想なのか．Andresは米国の生命保険会社のデータから，体重（kg）を身長（m）の二乗で割って求めたBody Mass Index（BMI）を身長とは無相関の肥満の指標として用い，各年代で最も死亡率の低いBMIをもとめた[4]．この結果死亡率を縦軸，BMIを横軸にとった時，きれいなU字を描くことに示した．BMIの小さいやせた人では，肺炎や結核などの感染症の発病率が高く，BMIの大きな太った人では糖尿病や心臓病などの発病率が高くなる．男女別に，年齢ごとにこのようなグラフを作成し，死亡率の最も低い肥満度を求めてみると，この理想的な肥満度の値は加齢とともに大きくなっている[5]．男女で大きな差はなく年齢とともにほぼ直線的に理想的なBMIの値が大きくなっていく．例えば，身長170cmの45歳の男性で67kg位の体重であると死亡率，疾患の罹患率が最も低くなる．

日本での検討では，生活習慣病の発生率の最も少ないのは，BMIが22.2であることが示されており[6]，この値は米国の40歳代における最も死亡率の低いBMIの値とほぼ同じ値である．日本人でも，理想的BMIは，米国

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表1 年齢別にみたBMIによる痩せの基準値

年齢	BMI
20～29歳	18
30～39歳	19
40～49歳	20
50～59歳	21
60～69歳	22
70歳以上	23

での場合と同様に，加齢とともに高くなっていると思われるが，残念ながら日本ではこうした加齢による，理想的肥満度の変化についての十分な検討は行われていない．

高齢者の栄養問題

1）老化に伴う生理学的変化

消化吸収という生体機能は原始的機能であり，基本的には予備力が大きい．しかし加齢によって消化吸収に関連する機能は少しずつ低下し，いろいろな疾患や病態を引き起こす．老化により唾液分泌が低下することが多い．唾液が出にくくなれば食物の咀嚼も悪くなる．また食物を飲み込みにくくなり，嚥下障害となる．さらに口腔内の衛生状態も悪くなり，慢性の口内炎や慢性の舌炎，歯槽膿漏の原因となる．口内炎や歯肉炎は入れ歯があわない場合にも起きやすい．口腔内の炎症があれば，不快感や疼痛のため食事が十分とれなくなる．

胃の支持組織の緊張低下により胃液が食道に逆流し，食道にびらんや潰瘍を形成する逆流性食道炎は老人に多い．胃の粘膜が萎縮し胃酸の分泌が悪くなる．鉄やビタミンの吸収が低下し，また胃酸は細菌の増殖を抑える作用があるが，酸が低下すれば消化管への細菌感染の危険が増加する．

消化液の分泌能の低下はとくに油脂類の消化に負担を与える．また歯の脱落や咀しゃく筋の筋力低下による咀しゃく能の低下により，堅い食品を避け，柔らかいものを好むようになる．柔らかい食品には糖質を主体とするものが多く，たんぱく質やカルシウムなどが不足してしまう．消化管の筋組織の筋力低下や支持組織の緊張低下に起因する消化管運動機能の低下によって便秘となりやすい．さらに消化管の栄養素の吸収能，肝臓における処理能力の低下もみられる．このような老化による変化は個人により進行の程度に差はあるとはいえ避けがたいものである．

2）高齢者の食欲不振

高齢者では若年者に比べて食欲が低下することが多い．これにはいくつかの要因がある．高齢者では心肺機能が低下し運動を十分にすることができなくなり，身体活動によるエネルギー消費が少なくなる．運動を行わないため骨格筋が萎縮し体脂肪が増加する．骨格筋は多くのエネルギーを消費するが脂肪組織ではエネルギーはほとんど消費されず，体脂肪率の上昇とともに全身の基礎代謝率は低下する．エネルギー要求量が低くなり，その結果，食欲が低下することが多い．感覚機能，特に食欲に密接に関わる味覚，臭覚，視覚などの機能の低下がいっそう食欲不振を増強させる．高齢者に多い心疾患に対して使われるジギタリス剤などには食欲を減退させる副作用が往々にしてみられる．また亜鉛欠乏は味覚障害を起こし食欲低下の原因となる．

高齢者の栄養状態の評価

1）血液検査による評価

血清アルブミンは高齢者の栄養状態を示す指標として最も有用なものである．健康な高齢者では加齢に伴う血清アルブミンの低下はみられない．血清アルブミンは生命予後の有用な指標でもある[7]．アルブミン値が3.5g/dl以下の状態では骨格筋の消耗が始まっている可能性が強い．高脂血症，特に高コレステロール血症は虚血性心疾患のリスクとなるが，血清コレステロール値が300mg/dlを越えるような場合は家族性の高脂血症であることがほとんどで，治療しない限り老年に達する前に心疾患などで死亡してしまうことが多い．しかし高齢者では低コレステロール血症がむしろ死亡や日常生活の活動能力が低下することにつながることが知られている[8]．

2）体格による評価

高齢者では生命予後を考えた場合，肥満よりもやせの方が重要である．肥満は糖尿病や高血圧の原因のひとつであり，肥満者では心臓病や脳卒中の発生率が高くなる．しかし肥満者の死亡が多いのは主に中年期である．高齢者では中年に比べて肥満は健康を害したりする危険や死亡に結びついたりすることが少ない．表1にBMIでの年齢別にみたやせの基準値を示した[9][10]．年齢が高くなるにつれて基準となるBMIの値が高くなっている．高齢者では椎間の狭小化，椎骨の圧迫骨折による脊椎前弯の増強などにより，身長が年齢とともに低くなっていく．このためBMIは本来あるべき値よりも大きくなっていることにも注意しなければならない．高度の肥満に伴う高血圧症や糖尿病などがないかぎり高齢者に食事制限を勧めるべきではない．高齢者では肥満よりもやせの重要性を認識すべきである．

おわりに

高齢者では一般成人と異なった視点からの栄養管理が必要である．加齢とともに肥満よりもやせのリスクが高くなる．やせた高齢者が寝たきりになると褥創ができやすく，また感染症も治りにくい．低栄養に十分に留意する必要がある．耐糖能は年齢とともに低下する．高齢者で食後血糖やHbA1cが高くなることは高頻度にみられる．40代，50代では糖尿病合併症の進行を抑えるためにも厳格な血糖のコントロールが必要だが，高齢者では過度な制限はむしろ栄養のバランスを崩し，低栄養をきたすこともある．高齢者では血圧も高くなることが多い．しかし食事療法で，無理な減塩を行えば食事が取れなくなってしまい，かえって健康を害することもある．高コレステロールは高齢者ではむしろ生命予後を良くしている．こうした高齢者の特性を考えて，栄養管理を行うことが重要であろう．

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Dietary habit and longevity

Hiroshi Shimokata

Abstract

Obesity is one of the most important causes of life-style related diseases, and recently its pathophysiology is emphasized as metabolic syndrome. Preventing obesity by good dietary habit is a key to achieve healthy longevity. However, a lean body is not always good for health. There is an ideal body size for each person. This ideal body size differs according to age. Especially in the elderly, to prevent weight loss is more important for maintaining health and longevity than to be obese. Malnutrition is a critical factor of diseases and death in the elderly. Problems in nutritional status, and dietary intake, and methods of nutritional assessment in the elderly are discussed. Ideal body size for health and longevity, the relationship of body fat distribution and intra-abdominal fat accumulation health, and the effects of rapid weight change are also discussed to clarify the association of dietary habit and nutrition with longevity.

