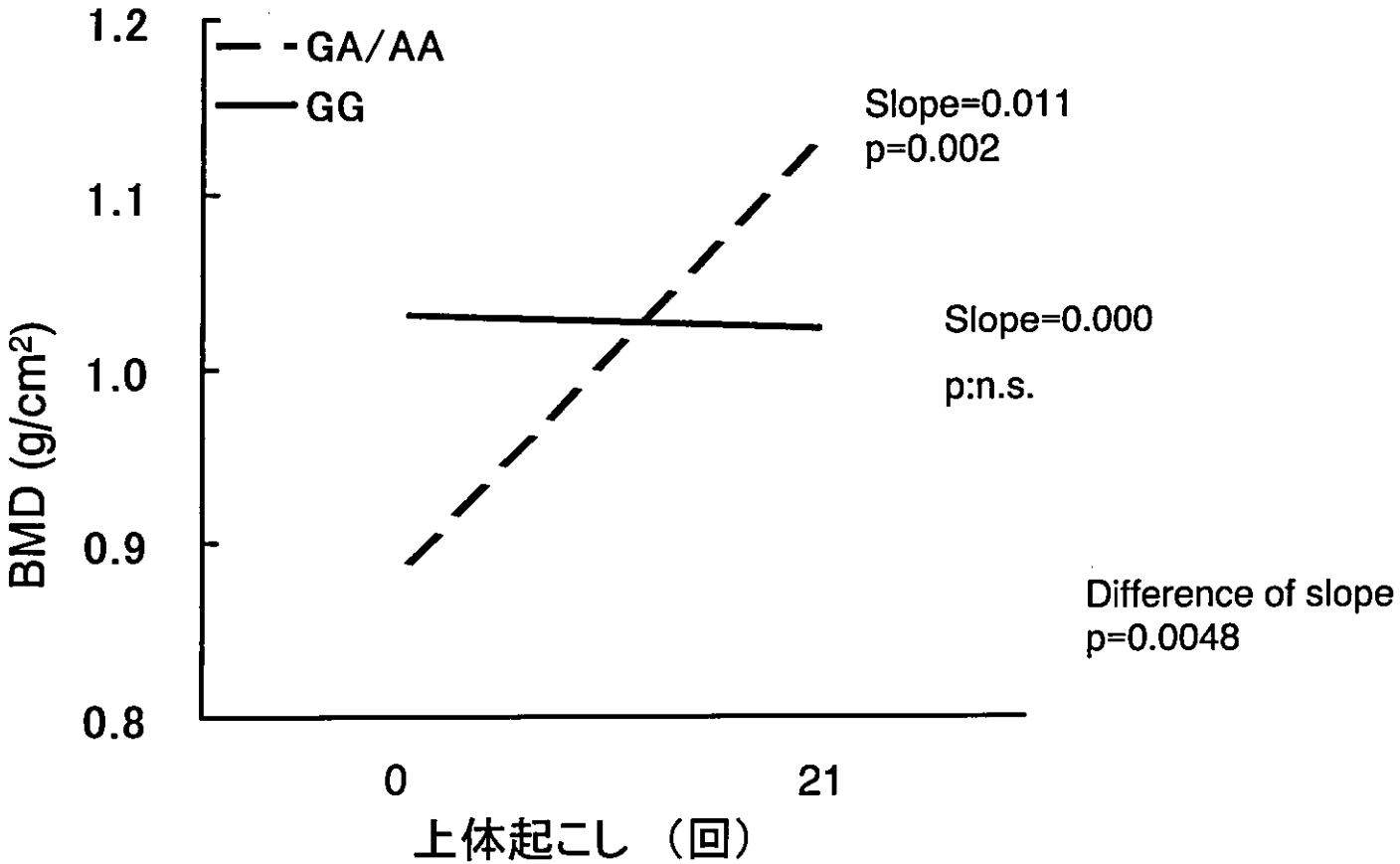
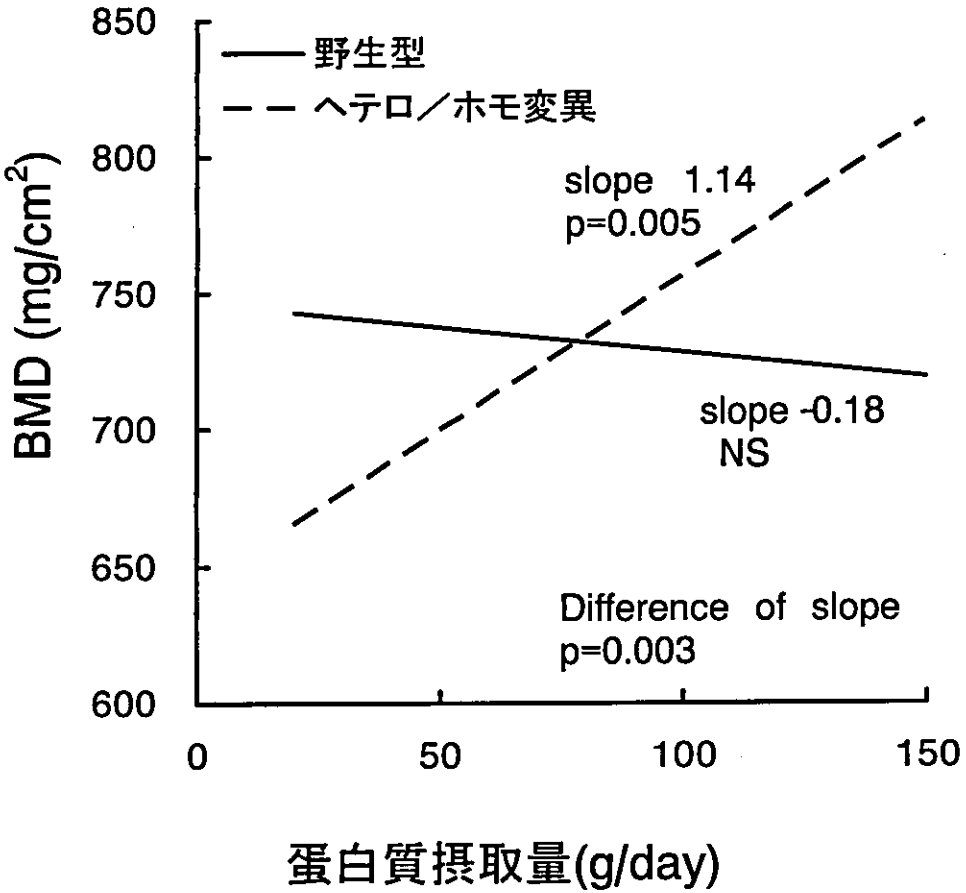
図4. Leptin Receptor (Gln223Arg) 遺伝子多型別の上体起こしと腰椎(L2-L4)骨密度との関連 (未閉経女性)



