# ORIGINAL ARTICLE

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# Association of polymorphisms of paraoxonase 1 and 2 genes, alone or in combination, with bone mineral density in community-dwelling Japanese

**Abstract** Oxidative stress may affect cellular functions in various pathological conditions, including osteoporosis. Paraoxonase 1 confers antioxidant properties on high-density lipoprotein, with which it is associated, by reducing the accumulation of lipid peroxidation products. We have now examined whether the $584A \rightarrow G$ (Gln192Arg) and $172T \rightarrow A$ (Leu55Met) polymorphisms of the paraoxonase 1 gene and the $959G \rightarrow C$ (Cys311Ser) polymorphism of the paraoxonase 2 gene are associated with bone mineral density (BMD) in community-dwelling Japanese (1,087–1,094 women and 1,112–1,125 men). The subjects were aged 40–79 years and were randomly recruited to a population-based prospective cohort study of aging and age-related diseases. BMD for the lumbar spine and right femoral neck was measured by dual-energy X-ray absorptiometry. Genotypes were determined with a fluorescence- or colorimetry-based allele-specific DNA primer-probe assay system. The $584A \rightarrow G$ and $172T \rightarrow A$ polymorphisms of the paraoxonase 1 gene and the $959G \rightarrow C$ polymorphism of the paraoxonase 2 gene were associated with BMD for the lumbar spine or femoral neck in postmenopausal women, with the $584GG$, $172TT$, and $959CC$ genotypes representing risk factors for reduced bone mass. None of these three polymorphisms was associated with BMD in premenopausal women or in men. Our results suggest that the paraoxonase 1 and 2 genes are candidate loci for reduced bone mass in postmenopausal Japanese women.

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## Introduction

Osteoporosis is characterized by a reduction in bone mineral density (BMD) and a deterioration in the microarchitecture of bone, both of which result in an increased susceptibility to fractures (Kanis et al. 1994). Although several environmental factors, such as diet and physical exercise, influence BMD, a genetic contribution to the etiology of osteoporosis has been recognized (Pocock et al. 1987). Genetic linkage analyses (Devoto et al. 1998; Johnson et al. 1997; Morrison et al. 1994) and candidate gene association studies (Morrison et al. 1994; Uitterlinden et al. 1998; Yamada et al. 2001) have thus implicated several loci and candidate genes in the regulation of bone mass and the pathogenesis of osteoporotic fractures. The genes that contribute to genetic susceptibility to osteoporosis, however, remain to be identified definitively.

Oxidative stress may affect cellular functions in various pathological conditions, including osteoporosis (Basu et al. 2001; Garrett et al. 1990). Recent evidence has suggested that lipid oxidation contributes to the development of osteoporosis (Parhami et al. 2000). In vitro studies indicate that lipid oxidation products promote osteoblastic differentiation of vascular cells and inhibit such differentiation of bone cells (Parhami et al. 1997). Oxidation products of low-density lipoprotein (LDL) also promote osteoporotic loss of bone by inducing progenitor marrow stromal cells to undergo adipogenic rather than osteogenic differentiation (Parhami et al. 1999).

Paraoxonase 1 (PON1) is a calcium-dependent esterase that is closely associated with high-density

lipoprotein (HDL)-containing apolipoprotein A-I and is thought to confer antioxidant properties on HDL by preventing the accumulation of lipid peroxidation products (Mackness et al. 1991). This property of PON1 accounts for its ability to protect against atherosclerosis. In addition to the PON1 gene (*PON1*), related genes designated *PON2* and *PON3* have been identified in the human genome and are linked with *PON1* on chromosome 7q21-q22 (Primo-Parmo et al. 1996). Two single nucleotide polymorphisms (SNPs), 584A → G (Gln192 Arg; GenBank accession no. M63012) and 172T → A (Leu55Met; accession no. M63012), of *PON1* and a 959G → C (Cys311Ser; accession no. L48513) SNP of *PON2* have been associated with coronary artery disease (Ruiz et al. 1995; Serrato and Marian 1995; Garin et al. 1997; Sanghera et al. 1998). Although PON1 catalyzes the reduction of oxidized LDL and thereby may affect bone remodeling, the possible relations of these SNPs to BMD have not been determined.

We have now examined whether the 584A → G (Gln192Arg) and 172T → A (Leu55Met) SNPs of *PON1* and the 959G → C (Cys311Ser) SNP of *PON2* are associated with BMD in women or men in a large-scale, population-based study.

## 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 (Shimokata et al. 2000). 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 the baseline age is 40–79 years, with similar numbers of participants in each decade (40s, 50s, 60s, 70s). The subjects will be followed up every 2 years.