Key words: *Longevity, Obesity, Diet, Aging, Nutrition*
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Association of candidate gene polymorphisms with bone mineral density in community-dwelling Japanese women and men

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Abstract. Although bone mineral density (BMD) is a complex trait that is influenced by both genetic and environmental factors, heritability studies in twins and families have shown that genetic factors account for 60-85% of the variance in BMD. We examined the relations of six candidate gene polymorphisms to BMD in community-dwelling women and men. The 2238 subjects (1110 women, 1128 men) were aged 40-79 years and were randomly recruited to a population-based prospective cohort study of aging and age-related diseases in Japan. BMD at the distal and proximal radius was measured by peripheral quantitative computed tomography, and BMD for the total body, lumbar spine (L2-L4), right femoral neck, and right trochanter was measured by dual-energy X-ray absorptiometry. Genotypes for the 1019C→T (Pro319Ser) polymorphism of *GJA4* and the 1462A→G (Lys469Glu) polymorphism of *ICAM1* were determined with a fluorescence-based allele-specific DNA primer assay system, and those for the 386G→A (Ala99Thr) polymorphism of *PLOD1*, the A→G polymorphism of *CNR2*, the 1583G→A (Arg528Lys) polymorphism of *ALAP*, and the -514C→T polymorphism of *LIPC* were determined by melting curve analysis. The polymorphisms of *ALAP* and *PLOD1* were associated with BMD in premenopausal women; those of *ICAM1* and *LIPC* with BMD in postmenopausal women; that of *CNR2* with BMD in premenopausal and postmenopausal women; and that of *GJA4* with BMD in men. Among these polymorphisms, those of *ICAM1*, *CNR2*, and *GJA4* were markedly associated with BMD. These results suggest that *ALAP*, *PLOD1*, *ICAM1*, *LIPC*, and *CNR2* are susceptibility loci for reduced bone mass in Japanese women and that *GJA4* constitutes such a locus in Japanese men. The polymorphisms of *ICAM1* and *CNR2* may confer susceptibility to postmenopausal osteoporosis in women, and that of *GJA4* to osteoporosis in men.

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Introduction

Osteoporosis, a major health problem of the elderly, is characterized by a reduction in bone mineral density (BMD) and a deterioration in the microarchitecture of bone, both of which result in predisposition to fractures (1). Although reproductive, nutritional, and lifestyle factors influence BMD, family and twin studies have suggested that BMD is largely (60-85%) heritable and under the control of multiple genes (2-4). Personalized prevention of osteoporosis and osteoporotic fractures is an important public health goal, for which one approach is to identify disease susceptibility genes. Although genetic linkage analyses (5-7) and candidate gene association studies (7-10) have implicated various loci and genes in predisposition to osteoporosis or fractures, the genes that confer susceptibility to this disease remain to be identified definitively. In addition, because of ethnic differences in gene polymorphisms as well as in lifestyle and other environmental factors, it is important to examine polymorphisms related to BMD in each ethnic group.

We have been attempting to identify genes significantly associated with BMD in Japanese women or men with a population-based approach. In the present study, we selected six candidate genes that might be expected to contribute to bone remodeling (Table I) and examined the relations of polymorphisms of these genes to BMD, even though there is no apparent biological link among these genes. Our aim was to identify a single polymorphism significantly associated with BMD for each gene. Among several polymorphisms previously identified, we selected those that might be expected to affect gene function. We thus examined the relations of these polymorphisms to BMD in community-dwelling Japanese women and men.

Materials and methods

Study population. The National Institute for Longevity Sciences - Longitudinal Study of Aging is a population-based prospective cohort study of aging and age-related diseases, the details of which have been described previously (11-15). Individuals with disorders known to cause abnormalities of bone metabolism, including diabetes mellitus, chronic renal failure, rheumatoid arthritis, as well as thyroid, parathyroid, adrenal, and other endocrine diseases, or those who had taken drugs that affect bone metabolism, such as estrogen,

Table I. The six gene polymorphisms examined in the present study.

Locus	Gene	Symbol	Polymorphism	dbSNP
1p36.3-36.2	Procollagen-lysine, 2-oxoglutarate 5-dioxygenase	*PLOD1*	386G→A (Ala99Thr)	rs7551175
1p36.11	Cannabinoid receptor 2	*CNR2*	A→G	rs2501431
1p35.1	Gap junction protein, α-4	*GJA4*	1019C→T (Pro319Ser)	rs1764391
5q15	Adipocyte-derived leucine aminopeptidase	*ALAP*	1583G→A (Arg528Lys)	rs30187
15q21-23	Lipase, hepatic	*LIPC*	-514C→T	rs1800588
19p13.3-13.2	Intercellular adhesion molecule 1	*ICAM1*	1462A→G (Lys469Glu)	rs5498

glucocorticoids, bisphosphonates, and vitamin D, were excluded from the present study. We thus examined the relations of gene polymorphisms to BMD in 2238 individuals (1110 women and 1128 men). Individuals whose genotypes were not successfully determined were also excluded from the analysis. In addition, to uncover potential differences between women according to menopausal status, we conducted all analyses for premenopausal and postmenopausal women separately. Menopausal status was evaluated with a detailed questionnaire, and menopause was defined as complete cessation of menstruation. Because of their small number (n=19), perimenopausal women were excluded from this analysis. The study protocol complied with the Declaration of Helsinki and was approved by the Committee on Ethics of Human Research of the National Institute for Longevity Sciences. Written informed consent was obtained from each subject.