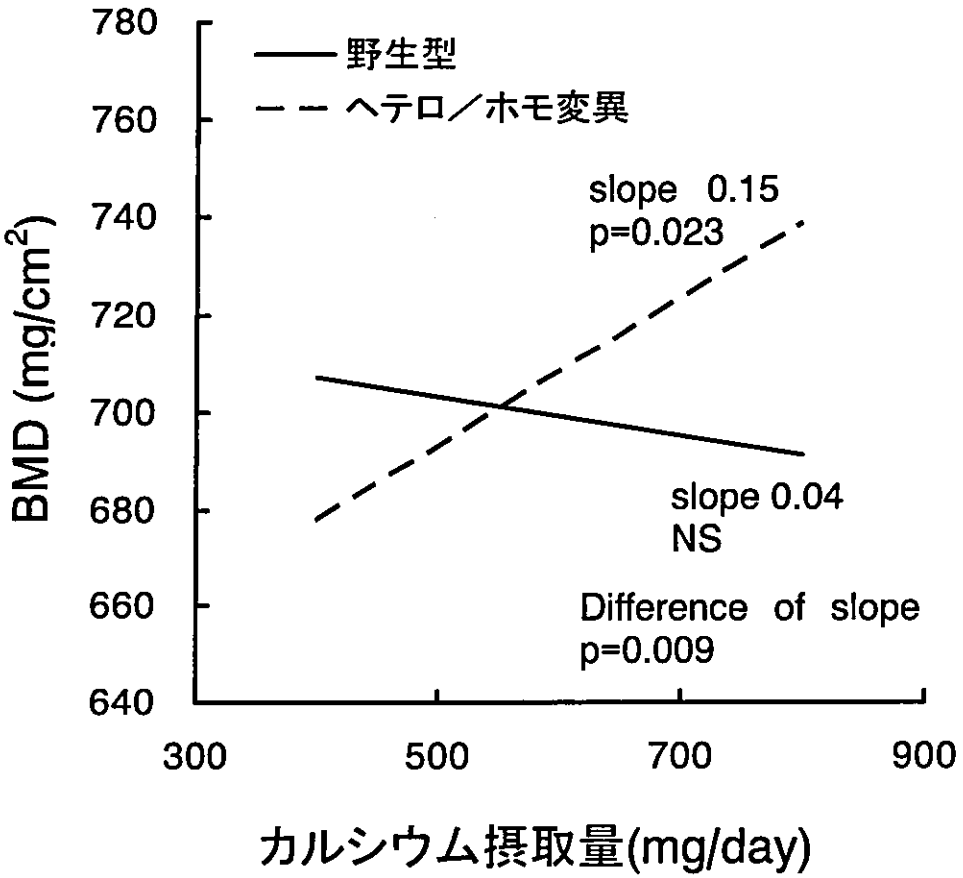
(年齢、BMIで調整)

図5. Leptin receptor (Gln223Arg)遺伝子多型別にみた
蛋白質摂取量による大腿骨頸部骨塩量との関連 （男性）



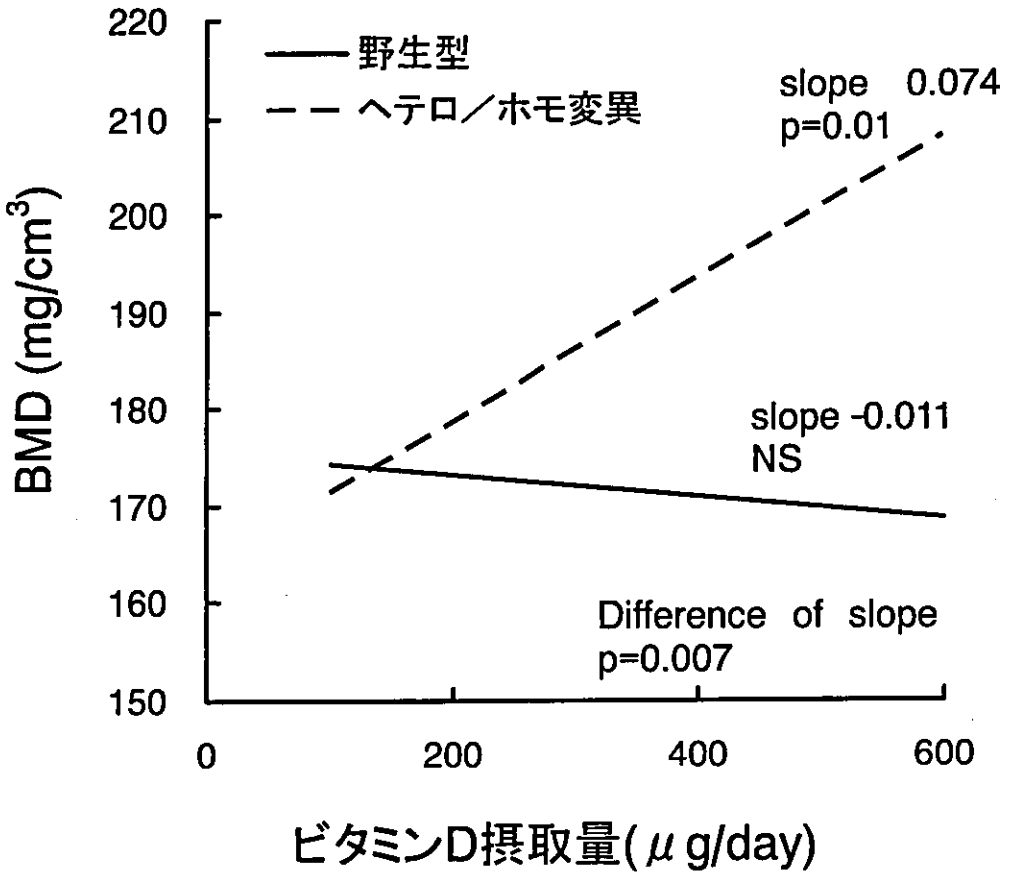
（年齢、BMI、エネルギー摂取量で調整）

図6. Interleukin-1 α (C-889T)遺伝子多型別にみた
カルシウム摂取量による大腿骨頸部骨塩量との関連(未閉経女性)



(年齢、BMI、エネルギー摂取量で調整)

図7. Werner helicase (1367Cys/Arg)遺伝子多型別にみた
ビタミンD摂取量による橈骨遠位部骨密度(pQCT D50)との関連 (閉経女性)



(年齢、BMI、エネルギー摂取量で調整)

表3 ロジスティック回帰分析結果(60歳未満男性)
結果変数:転倒歴2nd[無=0,有=1]

	Odds ratio	95%CI
D50(低)	0.66	0.36-1.20
握力(低)	1.47	0.81-2.69
主観的健康感(不良)	1.76	0.70-4.54
1st転倒歴(有)	7.33 ***	3.61-14.89
D100(低)	0.76	0.42-1.38
握力(低)	1.42	0.78-2.59
主観的健康感(不良)	1.78	0.70-4.50
1st転倒歴(有)	7.20 ***	3.56-14.57
P100(低)	0.63	0.34-1.18
握力(低)	1.37	0.76-2.51
主観的健康感(不良)	1.90	0.75-4.81
1st転倒歴(有)	7.49 ***	3.68-15.25
全身骨(低)	0.53 *	0.29-0.98
握力(低)	1.49	0.81-2.73
主観的健康感(不良)	1.69	0.66-4.32
1st転倒歴(有)	7.57 ***	3.69-15.52
腰椎(低)	0.60	0.33-1.10
握力(低)	1.51	0.83-2.77
主観的健康感(不良)	1.77	0.69-4.52
1st転倒歴(有)	7.41 ***	3.64-15.11
大腿骨頸部(低)	1.57	0.84-2.90
握力(低)	1.34	0.73-2.45
主観的健康感(不良)	1.73	0.68-4.37
1st転倒歴(有)	7.08 ***	3.50-14.34
大転子部(低)	1.24	0.67-2.31
握力(低)	1.37	0.75-2.51
主観的健康感(不良)	1.82	0.72-4.59
1st転倒歴(有)	7.24 ***	3.58-14.64
Ward三角(低)	1.33	0.72-2.44
握力(低)	1.37	0.75-2.51
主観的健康感(不良)	1.78	0.71-4.50
1st転倒歴(有)	7.30 ***	3.61-14.79