Measurement of BMD. BMD at the radius was measured by peripheral quantitative computed tomography (pQCT) with a Desiscan 1000 instrument (Scanco Medical, Bassersdorf, Switzerland) and was expressed as D50 (BMD for the inner 50% of the cross-sectional area of the distal radius, comprising mostly cancellous bone), D100 (BMD for the entire cross-sectional area of the distal radius, including both cancellous and cortical bone), and P100 (BMD for the entire cross-sectional area of the proximal radius, consisting mostly of cortical bone). BMD for the total body, lumbar spine (L2-L4), right femoral neck, and right trochanter was measured by dual-energy X-ray absorptiometry (DXA) with a QDR 4500 instrument (Hologic, Bedford, MA, USA). The coefficients of variation of the pQCT instrument for BMD values were 0.7% (D50), 1.0% (D100), and 0.6% (P100), and those of the DXA instrument were 0.9% (total body), 0.9% (L2-L4), 1.3% (femoral neck), and 1.0% (trochanter).

Determination of genotype. Genotypes for polymorphisms of *GJA4* and *ICAM1* were determined with a fluorescence-based allele-specific DNA primer assay system (Toyobo Gene Analysis, Tsuruga, Japan) (16). Primers and other conditions for genotyping are shown in Table II. The polymorphic region of each gene was amplified by the polymerase chain reaction (PCR) with allele-specific sense primers labeled at the 5' end with either fluorescein isothiocyanate (FITC) or Texas red and with an antisense primer labeled at the 5' end with biotin. The reaction mixture (25 μl) contained 20 ng of DNA, 5 pmol of each primer, 0.2 mmol/l of each deoxynucleoside triphosphate, 2.5 or 4.5 mmol/l $MgCl_2$, and 1 U of rTaq DNA polymerase (Toyobo, Osaka, Japan) in polymerase buffer. The amplification protocol comprised an initial denaturation at 95˚C for 5 min; 35 cycles of denaturation at 95˚C for 30 sec, annealing at 60˚C for 30 sec, and extension at 72˚C for 30 sec; and a final extension at 72˚C for 2 min. The amplified DNA was incubated with streptavidin-conjugated magnetic beads in the wells of a 96-well plate at room temperature, and the plate was then placed on a magnetic stand. The supernatants from each well were transferred to the wells of a 96-well plate containing 0.01 mol/l NaOH and were measured for fluorescence with a microplate reader (Fluoroscan Ascent; Dainippon Pharmaceutical, Osaka, Japan) at excitation and emission wavelengths of 485 and 538 nm, respectively, for FITC; and of 584 and 612 nm, respectively, for Texas red.

Genotypes for polymorphisms of *PLOD1*, *CNR2*, *ALAP*, and *LIPC* were determined by melting curve analysis (intercalater-mediated fluorescence resonance energy transfer probe method). The polymorphic region of each gene was amplified by PCR (Table II) in a reaction mixture (25 μl) containing 20 ng of DNA, 5 pmol of each primer, 0.2 mmol/l of each deoxynucleoside triphosphate, 2 or 3 mmol/l $MgCl_2$, and 1.25 U of rTaq DNA polymerase in polymerase buffer. The amplification protocol comprised an initial denaturation at 95˚C for 5 min; 45 or 50 cycles of denaturation at 95˚C for 30 sec, annealing at 65˚C for 30 sec, and extension at 72˚C for 30 sec; and a final extension at 72˚C for 2 min. A mixture (2 μl) of 10 pmol of probe and SYBR-Green was added to the PCR products, which were then transferred to a PRISM 7700 instrument (Applied Biosystems, Foster City, CA, USA) for measurement of melting temperature. The program for analytic melting comprised incubation at 95˚C for 30 sec, 40˚C for 1 min, and temperatures increasing to 80˚C over 10 min. The fluorescence signals were detected at excitation and emission wavelengths of 485 and 612 nm, respectively.

Statistical analysis. Data are presented as means ± SE. Statistical analysis was performed with SAS software (SAS Institute, Cary, NC, USA). Data were compared among three genotype groups by one-way analysis of variance and the Tukey-Kramer *post hoc* test, and between two groups (dominant or recessive model) by the unpaired Student's t-test. BMD values were compared among genotypes for each polymorphism with adjustment for age, height, and body weight by the least squares method in a general linear model. Allele frequencies were estimated by the gene-counting

Table II. Primers, probes, and other PCR conditions for genotyping.

Gene	Polymorphism	Sense primer with FITC	Sense primer with Texas red
GJA4	1019C→T (Pro319Ser)	CCTCAGAATGGCCAAAAxTC	CTCAGAATGGCCAAAAxCC
ICAM1	1462A→G (Lys469Glu)	AAGGGGAGGTCACCCGxGA	AGGGGAGGTCACCCGxAA

	Antisense primer with biotin	Annealing (°C)	Cycles	Mg^{2+} (mM)
GJA4	GCAGAGCTGCTGGGACGA	60	35	2.5
ICAM1	CTCACAGAGCACATTCACGGTCAC	60	35	4.5

Gene	Polymorphism	Sense primer	Antisense primer
PLOD1	386G→A (Ala99Thr)	AAGCACGCAGACAAGGAGGATCTG	GAGGGCCTCATTTTAGAATATTCTTCTATCTTC
CNR2	A→G	GGGCAGGTAGGAGACTAGTGCTGAGAG	CTCACCCGTGGAAGGGCACTG
ALAP	1583G→A (Arg528Lys)	CCCTTCATGTAGTGCTCTTGCTTCATG	GCATCAGGAAGGGGTGGATGTG
LIPC	-514C→T	GGGCATCTTTGCTTCTTCGTCAG	TTGGTGATGCTTGTGGTCAAAGTG

	Probe	Annealing (°C)	Cycles	Mg^{2+} (mM)
PLOD1	CATTCTCTTGGCAGACAGGTAG	65	45	2.0
CNR2	CACATGATGCCCAGGGTC	65	45	2.0
ALAP	CCCCTCTGCAGTGTCCAA	65	45	2.0
LIPC	TTCACCCCCATGTCAAAA	65	50	3.0

All primer and probe sequences are 5'→3'.

method, and the Chi-square test was used to identify significant departure from Hardy-Weinberg equilibrium. A P value of <0.05 was considered statistically significant.

Results

Relation of the 386G→A (Ala99Thr) polymorphism of PLOD1 to BMD. The distribution of 386G→A genotypes of *PLOD1* was in Hardy-Weinberg equilibrium, and age, height, and body weight did not differ among genotypes, for all women (Table III) or for premenopausal or postmenopausal women. Among all women, BMD for the femoral neck, with adjustment for age, height, and body weight, was greater in individuals with the *GG* genotype than in those with the *GA* genotype or in the combined group of *GA* and *AA* genotypes (Table III). BMD for the trochanter was also greater in individuals with the *GG* genotype than in the combined group of *GA* and *AA* genotypes. The differences in BMD for

Table III. BMD and other characteristics for all women (n=1109) according to the *PLOD1* genotype.[a]

Characteristic	*GG*	*GA*	*AA*	*GG + GA*	*GA + AA*
Number (%)	621 (56.0)	427 (38.5)	61 (5.5)	1048 (94.5)	488 (44.0)
Age (years)	59.3±0.4	59.0±0.5	60.8±1.4	59.2±0.3	59.3±0.5
Height (cm)	151.2±0.2	151.4±0.3	151.3±0.8	151.3±0.2	151.4±0.3
Body weight (kg)	52.7±0.3	52.4±0.4	53.2±1.0	52.6±0.3	52.5±0.4
BMD measured with pQCT (mg/cm³)					
D50	186.3±2.5	183.5±3.0	184.4±7.9	185.2±1.9	183.6±2.8
D100	485.2±3.6	486.8±4.3	484.8±11.4	485.9±2.8	486.5±4.1
P100	1158.0±5.8	1146.3±7.0	1164.1±18.3	1153.3±4.5	1148.6±6.5
BMD measured with DXA (g/cm²)					
Total body	0.968±0.003	0.960±0.004	0.965±0.011	0.966±0.003	0.961±0.004
L2-L4	0.868±0.005	0.861±0.006	0.863±0.016	0.865±0.004	0.861±0.006
Femoral neck	0.683±0.003	0.670±0.004[b]	0.684±0.011	0.677±0.003	0.671±0.004[c]
Trochanter	0.576±0.003	0.564±0.004	0.569±0.010	0.571±0.003	0.565±0.004[d]

[a]BMD is adjusted for age, height, and body weight. Data are means ± SE. [b]P=0.0442, [c]P=0.0320, [d]P=0.0266 versus *GG*.

Table IV. BMD and other characteristics for all women (n=1106) according to the *CNR2* genotype.[a]

Characteristic	*AA*	*AG*	*GG*	*AA* + *AG*	*AG* + *GG*
Number (%)	402 (36.3)	544 (49.2)	160 (14.5)	946 (85.5)	704 (63.7)
Age (years)	59.1±0.5	59.1±0.5	60.4±0.9	59.1±0.4	59.4±0.4
Height (cm)	151.6±0.3	151.4±0.3	150.5±0.5	151.5±0.2	151.2±0.2
Body weight (kg)	52.8±0.4[b]	53.0±0.4[c]	51.0±0.6	52.9±0.3[d]	52.5±0.3
BMD measured with pQCT (mg/cm^3)					
D50	192.9±3.1[e]	182.1±2.6[f]	176.3±4.9	186.7±2.0	180.8±2.3[g]
D100	491.4±4.5	484.8±3.8	476.7±7.1	487.6±2.9	483.0±3.4
P100	1154.0±7.2	1157.3±6.2	1140.5±11.5	1155.9±4.7	1153.5±5.4
BMD measured with DXA (g/cm^2)					
Total body	0.971±0.004[h]	0.965±0.004	0.952±0.007	0.967±0.003[i]	0.962±0.003
L2-L4	0.876±0.006[j]	0.863±0.005	0.846±0.010	0.869±0.004[k]	0.859±0.005[l]
Femoral neck	0.682±0.004	0.676±0.004	0.673±0.007	0.679±0.003	0.675±0.003
Trochanter	0.575±0.004	0.571±0.004	0.559±0.006	0.573±0.003	0.569±0.003

[a]BMD is adjusted for age, height, and body weight. Data are means ± SE. [b]P=0.0497, [c]P=0.0218, [d]P=0.0070, [e]P=0.0118, [h]P=0.0449, [i]P=0.0352, [j]P=0.0352, [k]P=0.0412 versus *GG*; [f]P=0.0220, [g]P=0.0018, [l]P=0.0382 versus *AA*.

the femoral neck and trochanter between individuals with the *GG* genotype and the combined group of *GA* and *AA* genotypes (expressed as a percentage of the larger value) were 1.8 and 1.9%, respectively. For premenopausal women, BMD for the femoral neck and that for the trochanter were greater in individuals with the *GG* genotype than in the combined group of *GA* and *AA* genotypes (data not shown). For postmenopausal women or for men, there was no difference in BMD among *PLOD1* genotypes (data not shown).

Relation of the A→G polymorphism of CNR2 to BMD. The distribution of A→G genotypes of *CNR2* was in Hardy-Weinberg equilibrium, and age and height did not differ among genotypes, for all women (Table IV). Body weight was greater in women with the *AA* genotype or the *AG* genotype or in the combined group of *AA* and *AG* genotypes than in women with the *GG* genotype. Among all women, BMD for D50 was greater in individuals with the *AA* genotype than in those with the *AG* genotype or the *GG* genotype or in the combined group of *AG* and *GG* genotypes (Table IV). BMD for the total body and that for the lumbar spine were greater in individuals with the *AA* genotype or in the combined group of *AA* and *AG* genotypes than in women with the *GG* genotype. BMD for the lumbar spine was also greater in individuals with the *AA* genotype than in the combined group of *AG* and *GG* genotypes. The differences in BMD for D50, total body, and lumbar spine between individuals with the *AA* genotype and those with the *GG* genotype were 8.6, 2.0, and 3.4%, respectively.

The distribution of A→G genotypes of *CNR2* was in Hardy-Weinberg equilibrium in premenopausal (Table V) and postmenopausal (Table VI) women. Age, height, or body weight did not differ among genotypes for premenopausal or postmenopausal women. For premenopausal women, BMD for D50 was greater in individuals with the *AA* genotype than in those with the *AG* genotype or in the combined group of *AG* and *GG* genotypes (Table V). BMD for the lumbar spine was also greater in individuals with the *AA* genotype than in the combined group of *AG* and *GG* genotypes. The differences in BMD for D50 and the lumbar spine between individuals with the *AA* genotype and the combined group of *AG* and *GG* genotypes were 6.4 and 2.7%, respectively.

For postmenopausal women, BMD for D50 was greater in individuals with the *AA* genotype or in the combined group of *AA* and *AG* genotypes than in individuals with the *GG* genotype, and was greater in individuals with the *AA* genotype than in the combined group of *AG* and *GG* genotypes (Table VI). BMD for the total body and that for the trochanter were greater in the combined group of *AA* and *AG* genotypes than in individuals with the *GG* genotype. The difference in BMD for D50 between individuals with the *AA* genotype and those with the *GG* genotype was 10.1%, and those for the total body and trochanter between the combined group of *AA* and *AG* genotypes and individuals with the *GG* genotype were 2.1 and 2.8%, respectively.

For men, the distribution of A→G genotypes of *CNR2* was in Hardy-Weinberg equilibrium, and BMD for the total body, femoral neck, or trochanter was greater in the combined group of *AG* and *GG* genotypes than in individuals with the *AA* genotype (data not shown).