***p<.001

表4 ロジスティック回帰分析結果(60歳未満女性)
結果変数:転倒歴2nd[無=0,有=1]

	Odds ratio	95%CI
D50(低)	0.6 †	0.36-1.00
握力(低)	1.24	0.75-2.05
主観的健康感(不良)	.89	0.34-8.69
1st転倒歴(有)	4.96 ***	2.83-8.68
D100(低)	0.63 †	0.37-1.05
握力(低)	1.21	0.73-1.99
主観的健康感(不良)	.89	0.34-2.30
1st転倒歴(有)	5.08 ***	2.89-8.92
P100(低)	0.61 †	0.36-1.02
握力(低)	1.19	0.72-1.97
主観的健康感(不良)	.91	0.35-2.34
1st転倒歴(有)	5.01 ***	2.86-8.78
全身骨(低)	1.13	0.67-1.89
握力(低)	1.13	0.67-1.89
主観的健康感(不良)	.87	0.33-2.28
1st転倒歴(有)	4.81 ***	2.76-8.39
腰椎(低)	0.78	0.46-1.31
握力(低)	1.23	0.74-2.06
主観的健康感(不良)	.91	0.35-2.36
1st転倒歴(有)	4.73 ***	2.72-8.22
大腿骨頸部(低)	1	0.60-1.66
握力(低)	1.17	0.70-1.95
主観的健康感(不良)	.88	0.34-2.29
1st転倒歴(有)	4.75 ***	2.73-8.27
大転子部(低)	0.81	0.49-1.35
握力(低)	1.21	0.73-2.01
主観的健康感(不良)	.87	0.33-2.26
1st転倒歴(有)	4.79 ***	2.75-8.35
Ward三角(低)	0.79	0.47-1.31
握力(低)	1.23	0.74-2.05
主観的健康感(不良)	.90	0.34-2.36
1st転倒歴(有)	4.78 ***	2.75-8.32

†p<.10 ***p<.001

表5 ロジスティック回帰分析結果(60歳以上男性)
結果変数:転倒歴2nd[無=0,有=1]

	Odds ratio	95%CI
D50(低)	0.88	0.56-1.41
握力(低)	1.33	0.84-2.11
主観的健康感(不良)	1.75 †	0.90-3.40
1st転倒歴(有)	4.68 ***	2.73-8.03
D100(低)	1.06	0.67-1.69
握力(低)	1.28	0.81-2.04
主観的健康感(不良)	1.79 †	0.92-3.47
1st転倒歴(有)	4.63 ***	2.70-7.94
P100(低)	0.87	0.55-1.38
握力(低)	1.32	0.84-2.09
主観的健康感(不良)	1.76 †	0.91-3.41
1st転倒歴(有)	4.62 ***	2.69-7.91
全身骨(低)	1.14	0.71-1.81
握力(低)	1.26	0.79-2.01
主観的健康感(不良)	1.81 †	0.93-3.52
1st転倒歴(有)	4.66	2.72-7.99
腰椎(低)	0.85	0.54-1.34
握力(低)	1.31	0.83-2.07
主観的健康感(不良)	1.75 †	0.90-3.39
1st転倒歴(有)	4.63 ***	2.70-7.94
大腿骨頸部(低)	0.89	0.56-1.41
握力(低)	1.38	0.84-2.12
主観的健康感(不良)	1.78 †	0.92-3.44
1st転倒歴(有)	4.64 ***	2.71-7.96
大転子部(低)	0.91	0.57-1.44
握力(低)	1.32	0.83-2.09
主観的健康感(不良)	1.78 †	0.92-3.44
1st転倒歴(有)	4.61 ***	2.69-7.91
Ward三角(低)	1.28	0.79-2.06
握力(低)	1.21	0.75-1.95
主観的健康感(不良)	1.81 †	0.94-3.51
1st転倒歴(有)	4.73 ***	2.75-8.13

†p<.10 ***p<.001

表6 ロジスティック回帰分析結果(60歳以上女性)
結果変数:転倒歴2nd[無=0,有=1]

	Odds ratio	95%Cl
D50(低)	1.53 †	0.93-2.53
握力(低)	.94	0.58-1.54
主観的健康感(不良)	1.24	0.61-2.53
1st転倒歴(有)	4.55 ***	2.73-7.59
D100(低)	1.53 †	0.93-2.50
握力(低)	.97	0.59-1.57
主観的健康感(不良)	1.16	0.57-2.36
1st転倒歴(有)	4.49 ***	2.69-7.49
P100(低)	1.34	0.83-2.16
握力(低)	1.01	0.62-1.63
主観的健康感(不良)	1.14	0.56-2.33
1st転倒歴(有)	4.52 ***	2.71-7.53
全身骨(低)	1.55 †	0.95-2.54
握力(低)	.94	0.57-1.53
主観的健康感(不良)	1.21	0.60-2.46
1st転倒歴(有)	4.56 ***	2.73-7.61
腰椎(低)	.99	0.61-1.61
握力(低)	1.02	0.63-1.66
主観的健康感(不良)	1.17	0.57-2.37
1st転倒歴(有)	4.64 ***	2.79-7.73
大腿骨頸部(低)	1.10	0.68-1.79
握力(低)	1.01	0.62-1.63
主観的健康感(不良)	1.16	0.57-2.36
1st転倒歴(有)	4.63 ***	2.78-7.70
大転子部(低)	1.03	0.63-1.67
握力(低)	1.01	0.62-1.66
主観的健康感(不良)	1.17	0.57-2.37
1st転倒歴(有)	4.63 ***	2.78-7.72
Ward三角(低)	2.04 **	1.24-3.34
握力(低)	.92	0.56-1.50
主観的健康感(不良)	1.19	0.58-2.41
1st転倒歴(有)	4.61 ***	2.75-7.72

†p<.10 **p<.05 ***p<.001

Ⅱ．研究成果の刊行に関する一覧表

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Ⅲ. 研究成果の
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Association of Polymorphisms of Interleukin-6, Osteocalcin, and Vitamin D Receptor Genes, Alone or in Combination, with Bone Mineral Density in Community-Dwelling Japanese Women and Men

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We examined whether the −634C→G, 298C→T, and 2C→T polymorphisms of the IL-6, osteocalcin, and vitamin D receptor (VDR) genes, respectively, were associated, alone or in combination, with bone mineral density (BMD) in community-dwelling Japanese women (between 1108 and 1113) or men (between 1116 and 1130) aged 40–79 yr. The −634C→G polymorphism of the IL-6 gene and the 298C→T polymorphism of the osteocalcin gene were associated with BMD in postmenopausal women, with the respective GG and TT genotypes representing risk factors for reduced bone mass. IL-6 and osteocalcin genotypes showed additive effects on BMD for postmenopausal women. The 2C→T polymorphism of the VDR gene was associated with BMD in men, with the CT genotype contributing to reduced BMD. These results suggest that the IL-6 and osteocalcin genes are susceptibility loci for reduced BMD in postmenopausal women and that the VDR gene constitutes such a locus in men. The combined IL-6 and osteocalcin genotypes may prove informative for the assessment of osteoporosis in women. (*J Clin Endocrinol Metab* 88: 3372–3378, 2003)

OSTEOPOROSIS IS CHARACTERIZED by a decrease in bone mineral density (BMD) and a deterioration in the microarchitecture of bone, both of which result in an increased susceptibility to fractures (1). Although several environmental factors, such as diet and physical exercise, influence BMD, a genetic contribution to the etiology of osteoporosis has been recognized (2). Genetic linkage studies (3, 4) and candidate gene association studies (5, 6) have implicated several loci and candidate genes in the regulation of bone mass and the pathogenesis of osteoporotic fractures. Such candidate genes also include those for IL-6, osteocalcin, and the vitamin D receptor (VDR).