Relation of the 1019C→T (Pro319Ser) polymorphism of GJA4 to BMD. The distribution of 1019C→T genotypes of *GJA4* was in Hardy-Weinberg equilibrium, and BMD for the femoral neck was greater in individuals with the *CT* genotype or in the combined group of *CT* and *TT* genotypes than in individuals with the *CC* genotype, for all women and for postmenopausal women (data not shown). For premenopausal women, there was no difference in BMD among *GJA4* genotypes (data not shown).

Table V. BMD and other characteristics for premenopausal women (n=276) according to the *CNR2* genotype.[a]

Characteristic	*AA*	*AG*	*GG*	*AA + AG*	*AG + GG*
Number (%)	106 (38.4)	140 (50.7)	30 (10.9)	246 (89.1)	170 (61.6)
Age (years)	46.8±0.4	46.1±0.4	44.8±0.8	46.4±0.3	45.9±0.4
Height (cm)	154.3±0.5	154.6±0.4	154.3±0.9	154.5±0.3	154.5±0.4
Body weight (kg)	54.7±0.8	54.6±0.7	52.0±1.5	54.6±0.5	54.1±0.6
BMD measured with pQCT (mg/cm^3)					
D50	255.7±5.4	237.8±4.6[b]	246.4±10.1	245.3±3.6	239.3±4.2[c]
D100	614.5±7.7	598.7±6.6	612.3±14.3	605.4±5.0	601.1±6.0
P100	1366.3±11.4	1354.0±9.7	1370.9±21.2	1359.2±7.4	1356.9±8.8
BMD measured with DXA (g/cm^2)					
Total body	1.102±0.008	1.085±0.007	1.100±0.015	1.092±0.005	1.088±0.006
L2-L4	1.042±0.011	1.011±0.010	1.027±0.021	1.024±0.007	1.014±0.009[d]
Femoral neck	0.776±0.009	0.763±0.008	0.790±0.016	0.769±0.006	0.768±0.007
Trochanter	0.666±0.008	0.652±0.007	0.659±0.015	0.658±0.005	0.653±0.006

[a]BMD is adjusted for age, height, and body weight. Data are means ± SE. [b]P=0.0334, [c]P=0.0173, [d]P=0.0480 versus *AA*.

Table VI. BMD and other characteristics for postmenopausal women (n=813) according to the *CNR2* genotype.[a]

Characteristic	*AA*	*AG*	*GG*	*AA + AG*	*AG + GG*
Number (%)	291 (35.8)	393 (48.3)	129 (15.9)	684 (84.1)	522 (64.2)
Age (years)	63.7±0.5	63.9±0.4	64.1±0.8	63.8±0.3	64.0±0.4
Height (cm)	150.5±0.4	150.1±0.3	149.6±0.5	150.3±0.2	150.0±0.3
Body weight (kg)	52.1±0.5	52.3±0.4	50.9±0.7	52.2±0.3	52.0±0.4
BMD measured with pQCT (mg/cm^3)					
D50	170.9±3.7[b]	161.9±3.2	153.6±5.6	165.7±2.5[c]	159.8±2.8[d]
D100	447.3±5.4	443.9±4.6	434.0±8.1	445.4±3.5	441.5±4.0
P100	1078.2±8.9	1086.4±7.6	1066.4±13.5	1082.9±5.8	1081.5±6.6
BMD measured with DXA (g/cm^2)					
Total body	0.924±0.005	0.921±0.004	0.903±0.008	0.922±0.003[e]	0.917±0.004
L2-L4	0.817±0.007	0.810±0.006	0.789±0.011	0.813±0.005	0.805±0.006
Femoral neck	0.649±0.005	0.644±0.004	0.635±0.007	0.646±0.003	0.642±0.004
Trochanter	0.542±0.005	0.543±0.004	0.527±0.007	0.542±0.003[f]	0.539±0.004

[a]BMD is adjusted for age, height, and body weight. Data are means ± SE. [b]P=0.0290, [c]P=0.0497, [e]P=0.0237, [f]P=0.0445 versus *GG*; [d]P=0.0179 versus *AA*.

The distribution of 1019C→T genotypes of *GJA4* was in Hardy-Weinberg equilibrium, and age, height, or body weight did not differ among genotypes for men (Table VII). BMD for the total body was greater in individuals with the *CT* genotype or in the combined group of *CC* and *CT* genotypes than in individuals with the *TT* genotype. BMD for the lumbar spine and that for the femoral neck were greater in individuals with the *CC* genotype or the *CT* genotype or in the combined group of *CC* and *CT* genotypes than in individuals with the *TT* genotype. BMD for the trochanter was also greater in the combined group of *CC* and *CT* genotypes than in individuals with the *TT* genotype. The differences in BMD for the total body, lumbar spine, femoral neck, and trochanter between the combined group of *CC* and *CT* genotypes and individuals with the *TT* genotype were 3.1, 8.3, 6.4, and 4.6%, respectively.

Relation of the 1583G→A (Arg528Lys) polymorphism of ALAP to BMD. The distribution of 1583G→A genotypes of *ALAP* was in Hardy-Weinberg equilibrium, and age, height, and body weight did not differ among genotypes, for all women, premenopausal women (Table VIII), or postmenopausal women. For premenopausal women, BMD for the total body, femoral neck, or trochanter was greater in individuals with the *GG* genotype than in the combined group of *GA* and *AA* genotypes (Table VIII). The differences in BMD for the total body, femoral neck, and trochanter between individuals with the *GG* genotype and the combined

Table VII. BMD and other characteristics for all men (n=1128) according to the *GJA4* genotype.[a]

Characteristic	CC	CT	TT	CC + CT	CT + TT
Number (%)	729 (64.6)	357 (31.7)	42 (3.7)	1086 (96.3)	399 (35.4)
Age (years)	59.2±0.4	59.0±0.6	61.0±1.7	59.1±0.3	59.2±0.5
Height (cm)	164.5±0.2	164.8±0.3	164.8±1.0	164.6±0.2	164.8±0.3
Body weight (kg)	62.4±0.3	62.6±0.5	61.5±1.4	62.5±0.3	62.5±0.5
BMD measured with pQCT (mg/cm^3)					
D50	266.3±2.5	268.7±3.5	257.3±10.3	267.1±2.0	267.5±3.3
D100	541.2±3.4	541.9±4.9	533.4±14.2	541.4±2.8	541.0±4.6
P100	1184.3±5.2	1188.0±7.4	1185.8±21.6	1185.5±4.2	1187.8±7.0
BMD measured with DXA (g/cm^2)					
Total body	1.087±0.003	1.091±0.005[b]	1.055±0.014	1.089±0.003[c]	1.087±0.005
L2-L4	0.987±0.006[d]	0.983±0.008[e]	0.904±0.023	0.986±0.005[f]	0.975±0.007
Femoral neck	0.754±0.004[g]	0.758±0.005[h]	0.707±0.015	0.755±0.003[i]	0.753±0.005
Trochanter	0.669±0.004	0.671±0.005	0.639±0.015	0.670±0.003[j]	0.668±0.005

[a]BMD is adjusted for age, height, and body weight. Data are means ± SE. [b]P=0.0370, [c]P=0.0181, [d]P=0.0011, [e]P=0.0029, [f]P=0.0004, [g]P=0.0085, [h]P=0.0042, [i]P=0.0020, [j]P=0.0446 versus *TT*.

Table VIII. BMD and other characteristics for premenopausal women (n=276) according to the *ALAP* genotype.[a]

Characteristic	*GG*	*GA*	*AA*	*GG + GA*	*GA + AA*
Number (%)	80 (29.0)	127 (46.0)	69 (25.0)	207 (75.0)	196 (71.0)
Age (years)	46.4±0.5	45.9±0.4	46.7±0.6	46.1±0.3	46.2±0.3
Height (cm)	154.5±0.5	154.3±0.4	154.6±0.6	154.4±0.3	154.4±0.3
Body weight (kg)	54.2±0.9	54.8±0.7	53.5±1.0	54.6±0.6	54.4±0.6
BMD measured with pQCT (mg/cm^3)					
D50	250.7±6.2	241.8±5.0	247.6±6.6	245.3±3.9	243.9±4.0
D100	609.9±8.7	603.4±7.0	607.5±9.4	605.9±5.5	604.9±5.6
P100	1357.1±12.9	1359.2±10.4	1370.7±13.9	1358.4±8.1	1363.3±8.3
BMD measured with DXA (g/cm^2)					
Total body	1.113±0.009	1.086±0.007	1.085±0.010	1.097±0.006	1.086±0.006[b]
L2-L4	1.036±0.013	1.017±0.010	1.024±0.014	1.025±0.008	1.020±0.008
Femoral neck	0.789±0.010	0.761±0.008	0.771±0.011	0.772±0.006	0.764±0.006[c]
Trochanter	0.676±0.009	0.653±0.007	0.646±0.010	0.662±0.006	0.650±0.006[d]

[a]BMD is adjusted for age, height, and body weight. Data are means ± SE. [b]P=0.0107, [c]P=0.0380, [d]P=0.0191 versus *GG*.

group of *GA* and *AA* genotypes were 2.4, 3.2, and 3.8%, respectively. There was no difference in BMD among *ALAP* genotypes for all women or postmenopausal women (data not shown).

For men, the distribution of 1583G→A genotypes of *ALAP* was in Hardy-Weinberg equilibrium, and BMD for the femoral neck was greater in individuals with the *GG* genotype than in those with the *GA* genotype or in the combined group of *GA* and *AA* genotypes (data not shown).

Relation of the -514C→T polymorphism of LIPC to BMD. The distribution of -514C→T genotypes of *LIPC* was in Hardy-Weinberg equilibrium, and age, height, and body weight did not differ among genotypes, for all women (Table IX), premenopausal women, or postmenopausal women (Table X). For all women, BMD for D50 was greater in the combined group of *CC* and *CT* genotypes than in individuals with the *TT* genotype (Table IX). BMD for the total body was greater in individuals with the *CT* genotype than in those with the *CC* genotype or the *TT* genotype, and was greater in the combined group of *CC* and *CT* genotypes than in individuals with the *TT* genotype. BMD for the trochanter was greater in individuals with the *CT* genotype or in the combined group of *CC* and *CT* genotypes than in individuals with the *TT* genotype. The differences in BMD for D50, total body, and trochanter between the combined group of *CC* and *CT* geno-

Table IX. BMD and other characteristics for all women (n=1110) according to the *LIPC* genotype.[a]

Characteristic	*CC*	*CT*	*TT*	*CC* + *CT*	*CT* + *TT*
Number (%)	250 (22.5)	558 (50.3)	302 (27.2)	808 (72.8)	860 (77.5)
Age (years)	59.2±0.7	59.3±0.5	59.2±0.6	59.3±0.4	59.3±0.4
Height (cm)	151.2±0.4	151.3±0.3	151.4±0.3	151.3±0.2	151.4±0.2
Body weight (kg)	52.7±0.5	52.6±0.3	52.6±0.5	52.6±0.3	52.6±0.3
BMD measured with pQCT (mg/cm^3)					
D50	186.2±3.9	188.3±2.6	178.6±3.5	187.6±2.2[b]	184.8±2.1
D100	485.9±5.6	490.1±3.8	478.2±5.1	488.8±3.1	485.9±3.1
P100	1148.2±9.1	1159.1±6.1	1149.2±8.3	1155.7±5.1	1155.6±4.9
BMD measured with DXA (g/cm^2)					
Total body	0.956±0.005	0.974±0.004[c,d]	0.956±0.005	0.968±0.003[e]	0.968±0.003
L2-L4	0.859±0.008	0.873±0.005	0.856±0.007	0.869±0.004	0.867±0.004
Femoral neck	0.674±0.005	0.681±0.004	0.675±0.005	0.679±0.003	0.679±0.003
Trochanter	0.568±0.005	0.577±0.003[f]	0.562±0.005	0.574±0.003[g]	0.572±0.003

[a]BMD is adjusted for age, height, and body weight. Data are means ± SE. [b]P=0.0310, [d]P=0.0098, [e]P=0.0324, [f]P=0.0353, [g]P=0.0317 versus *TT*; [c]P=0.0176 versus *CC*.

Table X. BMD and other characteristics for postmenopausal women (n=815) according to the *LIPC* genotype.[a]

Characteristic	*CC*	*CT*	*TT*	*CC* + *CT*	*CT* + *TT*
Number (%)	181 (22.2)	406 (49.8)	228 (28.0)	587 (72.0)	634 (77.8)
Age (years)	64.2±0.6	64.1±0.4	63.3±0.6	64.1±0.4	63.8±0.3
Height (cm)	149.7±0.5	150.3±0.3	150.3±0.4	150.1±0.3	150.3±0.2
Body weight (kg)	52.2±0.6	51.7±0.4	52.4±0.5	51.8±0.4	52.0±0.3
BMD measured with pQCT (mg/cm^3)					
D50	164.7±4.7	167.6±3.2	156.1±4.2	166.7±2.6[b]	163.4±2.5
D100	442.3±6.8	447.7±4.6	436.2±6.0	446.1±3.8	443.6±3.6
P100	1073.7±11.3	1087.7±7.5	1071.3±10.0	1083.4±6.3	1081.8±6.0
BMD measured with DXA (g/cm^2)					
Total body	0.907±0.006	0.929±0.004[c,d]	0.911±0.006	0.922±0.004	0.922±0.003[e]
L2-L4	0.799±0.010	0.818±0.006	0.801±0.008	0.812±0.005	0.812±0.005
Femoral neck	0.639±0.006	0.649±0.004	0.641±0.006	0.646±0.003	0.646±0.003
Trochanter	0.537±0.006	0.545±0.004	0.533±0.005	0.543±0.003	0.541±0.003

[a]BMD is adjusted for age, height, and body weight. Data are means ± SE. [b]P=0.0334, [d]P=0.0348 versus *TT*; [c]P=0.0173, [e]P=0.0439 versus *CC*.

types and individuals with the *TT* genotype were 4.8, 1.2, and 2.1%, respectively.