IL-6 is a multifunctional cytokine that is important in the development of postmenopausal osteoporosis (7). Sibling-pair analysis has provided evidence of linkage between the IL-6 gene locus and reduced BMD in postmenopausal Japanese women (8). Three polymorphisms of the IL-6 gene have been identified in Japanese, among which a C→G substitution at nucleotide −634 in the promoter region has been associated with radial BMD (9). A variable number of tandem repeats polymorphism in the 3′ flanking region of the IL-6 gene has also been associated with BMD in postmenopausal Caucasian women (10).

Osteocalcin is an extracellular matrix protein that is abundant in bone. Characterization of osteocalcin-deficient mice demonstrated that this protein functions as a negative regulator of bone formation (11). A C→T polymorphism at nucleotide 298 in the promoter region of the osteocalcin gene was identified and shown to be associated with BMD in a small Japanese population (12).

A *Bsm*I restriction fragment length polymorphism (RFLP) of the VDR gene was shown to be associated with BMD in Australian women (13). A 2C→T polymorphism (ACG→ATG) at the translation initiation site of the VDR gene has also been associated with BMD in postmenopausal Mexican-American (14) and Japanese (15) women, but not in premenopausal French women (16). The 2C→T polymorphism was also associated with BMD in men (17, 18) and with calcium absorption and BMD in girls and boys of various ethnic ancestries (19).

Although the association of these various single nucleotide polymorphisms (SNPs) with BMD suggests that the IL-6, osteocalcin, and VDR genes might be susceptibility loci for osteoporosis in women, large-scale population-based studies identifying the association of these SNPs with BMD simultaneously are required to clarify their roles in determination of this parameter. In addition, the relation of these SNPs to BMD in men has not been definitively identified. We have now examined whether the −634C→G SNP of the IL-6 gene, the 298C→T SNP of the osteocalcin gene, and the 2C→T SNP of the VDR gene are associated with BMD in women or men in a large-scale population-based study.

Abbreviations: BMD, Bone mineral density; BMI, body mass index; DXA, dual-energy X-ray absorptiometry; PCR, polymerase chain reaction; pQCT, peripheral quantitative computed tomography; RFLP, restriction fragment length polymorphism; SNP, single nucleotide polymorphism; VDR, vitamin D receptor.

Subjects and Methods

Study population

The National Institute for Longevity Sciences–Longitudinal Study of Aging (NILS-LSA) is a population-based prospective cohort study of

aging and age-related diseases (20). The subjects of the NILS-LSA are stratified by both age and gender, and are randomly selected from resident registrations in the city of Obu and town of Higashiura in central Japan. The lifestyle of residents of this area is typical of that of individuals in most regions of Japan. The numbers of men and women recruited are similar, and age at the baseline is 40–79 yr, with similar numbers of participants in each decade (40s, 50s, 60s, and 70s). The subjects will be followed up every 2 yr. All participants are subjected at a special center to a detailed examination, which includes not only medical evaluation but also assessment of exercise physiology, body composition, nutrition, and psychology. We have examined the association of BMD at various sites with the −634C→G SNP of the IL-6 gene in 2239 participants (1113 women, 1126 men), with the 298C→T SNP of the osteocalcin gene in 2224 participants (1108 women, 1116 men), and with the 2C→T SNP of the VDR gene in 2238 participants (1108 women, 1130 men). The study protocol was approved by the Committee on Ethics of Human Research of National Chubu Hospital and the NILS, and written informed consent was obtained from each subject.

Measurement of BMD

BMD of the nondominant radius was measured by peripheral quantitative computed tomography (pQCT) (Desiscan 1000, Scanco Medical, Bassersdorf, Switzerland), with the forearm positioned in a cast during measurement. For measurement of BMD of the distal radius, the examination was initiated at a position 6.0 mm from the distal end of the radius and progressed proximally with 10 tomographs (slice thickness, 1 mm; interslice distance, 0.5 mm), and the average BMD was calculated. D50 represented distal radius BMD for the inner 50% of the cross-sectional area, comprising mostly cancellous bone, and D100 represented that for the entire cross-sectional area, including both cancellous and cortical bone. BMD for the proximal radius was measured at the diaphysis; the examination was initiated at a site 27.5 mm from the last slice of the distal radius and proceeded proximally with six tomographs, and the average BMD was calculated. P100 represented proximal radius BMD for the entire cross-sectional area, consisting mostly of cortical bone. BMD for total body, lumbar spine (L2–L4), right femoral neck, right trochanter, and right Ward's triangle was measured by dual-energy x-ray absorptiometry (DXA) (QDR 4500; Hologic, Inc., Bedford, MA). The coefficients of variance 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), 1.0% (trochanter), and 2.5% (Ward's triangle).

Determination of IL-6, osteocalcin, and VDR genotypes

IL-6, osteocalcin, and VDR genotypes were determined with a fluorescence- or colorimetry-based allele-specific DNA primer assay system (Toyobo Gene Analysis, Tsuruga, Japan). For determination of IL-6 genotype, the polymorphic region of the gene was amplified by the polymerase chain reaction (PCR) with allele-specific sense primers labeled at the 5′ end either with fluorescein isothiocyanate (5′-GGCAGTTCTACAACAGCXCC-3′) or with Texas red (5′-GCAGTTCTACAACAGCXGC-3′) and an antisense primer labeled at the 5′ end with biotin (5′-CTGTGTTCTGGCTCTCCCTG-3′). For determination of osteocalcin or VDR genotype, the polymorphic region of the gene was amplified by PCR with allele-specific sense primers (5′-CAGCTCCCAACCACAATATCCXTT-3′ or 5′-CAGCTCCCAACCACAATATCCXCT-3′ for osteocalcin genotype, and 5′-CTTGCTGTTCTTACAGGGXTG-3′ or 5′-CTTGCTGTTCTTACAGGGXCG-3′ for VDR genotype) and an antisense primer labeled at the 5′ end with biotin (5′- GTGTGAGGGCTCTCATGGTGT-3′ for osteocalcin genotype, 5′-AAGTGCTGGCCGCCATTG-3′ for VDR genotype). The reaction mixture (25 μl) contained 20 ng of DNA, 5 pmol of each primer, 0.2 mmol/liter of each deoxynucleoside triphosphate, 1–4 mmol/liter $MgCl_2$, and 1 U DNA polymerase (rTaq or KODplus; Toyobo, Osaka, Japan) in the respective DNA polymerase buffer. The amplification protocol comprised initial denaturation at 95 C for 5 min; 35 (IL-6 genotype), 45 (osteocalcin genotype), or 40 (VDR genotype) cycles of denaturation at 95 C for 30 sec, annealing at 55–65 C for 30 sec, and extension at 72 C for 30 sec; and a final extension at 72 C for 2 min.

For determination of IL-6 genotype, 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 were then collected from each well, transferred to the wells of a 96-well plate containing 0.01 mol/liter NaOH, and 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 fluorescein isothiocyanate and of 584 and 612 nm, respectively, for Texas red.