For postmenopausal women, BMD for D50 was greater in the combined group of *CC* and *CT* genotypes than in individuals with the *TT* genotype (Table X). BMD for the total body was greater in individuals with the *CT* genotype than in those with the *CC* genotype or the *TT* genotype, and was greater in the combined group of *CT* and *TT* genotypes than in individuals with the *CC* genotype. The difference in BMD for D50 between the combined group of *CC* and *CT* genotypes and individuals with the *TT* genotype and that for the total body between the combined group of *CT* and *TT* genotypes and individuals with the *CC* genotype were 6.4 and 1.6%, respectively.

For premenopausal women or men, no relation was detected between *LIPC* genotype and BMD (data not shown).

Relation of the 1462A→G (Lys469Glu) polymorphism of ICAM1 to BMD. The distribution of 1462A→G genotypes of *ICAM1* was in Hardy-Weinberg equilibrium, and age and height did not differ among genotypes for all women (Table XI), premenopausal women, or postmenopausal women (Table XII). For all women and postmenopausal women, but not premenopausal women, body weight was greater in individuals with the *GG* genotype than in the combined group of *AA* and *AG* genotypes. For all women, BMD for D50 was greater in individuals with the *GG* genotype than in those with the *AA* genotype or in the combined group of *AA* and

Table XI. BMD and other characteristics for all women (n=1096) according to the *ICAM1* genotype.[a]

Characteristic	AA	AG	GG	AA + AG	AG + GG
Number (%)	364 (33.2)	536 (48.9)	196 (17.9)	900 (82.1)	732 (66.8)
Age (years)	60.0±0.6	58.9±0.5	59.1±0.8	59.3±0.4	59.0±0.4
Height (cm)	151.1±0.3	151.3±0.3	151.7±0.4	151.2±0.2	151.4±0.2
Body weight (kg)	52.3±0.4	52.4±0.4	53.9±0.6	52.3±0.3[b]	52.8±0.3
BMD measured with pQCT (mg/cm³)					
D50	177.6±3.2[c]	186.6±2.7	194.9±4.4	182.9±2.1[d]	188.8±2.3[e]
D100	481.8±4.6[f]	483.1±3.9[g]	500.8±6.4	482.6±3.0[h]	487.9±3.3
P100	1143.5±7.5	1154.3±6.2	1169.9±10.3	1149.9±4.8	1158.5±5.3
BMD measured with DXA (g/cm²)					
Total body	0.960±0.004	0.965±0.004	0.973±0.006	0.963±0.003	0.967±0.003
L2-L4	0.856±0.007	0.870±0.006	0.869±0.009	0.864±0.004	0.870±0.005
Femoral neck	0.677±0.004	0.677±0.004	0.679±0.006	0.677±0.003	0.678±0.003
Trochanter	0.564±0.004	0.574±0.004	0.576±0.006	0.570±0.003	0.574±0.003

[a]BMD is adjusted for age, height, and body weight. Data are means ± SE. [b]P=0.0148, [c]P=0.0046, [d]P=0.0142, [f]P=0.0439, [g]P=0.0489, [h]P=0.0101 versus *GG*; [e]P=0.0046 versus *AA*.

Table XII. BMD and other characteristics for postmenopausal women (n=807) according to the *ICAM1* genotype.[a]

Characteristic	*AA*	*AG*	*GG*	*AA + AG*	*AG + GG*
Number (%)	274 (34.0)	386 (47.8)	147 (18.2)	660 (81.8)	533 (66.0)
Age (years)	64.3±0.5	63.9±0.4	63.1±0.7	64.1±0.3	63.7±0.4
Height (cm)	150.0±0.4	150.0±0.3	150.8±0.5	150.0±0.2	150.2±0.3
Body weight (kg)	51.6±0.5	51.7±0.4	53.5±0.7	51.7±0.3[b]	52.2±0.4
BMD measured with pQCT (mg/cm³)					
D50	156.3±3.8[c]	163.9±3.3	176.0±5.2	160.7±2.5[d]	167.3±2.8[e]
D100	438.1±5.5	440.3±4.7	459.4±7.5	439.3±3.6[f]	445.6±4.0
P100	1068.4±9.1	1080.4±7.8	1097.6±12.5	1075.3±5.9	1085.2±6.6
BMD measured with DXA (g/cm²)					
Total body	0.910±0.005[g]	0.919±0.004	0.936±0.007	0.915±0.003[h]	0.923±0.004[i]
L2-L4	0.796±0.008	0.813±0.007	0.823±0.011	0.806±0.005	0.816±0.006[j]
Femoral neck	0.639±0.005	0.645±0.004	0.652±0.007	0.643±0.003	0.647±0.004
Trochanter	0.531±0.005[k]	0.543±0.004	0.551±0.007	0.538±0.003	0.545±0.004[l]

[a]BMD is adjusted for age, height, and body weight. Data are means ± SE. [b]P=0.0147, [c]P=0.0068, [d]P=0.0083, [f]P=0.0166, [g]P=0.0073, [h]P=0.0066, [k]P=0.0436 versus *GG*; [e]P=0.0205, [i]P=0.0319, [j]P=0.0441, [l]P=0.0220 versus *AA*.

AG genotypes, and was greater in the combined group of *AG* and *GG* genotypes than in individuals with the *AA* genotype (Table XI). BMD for D100 was greater in individuals with the *GG* genotype than in those with the *AA* genotype or the *AG* genotype or in the combined group of *AA* and *AG* genotypes. The differences in BMD for D50 and D100 between individuals with the *GG* genotype and those with the *AA* genotype were 8.9 and 3.8%, respectively.

For postmenopausal women, BMD for D50 and that for the total body were greater in individuals with the *GG* genotype than in those with the *AA* genotype or in the combined group of *AA* and *AG* genotypes, and were greater in the combined group of *AG* and *GG* genotypes than in individuals with the *AA* genotype (Table XII). BMD for D100 was greater in individuals with the *GG* genotype than in the combined group of *AA* and *AG* genotypes. BMD for the lumbar spine was greater in the combined group of *AG* and *GG* genotypes than in individuals with the *AA* genotype. BMD for the trochanter was greater in individuals with the *GG* genotype or in the combined group of *AG* and *GG* genotypes than in individuals with the *AA* genotype. The differences in BMD for D50, total body, and trochanter between individuals with the *GG* genotype and those with the *AA* genotype were 11.2, 2.8, and 3.6%, respectively. The difference in BMD for D100

between individuals with the *GG* genotype and the combined group of *AA* and *AG* genotypes and that for the lumbar spine between the combined group of *AG* and *GG* genotypes and individuals with the *AA* genotype were 4.4 and 2.5%, respectively. For premenopausal women, no relation was detected between *ICAM1* genotype and BMD.

For men, the distribution of A→G genotypes of *ICAM1* was in Hardy-Weinberg equilibrium, and BMD for P100 was greater in the combined group of *AA* and *AG* genotypes than in individuals with the *GG* genotype (data not shown).

Discussion

We have examined the relations of six candidate gene polymorphisms to BMD at various sites in community-dwelling Japanese women and men. Our results showed that the polymorphisms of *ALAP* and *PLOD1* were associated with BMD in premenopausal women; those of *ICAM1* and *LIPC* with BMD in postmenopausal women; that of *CNR2* with BMD in premenopausal and postmenopausal women; and that of *GJA4* with BMD in men. Among these polymorphisms, those of *ICAM1*, *CNR2*, and *GJA4* were markedly associated with BMD. These observations thus suggest that *ALAP*, *PLOD1*, *ICAM1*, *LIPC*, and *CNR2* are susceptibility loci for reduced bone mass in Japanese women and that *GJA4* constitutes such a locus in Japanese men. The polymorphisms of *ICAM1* and *CNR2* may confer susceptibility to postmenopausal osteoporosis in women, whereas that of *GJA4* may confer susceptibility to osteoporosis in men.

Association of the 386G→A (Ala99Thr) polymorphism of PLOD1 with BMD. *PLOD1* is located within a quantitative trait locus for regulation of BMD on chromosome 1p36 (17) and is a strong candidate gene for the regulation of BMD. *PLOD1* encodes the enzyme procollagen-lysine, 2-oxoglutarate 5-dioxygenase, which catalyzes the hydroxylation of lysine residues during the posttranslational modification of type I collagen, the major protein of bone matrix. Tasker *et al* (18) detected an association between BMD for the lumbar spine and the 386G→A (Ala99Thr) polymorphism of *PLOD1* in a population-based cohort of 678 Scottish women. Heterozygotes for this polymorphism had a reduced BMD and an increased hydroxylysylpyridinoline to lysylpyridinoline ratio compared with either group of homozygotes, suggesting a functional effect of this polymorphism on enzyme activity. Spotila *et al* (19) obtained evidence for an allelic association between a T→G polymorphism in intron 6 of *PLOD1* and BMD for the lumbar spine. This polymorphism and the 386G→A (Ala99Thr) polymorphism were in complete linkage disequilibrium. We have now shown that the 386G→A (Ala99Thr) polymorphism of *PLOD1* was associated with BMD for the femoral neck and trochanter for all women and premenopausal women, with the *A* allele being associated with reduced BMD. Our present results and the previous observations (18,19) thus suggest that *PLOD1* may be a susceptibility gene for reduced BMD in women.

Association of the A→G polymorphism of CNR2 with BMD. Two cannabinoid receptors, CB1 and CB2, encoded by *CNR1* and *CNR2*, respectively, are highly homologous, belong to the family of G protein-coupled seven-transmembrane domain receptors, and bind and are activated by endocannabinoids. *CNR1* is expressed predominantly in the brain and peripheral neurons (20), whereas *CNR2* is expressed mainly in immune cells (21). Mice with a targeted deletion of *CNR1* have an increased bone mass (22), whereas *CNR2* knockout mice have a decreased bone mass resembling human osteoporosis (23). These mouse genetic data implicate the endocannabinoid system in the regulation of bone mass. Furthermore, given that *CNR2* is located at chromosomal region 1p36, which has been implicated in osteoporosis (17), *CNR2* is a strong candidate determinant of susceptibility to osteoporosis. Karsak *et al* (24) detected an association of single polymorphisms and haplotypes encompassing *CNR2* on chromosome 1p36 with osteoporosis. We have now shown that the A→G polymorphism of *CNR2* was associated with BMD for the distal radius, total body, and lumbar spine in all women, with BMD for the distal radius and lumbar spine in premenopausal women, and with BMD for the distal radius, total body, and trochanter in postmenopausal women, with the *G* allele being related to reduced BMD. Our present results and the previous association study (24), as well as the observations with CB2-deficient mice (23), thus suggest that *CNR2* is a susceptibility gene for reduced BMD in women.

Association of the 1019C→T (Pro319Ser) polymorphism of GJA4 with BMD. Gap junction protein, α-4 (connexin37) is a gap junction protein in the arterial endothelium and contributes to the growth and regeneration after injury of endothelial cells (25). It forms functional intercellular channels with a voltage dependence and unitary conductance properties that are distinct from those of other channels (26). The 1019C→T (Pro319Ser) polymorphism of *GJA4* was previously associated with myocardial infarction (16) and coronary heart disease (27), with the *T* allele representing a risk factor for these conditions. Wong *et al* (28) recently showed that GJA4 protects against excessive monocyte recruitment in atherosclerosis, revealing an anti-inflammatory role for this protein *in vivo*. These researchers also showed that mononuclear cells expressing the 1019T (Ser319) allele of the *GJA4* polymorphism exhibited stronger adhesion than those expressing the 1019C (Pro319) allele, consistent with the observations that the 1019T (Ser319) allele is associated with an increased risk of myocardial infarction (16) and coronary heart disease (27). The anti-adhesive effect of GJA4 was shown to be mediated by release of ATP into the extracellular space. GJA4 hemichannels may thus control initiation of the development of atherosclerotic plaques by regulating monocyte adhesion (28). We have now shown that the 1019C→T (Pro319Ser) polymorphism of *GJA4* was associated with BMD for the total body, lumbar spine, femoral neck, and trochanter in men, with the *T* allele being associated with reduced BMD. This is the first demonstration of an association of *GJA4* with BMD, although the underlying molecular mechanism of the effect of this polymorphism on bone remodeling remains to be elucidated.

Association of the 1583G→A (Arg528Lys) polymorphism of ALAP with BMD. Adipocyte-derived leucine aminopeptidase