For determination of osteocalcin or VDR genotype, amplified DNA was denatured with 0.3 mol/liter NaOH and then subjected to hybridization at 37 C for 30 min in hybridization buffer containing 35–40% formamide with allele-specific capture probes (5′-CACAATATCCXTTGGGGTTT-3′ or 5′-CACAATATCCXCTGGGGTT-3′ for osteocalcin genotype, and 5′-TACAGGGXTGGAGGCAATG-3′ or 5′-TACAGGGXCGGAGGCAATG-3′ for VDR genotype) fixed to the bottom of the wells of a 96-well plate. After thorough washing of the wells, alkaline phosphatase-conjugated streptavidin was added to each, and the plate was incubated at 37 C for 15 min with agitation. The wells were washed again, and, after the addition of a solution containing 0.8 mmol/liter 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium (monosodium salt) and 0.4 mmol/liter 5-bromo-4-chloro-3-indolyl phosphate *p*-toluidine salt, absorbance at 450 nm was measured.

To confirm the accuracy of genotyping by this method, we selected 50 DNA samples at random and subjected them to PCR-RFLP analysis or direct DNA sequencing of PCR products. In each instance, the genotype determined by the allele-specific DNA primer assay system was identical to that determined by PCR-RFLP analysis or DNA sequencing.

Statistical analysis

Quantitative data were compared by one-way ANOVA and the Tukey-Kramer *post hoc* test. BMD values were analyzed with adjustment for age and body mass index (BMI) by the least-squares method in a general linear model. Qualitative data were analyzed by the χ^2 test. Allele frequencies were estimated by the gene-counting method, and the χ^2 test was used to identify significant departure from Hardy-Weinberg equilibrium. $P \leq 0.05$ was considered statistically significant.

Results

The relation of the −634C→G SNP in the promoter region of the IL-6 gene to BMD was examined. The distributions of IL-6 genotypes with regard to this SNP in women (Table 1) and in men (Table 2) were in Hardy-Weinberg equilibrium. Age and BMI did not differ among IL-6 genotypes in women (Table 1). For men, age did not differ among IL-6 genotypes, but BMI was significantly greater for those with the GG genotype than for those with the CG genotype (Table 2). Among all women, BMD for the total body or lumbar spine was smaller in those with the GG genotype than in those with the CC or CG genotypes, with adjustment for age and BMI (Table 1). To examine the influence of menopause on the relation between −634C→G genotype and BMD, we analyzed BMD and other characteristics for premenopausal and postmenopausal women independently. Given that the number of perimenopausal women was small (n = 17), these subjects were excluded from the analysis. For premenopausal women, BMD was not associated with IL-6 genotype. In contrast, for postmenopausal women, BMD for the distal radius (D50 and D100), total body, lumbar spine, femoral neck, trochanter, or Ward's triangle was significantly lower in those with the GG genotype than in those with the CC or CG genotypes (Table 1). The differences in BMD between the CC and GG genotypes (expressed as a percentage of the corresponding larger value) in postmenopausal women were 13.7% (D50), 7.2% (D100), 4.3% (total body), 8.4% (lumbar spine), 5.3% (femoral neck), 5.2% (trochanter), and 8.6%

TABLE 1. BMD and other characteristics of women according to IL-6 genotype

Characteristic	Total (n = 1113)			Premenopausal (n = 279)			Postmenopausal (n = 817)		
	CC	CG	GG	CC	CG	GG	CC	CG	GG
Number (%)	638 (57.3)	406 (36.5)	69 (6.2)	170 (60.9)	91 (32.6)	18 (6.5)	455 (55.7)	311 (38.1)	51 (6.2)
Age (yr)	59.1 ± 0.4	59.4 ± 0.5	60.4 ± 1.3	46.4 ± 0.4	45.8 ± 0.5	46.2 ± 1.1	64.1 ± 0.4	63.5 ± 0.5	65.4 ± 1.2
BMI (kg/m^2)	22.9 ± 0.1	22.9 ± 0.2	23.1 ± 0.4	22.6 ± 0.2	23.1 ± 0.3	22.6 ± 0.8	23.0 ± 0.2	22.8 ± 0.2	23.2 ± 0.5
BMD values measured by pQCT (mg/cm^3)									
D50	185.5 ± 2.5	184.3 ± 3.1	171.7 ± 7.6	244.9 ± 4.2	244.8 ± 5.8	255.8 ± 13.5	165.1 ± 3.0	164.5 ± 3.6	142.4 ± 9.0[a,b]
D100	486.2 ± 3.6	483.8 ± 4.5	465.2 ± 11.0	607.8 ± 6.0	598.8 ± 8.3	616.5 ± 19.3	444.0 ± 4.3	446.1 ± 5.2	412.2 ± 13.0[a,b]
P100	1153.1 ± 5.8	1148.4 ± 7.3	1126.7 ± 17.8	1360.1 ± 9.2	1354.8 ± 12.7	1376.5 ± 29.5	1081.7 ± 7.1	1080.4 ± 8.6	1039.8 ± 21.5
BMD values measured by DXA (g/cm^2)									
Total body	0.968 ± 0.003	0.961 ± 0.004	0.938 ± 0.010[a]	1.093 ± 0.006	1.098 ± 0.009	1.089 ± 0.020	0.924 ± 0.004	0.916 ± 0.005	0.884 ± 0.012[b,c]
L2–L4	0.869 ± 0.005	0.867 ± 0.006	0.807 ± 0.015[a,d]	1.026 ± 0.009	1.036 ± 0.012	0.974 ± 0.027	0.813 ± 0.006	0.813 ± 0.007	0.745 ± 0.018[e,f]
Femoral neck	0.678 ± 0.003	0.679 ± 0.004	0.655 ± 0.010	0.770 ± 0.007	0.775 ± 0.010	0.777 ± 0.022	0.646 ± 0.004	0.646 ± 0.005	0.612 ± 0.012[a,b]
Trochanter	0.572 ± 0.003	0.571 ± 0.004	0.554 ± 0.010	0.654 ± 0.006	0.665 ± 0.009	0.665 ± 0.020	0.543 ± 0.004	0.539 ± 0.005	0.515 ± 0.012[a]
Ward's triangle	0.508 ± 0.005	0.507 ± 0.006	0.480 ± 0.014	0.657 ± 0.009	0.665 ± 0.013	0.658 ± 0.028	0.456 ± 0.005	0.453 ± 0.007	0.417 ± 0.016[a]

Data are expressed as means ± SE. BMD values were adjusted for age and BMI.
[a] $P \leq 0.05$ *vs.* CC; [b] $P \leq 0.05$ *vs.* CG; [c] $P \leq 0.005$ *vs.* CC; [d] $P \leq 0.001$ *vs.* CC; [e] $P \leq 0.001$ *vs.* CC; [f] $P \leq 0.005$ *vs.* CG.

TABLE 2. BMD and other characteristics of men (n = 1126) according to IL-6 genotype

Characteristic	CC	CG	GG
Number (%)	664 (59.0)	392 (34.8)	70 (6.2)
Age (yr)	59.7 ± 0.4	58.7 ± 0.6	57.2 ± 1.3
BMI (kg/m^2)	23.0 ± 0.1	22.7 ± 0.1	23.7 ± 0.3[a]
BMD values measured by pQCT (mg/cm^3)			
D50	268.4 ± 2.6	263.6 ± 3.4	265.6 ± 7.8
D100	543.4 ± 3.6	537.7 ± 4.6	534.6 ± 10.9
P100	1189.6 ± 5.5	1180.5 ± 7.2	1172.9 ± 16.7
BMD values measured by DXA (g/cm^2)			
Total body	1.088 ± 0.004	1.088 ± 0.005	1.077 ± 0.011
L2–L4	0.985 ± 0.006	0.982 ± 0.008	0.966 ± 0.018
Femoral neck	0.756 ± 0.004	0.752 ± 0.005	0.736 ± 0.012
Trochanter	0.670 ± 0.004	0.668 ± 0.005	0.652 ± 0.012
Ward's triangle	0.556 ± 0.005	0.549 ± 0.006	0.541 ± 0.014

Data are expressed as means ± SE. BMD values were adjusted for age and BMI.
[a] $P \leq 0.05$ *vs.* CG.

(Ward's triangle). No significant difference in BMD among IL-6 genotypes was detected for men (Table 2).

The relation of the 298T→C SNP in the promoter region of the osteocalcin gene to BMD was examined. The distributions of osteocalcin genotypes with regard to this SNP were in Hardy-Weinberg equilibrium in women (Table 3) and in men (Table 4). Age did not differ among osteocalcin genotypes for all women or for premenopausal women, but it was greater for postmenopausal women with the TT genotype than for those with the CC genotype (Table 3). For men, age did not differ among osteocalcin genotypes (Table 4). BMI did not differ among osteocalcin genotypes in all, premenopausal, or postmenopausal women or in men. Among women, BMD for the total body, lumbar spine, or Ward's triangle was greater in those with the CC genotype than in those with the TT or CT genotypes (Table 3). For premenopausal women, BMD was not associated with osteocalcin genotype. In contrast, for postmenopausal women, BMD for the total body, lumbar spine, femoral neck, trochanter, or Ward's triangle was greater in those with the CC genotype than in those with the TT or CT genotypes. The differences in BMD between the CC and TT genotypes in postmenopausal women were 4.4% (total body), 8.3% (lumbar spine), 5.3% (femoral neck), 6.5% (trochanter), and 14.7% (Ward's triangle). For men, BMD did not differ significantly among osteocalcin genotypes (Table 4).

The relation of the 2C→T SNP in the translation initiation codon of the VDR gene to BMD was examined. The distributions of VDR genotypes with regard to this SNP in women (Table 5) and in men (Table 6) were in Hardy-Weinberg equilibrium. Age and BMI did not differ among VDR genotypes in women (Table 5) or in men (Table 6). BMD did not differ among VDR genotypes for all women or for premenopausal or postmenopausal women (Table 5, data not shown). Among men, BMD for the distal radius (D50), femoral neck, or Ward's triangle was smaller in those with the CT genotype than in those with the CC genotype (Table 6). The differences in BMD between the CC and CT genotypes in men were 3.6% (D50), 2.1% (femoral neck), and 3.4% (Ward's triangle).

The effects of IL-6, osteocalcin, and VDR genotypes as well as of other characteristics on BMD at various sites were analyzed by multivariate regression analysis (Table 7). For

TABLE 3. BMD and other characteristics of women according to osteocalcin genotype

Characteristic	Total (n = 1108)			Premenopausal (n = 278)			Postmenopausal (n = 813)		
	CC	CT	TT	CC	CT	TT	CC	CT	TT
Number (%)	50 (4.5)	357 (32.2)	701 (63.3)	12 (4.3)	86 (30.9)	180 (64.8)	38 (4.7)	265 (32.6)	510 (62.7)
Age (yr)	57.5 ± 1.5	59.0 ± 0.6	59.5 ± 0.4	46.7 ± 1.3	46.1 ± 0.5	46.2 ± 0.3	60.9 ± 1.4	63.3 ± 0.5	64.4 ± 0.4[a]
BMI (kg/m^2)	23.7 ± 0.5	22.9 ± 0.2	22.8 ± 0.1	24.1 ± 0.9	23.0 ± 0.3	22.5 ± 0.2	23.5 ± 0.5	22.8 ± 0.2	23.0 ± 0.1
BMD values measured by pQCT (mg/cm^3)									
D50	195.8 ± 8.7	189.5 ± 3.3	181.0 ± 2.4	240.8 ± 16.4	252.5 ± 6.0	241.9 ± 4.1	182.3 ± 10.1	168.5 ± 3.9	159.9 ± 2.8
D100	494.5 ± 12.5	489.2 ± 4.8	481.4 ± 3.4	620.7 ± 23.2	613.5 ± 8.5	601.0 ± 5.9	456.0 ± 14.6	447.7 ± 5.6	440.1 ± 4.1
P100	1175.4 ± 20.3	1152.0 ± 7.7	1148.9 ± 5.5	1404.2 ± 35.7	1359.7 ± 13.0	1358.5 ± 9.0	1103.3 ± 24.1	1082.2 ± 9.3	1076.9 ± 6.7
BMD values measured by DXA (g/cm^2)									
Total body	0.993 ± 0.012	0.968 ± 0.005	0.959 ± 0.003[b]	1.109 ± 0.024	1.099 ± 0.009	1.090 ± 0.006	0.955 ± 0.014	0.924 ± 0.005	0.913 ± 0.004[b]
L2–L4	0.910 ± 0.018	0.867 ± 0.007	0.860 ± 0.005[a]	1.029 ± 0.034	1.027 ± 0.013	1.024 ± 0.009	0.875 ± 0.021	0.814 ± 0.008[a]	0.802 ± 0.006[b]
Femoral neck	0.694 ± 0.012	0.681 ± 0.005	0.674 ± 0.003	0.745 ± 0.027	0.781 ± 0.010	0.769 ± 0.007	0.676 ± 0.014	0.648 ± 0.005	0.640 ± 0.004[a]
Trochanter	0.588 ± 0.012	0.574 ± 0.004	0.567 ± 0.003	0.626 ± 0.024	0.665 ± 0.009	0.656 ± 0.006	0.573 ± 0.013	0.543 ± 0.005	0.536 ± 0.004[a]
Ward's triangle	0.554 ± 0.016	0.510 ± 0.006[a]	0.501 ± 0.004[c]	0.639 ± 0.035	0.672 ± 0.013	0.654 ± 0.009	0.524 ± 0.019	0.454 ± 0.007[c]	0.447 ± 0.005[d]

Data are expressed as means ± SE. BMD values were adjusted for age and BMI.
[a] $P \leq 0.05$ *vs.* CC; [b] $P \leq 0.01$ *vs.* CC; [c] $P \leq 0.005$ *vs.* CC; [d] $P \leq 0.001$ *vs.* CC.

TABLE 4. BMD and other characteristics of men (n = 1116) according to osteocalcin genotype

Characteristic	CC	CT	TT
n (%)	56 (5.0)	380 (34.1)	680 (60.9)
Age (yr)	60.4 ± 1.5	58.8 ± 0.6	59.2 ± 0.4
BMI (kg/m^2)	22.3 ± 0.4	23.0 ± 0.1	22.9 ± 0.1
BMD values measured by pQCT (mg/cm^3)			
D50	260.6 ± 8.9	272.3 ± 3.4	264.0 ± 2.5
D100	539.6 ± 12.3	545.9 ± 4.8	538.5 ± 3.5
P100	1208.6 ± 18.9	1187.3 ± 7.3	1182.1 ± 5.4
BMD values measured by DXA (g/cm^2)			
Total body	1.096 ± 0.012	1.095 ± 0.005	1.082 ± 0.003
L2–L4	0.968 ± 0.020	0.992 ± 0.008	0.978 ± 0.006
Femoral neck	0.743 ± 0.013	0.761 ± 0.005	0.749 ± 0.004
Trochanter	0.651 ± 0.013	0.673 ± 0.005	0.666 ± 0.004
Ward's triangle	0.538 ± 0.016	0.557 ± 0.006	0.551 ± 0.005

Data are expressed as means ± SE. BMD values were adjusted for age and BMI.

TABLE 5. BMD and other characteristics of women (n = 1108) according to VDR genotype

Characteristic	CC	CT	TT
Number (%)	457 (41.3)	504 (45.5)	147 (13.3)
Age (yr)	59.9 ± 0.5	58.7 ± 0.5	59.3 ± 0.9
BMI (kg/m^2)	22.8 ± 0.2	23.1 ± 0.1	22.7 ± 0.3
BMD values measured by pQCT (mg/cm^3)			
D50	184.3 ± 2.9	184.2 ± 2.8	190.0 ± 5.1
D100	486.2 ± 4.2	484.3 ± 4.0	487.4 ± 7.4
P100	1153.5 ± 6.8	1151.1 ± 6.5	1159.9 ± 12.0
BMD values measured by DXA (g/cm^2)			
Total body	0.966 ± 0.004	0.965 ± 0.004	0.965 ± 0.007
L2–L4	0.871 ± 0.006	0.861 ± 0.006	0.866 ± 0.011
Femoral neck	0.678 ± 0.004	0.681 ± 0.004	0.670 ± 0.007
Trochanter	0.570 ± 0.004	0.573 ± 0.004	0.566 ± 0.007
Ward's triangle	0.507 ± 0.005	0.509 ± 0.005	0.500 ± 0.010

Data are expressed as means ± SE. BMD values were adjusted for age and BMI.

women, IL-6 genotype significantly affected BMD for the total body and lumbar spine, and osteocalcin genotype significantly contributed to BMD for the distal radius (D50), total body, lumbar spine, femoral neck, trochanter, and Ward's triangle. For men, osteocalcin genotype significantly influenced BMD for the total body, and VDR genotype significantly contributed to BMD for the distal radius (D50), femoral neck, trochanter, and Ward's triangle.

To determine whether the −634C→G SNP of the IL-6 gene and the 298C→T SNP of the osteocalcin gene exert an additive effect on BMD in women, we examined the association between the combined genotype and BMD. The distribution of the combined IL-6 and osteocalcin genotypes for women is shown in Table 8. The IL-6 and osteocalcin genes are located on chromosomes 7p21 and 1q25-q31, respectively, and no significant relation between the distributions of the corresponding genotypes was detected. Given the small number of subjects with the GG/CC genotype (n = 4), it was excluded from the analysis of combined genotype. Age and BMI did not differ among the remaining eight combined genotypes in premenopausal (data not shown) or postmenopausal (Table 9) women. For premenopausal women, there were no differences in BMD at the sites examined among combined genotypes (data not shown). For postmenopausal women, BMD for the total body, lumbar spine, or Ward's

TABLE 6. BMD and other characteristics of men (n = 1130) according to VDR genotype

Characteristic	CC	CT	TT
Number (%)	448 (39.7)	520 (46.0)	162 (14.3)
Age (yr)	58.7 ± 0.5	59.7 ± 0.5	59.2 ± 0.9
BMI (kg/m^2)	22.9 ± 0.1	22.9 ± 0.1	23.0 ± 0.2
BMD values measured by pQCT (mg/cm^3)			
D50	273.2 ± 3.1	263.5 ± 2.9[a]	261.1 ± 5.1
D100	546.4 ± 4.4	537.2 ± 4.1	537.9 ± 7.2
P100	1190.9 ± 6.7	1181.7 ± 6.2	1183.1 ± 11.0
BMD values measured by DXA (g/cm^2)			
Total body	1.094 ± 0.004	1.082 ± 0.004	1.089 ± 0.007
L2–L4	0.988 ± 0.007	0.979 ± 0.007	0.985 ± 0.012
Femoral neck	0.763 ± 0.005	0.747 ± 0.004[a]	0.748 ± 0.008
Trochanter	0.677 ± 0.005	0.663 ± 0.004	0.664 ± 0.008
Ward's triangle	0.563 ± 0.006	0.544 ± 0.005[a]	0.548 ± 0.009

Data are expressed as means ± SE. BMD values were adjusted for age and BMI.
[a] $P \leq 0.05$ *vs.* CC.

triangle was lower in those with the GG/*TT* genotype than in those with the CC/CC, CC/CT, CC/*TT*, CG/CC, CG/CT, or CG/*TT* genotypes. The differences in BMD for the total body between the GG/*TT* and CC/CC genotypes, for the lumbar spine between GG/*TT* and CG/CC genotypes, and for Ward's triangle between GG/*TT* and CC/CC genotypes in postmenopausal women were 8.6, 17.7, and 24.0%, respectively.

Discussion

Given that selection bias can influence the results of association studies, it is important that study populations be genetically and ethnically homogeneous. Our study population was recruited randomly from individuals resident in Obu city and Higashiura town in central Japan, where the population is thought to share the same ethnic ancestry and to possess a homogeneous genetic background (20). We also showed that the genotype distributions of the IL-6, osteocalcin, and VDR genes were in Hardy-Weinberg equilibrium both for women and for men. Our study population therefore appeared genetically homogeneous, and we thus appeared to avoid admixture and selection bias.

For the −634C→G SNP of the IL-6 gene, BMD was reduced in postmenopausal women with the GG genotype compared with that in those with the CC or CG genotypes, consistent with the results of a previous study of 470 postmenopausal Japanese women (9). In this previous study, however, only radial BMD was measured (9). Our results now show that IL-6 genotype is associated with BMD not only of the distal radius but also of the total body, lumbar spine, femoral neck, trochanter, and Ward's triangle in a large population of postmenopausal Japanese women.

For the 298C→T SNP of the osteocalcin gene, BMD decreased according to the rank order of genotypes CC > *CT* > *TT* in postmenopausal women. This observation differs from previous results obtained with 160 postmenopausal Japanese women showing that BMD of the lumbar spine increased according to the rank order of genotypes CC < *CT* < *TT*, although the observed differences were not statistically significant (12). Our present results demonstrate an association of osteocalcin genotype with BMD for the total body, femoral neck, trochanter, and Ward's triangle as well as for the lum-

TABLE 7. Effects of IL-6, osteocalcin, and VDR genotypes as well as of other characteristics on BMD for women and men determined by multivariate regression analysis

	Women						Men				
BMD value	Age (yr)	BMI (kg/m^2)	Menstrual state	IL-6 genotype	Osteocalcin genotype	VDR genotype	Age (yr)	BMI (kg/m^2)	IL-6 genotype	Osteocalcin genotype	VDR genotype
D50	0.325 (<0.001)	0.017 (<0.001)	0.010 (<0.001)	NS	0.004 (0.009)	NS	0.105 (<0.001)	0.019 (<0.001)	NS	NS	0.005 (0.014)
D100	0.492 (<0.001)	0.007 (<0.001)	0.011 (<0.001)	NS	NS	NS	0.155 (<0.001)	0.007 (0.003)	NS	NS	NS
P100	0.549 (<0.001)	0.003 (0.005)	0.005 (0.001)	NS	NS	NS	0.165 (<0.001)	NS	NS	NS	NS
Total body	0.555 (<0.001)	0.010 (<0.001)	0.011 (<0.001)	0.003 (0.011)	0.004 (0.001)	NS	0.050 (<0.001)	0.075 (<0.001)	NS	0.004 (0.027)	NS
L2–L4	0.396 (<0.001)	0.057 (<0.001)	0.022 (<0.001)	0.004 (0.007)	0.003 (0.010)	NS	NS	0.137 (<0.001)	NS	NS	NS
Femoral neck	0.405 (<0.001)	0.063 (<0.001)	0.002 (0.046)	NS	0.002 (0.043)	NS	0.083 (<0.001)	0.157 (<0.001)	NS	NS	0.004 (0.018)
Trochanter	0.386 (<0.001)	0.082 (<0.001)	NS	NS	0.002 (0.035)	NS	0.016 (<0.001)	0.176 (<0.001)	NS	NS	0.003 (0.051)
Ward's triangle	0.498 (<0.001)	0.031 (<0.001)	0.003 (0.006)	NS	0.004 (0.004)	NS	0.220 (<0.001)	0.060 (<0.001)	NS	NS	0.003 (0.041)

Data are R^2 (P) values from multivariate regression analysis of age, BMI, menstrual state (0 = premenopause; 1 = postmenopause), IL-6 genotype (0 = CC; 1 = CG; 2 = GG), osteocalcin genotype (0 = CC; 1 = *CT*; 2 = *TT*), and VDR genotype (0 = CC; 1 = CT; 2 = TT). NS, Not significant.

TABLE 8. Distribution of IL-6 and osteocalcin genotypes among women

Osteocalcin genotype	IL-6 genotype			Total
	CC	CG	GG	
CC	28 (2.58%)	18 (1.66%)	4 (0.37%)	50 (4.60%)
CT	196 (18.03%)	135 (12.42%)	19 (1.75%)	350 (32.20%)
TT	393 (36.15%)	248 (22.82%)	46 (4.23%)	687 (63.20%)
Total	617 (56.76%)	401 (36.89%)	69 (6.35%)	1087 (100%)

bar spine in a large female population, with the CC genotype exhibiting the highest BMD and the *TT* genotype the lowest BMD.

The 2C→T SNP at the translation initiation site of the VDR gene has previously been shown to be associated with BMD in postmenopausal Japanese women, with the *TT* genotype implicated as a risk factor for reduced BMD (15). However, we did not detect an association between this SNP and BMD for community-dwelling women. This SNP was also previously associated with BMD in small populations of Caucasian men, with the *T* allele being a predisposing factor to reduced bone mass (17, 18). Our results demonstrate that BMD for the distal radius (D50), femoral neck, or Ward's triangle was significantly reduced in men with the *CT* genotype compared with that in those with the CC genotype.

Analysis of combined IL-6 and osteocalcin genotypes revealed that the largest differences in BMD for the total body, lumbar spine, and Ward's triangle in postmenopausal women were 8.6, 17.7, and 24.0%, respectively, indicating that the effects of the two SNPs on BMD are additive. These results thus suggest that combined genotypes may be more useful for predicting bone mass in postmenopausal women.

The molecular mechanisms that underlie the association of the −634C→G SNP of the IL-6 gene and the 298C→T SNP of the osteocalcin gene with BMD in postmenopausal women remain unclear. The effects of these SNPs on the transcriptional activity of the corresponding gene promoters have not been determined. The 2C→T SNP of the VDR gene was shown to affect the molecular mass of the encoded protein (*T* allele, 50 kDa; *C* allele, 49.5 kDa) as well as the transcriptional activation of the gene by vitamin D (*T* allele < *C* allele) (15). These observations, however, were not independently confirmed (21). The functional impact of this polymorphism of the VDR gene thus remains to be determined.

Given the multiple comparisons of genotypes with BMD in the present study, it is not possible to completely exclude potential statistical errors such as false positives. It is also possible that the SNPs examined in our study are in linkage disequilibrium with polymorphisms of other nearby genes that are located at chromosome 7p21 (IL-6 gene), 1q25-q31 (osteocalcin gene), or 12q12-q14 (VDR gene) and that they are actually responsible for the association with BMD. However, our present results suggest that the IL-6 and osteocalcin genes are susceptibility loci for reduced bone mass in postmenopausal Japanese women and that the VDR gene constitutes such a locus in Japanese men. The combined IL-6 and osteocalcin genotypes exhibited an additive effect on BMD and may thus prove informative for the assessment of osteoporosis in women.

TABLE 9. BMD and other characteristics of postmenopausal women (n = 807) according to combined IL-6 and osteocalcin genotypes

Characteristic	Genotype (IL-6/osteocalcin)								
	CC/CC	CC/CT	CC/TT	CG/CC	CG/CT	CG/TT	GG/CT	GG/TT
Number (%)	20 (2.48)	143 (17.72)	287 (35.56)	14 (1.73)	111 (13.75)	185 (22.92)	10 (1.24)	37 (4.58)
Age (yr)	61.8 ± 1.9	63.1 ± 0.7	64.6 ± 0.5	59.6 ± 2.3	63.6 ± 0.8	63.6 ± 0.6	63.0 ± 2.7	66.4 ± 1.4
BMI (kg/m^2)	22.6 ± 0.7	23.1 ± 0.3	23.0 ± 0.2	24.8 ± 0.9	22.6 ± 0.3	22.8 ± 0.2	21.9 ± 1.1	23.5 ± 0.5
BMD values measured by pQCT (mg/cm^3)								
D50	187.0 ± 13.9	169.1 ± 5.3	162.2 ± 3.8	174.3 ± 16.7	170.4 ± 6.0	160.5 ± 4.7	137.3 ± 19.7	138.7 ± 10.7
D100	473.0 ± 20.1	445.3 ± 7.6	443.0 ± 5.4	440.0 ± 24.1	452.4 ± 8.7	442.9 ± 6.8	431.6 ± 28.4	404.5 ± 15.4
P100	1129.0 ± 33.2	1074.8 ± 12.6	1084.6 ± 8.9	1081.4 ± 39.8	1092.0 ± 14.3	1073.7 ± 11.2	1070.5 ± 46.9	1029.4 ± 25.5
BMD values measured by DXA (g/cm^2)								
Total body	0.954 ± 0.019[a]	0.933 ± 0.007[a]	0.917 ± 0.005[b]	0.946 ± 0.022	0.916 ± 0.008	0.915 ± 0.006	0.882 ± 0.028	0.872 ± 0.014
L2–L4	0.864 ± 0.028[a]	0.820 ± 0.011[a]	0.807 ± 0.007[b]	0.891 ± 0.034[a]	0.813 ± 0.012[b]	0.808 ± 0.009[b]	0.737 ± 0.042	0.733 ± 0.021
Femoral neck	0.674 ± 0.019	0.653 ± 0.007	0.641 ± 0.005	0.682 ± 0.022	0.644 ± 0.008	0.645 ± 0.006	0.609 ± 0.028	0.607 ± 0.014
Trochanter	0.576 ± 0.018	0.551 ± 0.007	0.537 ± 0.005	0.569 ± 0.022	0.539 ± 0.008	0.538 ± 0.006	0.484 ± 0.027	0.515 ± 0.013
Ward's triangle	0.530 ± 0.026[a,c]	0.461 ± 0.010	0.450 ± 0.007	0.516 ± 0.031[b]	0.449 ± 0.011	0.452 ± 0.008	0.427 ± 0.038	0.403 ± 0.019

Data are expressed as means ± SE. BMD values were adjusted for age and BMI.
[a] $P \leq 0.005$ *vs.* GG/TT; [b] $P \leq 0.05$ *vs.* GG/TT; [c] $P \leq 0.05$ *vs.* CC/TT.