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. Individuals with disorders known to cause abnormalities of bone metabolism, including diabetes mellitus, renal diseases, rheumatoid arthritis, as well as thyroid, parathyroid, and other endocrinologic diseases, were excluded from the study. Women who had taken drugs such as estrogen, progesterone, glucocorticoids, and bisphosphonates were also excluded. We examined the possible association of BMD with the 584A → G (Gln192Arg) SNP of *PON1* in 2,199 participants (1,087 women, 1,112 men), with the 172T → A (Leu55Met) SNP of *PON1* in 2,210 participants (1,092 women, 1,118 men), and with the 959G → C (Cys311Ser) SNP of *PON2* in 2,219 participants (1,094 women, 1,125 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 for the lumbar spine (L2–L4) and right femoral neck was measured by dual-energy X-ray absorptiometry (DEXA) (QDR 4500; Hologic, Bedford, MA, USA). The coefficients of variance of the DEXA instrument were 0.9% for L2–L4 and 1.3% for the femoral neck.

### Determination of genotypes

Genotypes were determined with a fluorescence- or colorimetry-based allele-specific DNA primer-probe assay system (Toyobo Gene Analysis, Tsuruga, Japan).

For determination of genotype of the 584A → G (Gln192Arg) SNP, the polymorphic region of *PON1* was amplified by the polymerase chain reaction (PCR) with a sense primer labeled at the 5' end with biotin (5'-GAATGATATTGTTGCTGTGGGACC-3') and allele-specific antisense primers (5'-AACCCAAATACATCTCCC-AGGAXTG-3' or 5'-ACCCAAATACATCTCCCAGGAXCG-3').

For determination of 172T → A (Leu55Met) genotype, the polymorphic region of *PON1* was similarly amplified with a sense primer (5'-TCTGGCAGAAACTGGCTCTGAA-3') and an antisense primer labeled at the 5' end with biotin (5'-GCTA-ATGAAAGCCAGTCCATTA-3').

For determination of 959G → C (Cys311Ser) genotype, the polymorphic region of *PON2* was amplified by PCR with allele-specific sense primers labeled at the 5' end with either fluorescein isothiocyanate (5'- CGCATCCAGAACATTCTAXGT-3') or Texas red (5'- CCGCATCCAGAACATTCTAXCT-3') and with an antisense primer labeled at the 5' end with biotin (5'-GGCA-TAAACTGTAGTCACTGTAGGC-3').

The reaction mixtures (25 μl) contained 20 ng of DNA, 5 pmol of each primer, 0.2 mmol/l of each deoxynucleoside triphosphate, 1.4 mmol/l MgSO₄ (584A → G and 172T → A genotypes) or 1.8 mmol/l MgCl₂ (959G → C genotype), and 1 U of 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 cycles (584A → G and 959G → C genotypes) or 40 cycles (172T → A genotype) of denaturation at 95°C for 30 s, annealing at 63°C (584A → G genotype), 55°C (172T → A genotype), or 60°C (959G → C genotype) for 30 s, and extension at 68°C for 30 s; and a final extension at 68°C for 2 min.

For determination of 584A → G and 172T → A genotypes, amplified DNA was denatured with 0.3 mol/l NaOH and then subjected to hybridization at 37°C for 30 min in hybridization buffer containing 35% (584A → G genotype) or 32.5% (172T → A genotype) formamide with allele-specific capture probes (5'-ACATCTCCCAGGAXTGTAAGTAG-3' or 5'-ACA-TCTCCCAGGAXCGTAAGTAG-3' for 584A → G genotype, and 5'-GAAGACTTGGAGATACTGCC-3' or 5'-GAAGACATG-GAGATACTGCC-3' for 172T → A 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 again washed, and after the addition of a solution containing 0.8 mmol/l 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium (monosodium salt) and 0.4 mmol/l 5-bromo-4-chloro-3-indolyl phosphate *p*-toluidine salt, absorbance at 450 nm was measured.

For determination of 959G → C 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/l 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.

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

Assay for serum activity of PON1

Venous blood was collected from subjects after an overnight fast. Blood samples were centrifuged at $1600 \times g$ for 15 min at 4°C, and serum was separated and stored at −80°C until assayed. The serum activity of PON1 was measured as previously described (Mackness et al. 1997). The intra- and interassay coefficients of variance were < 2.6 and < 2.3%, respectively.

Statistical analysis

Quantitative data were compared among three groups by one-way analysis of variance and the Tukey-Kramer post hoc test, and between two groups by the unpaired Student's $t$ test. BMD values were analyzed with or without adjustment for age by the least squares method in a general linear model (SAS, SAS Institute, Cary, NC, USA). Allele frequencies were estimated by the gene-counting method, and the chi-square test was used to identify significant departure from Hardy-Weinberg equilibrium. Haplotype analysis was performed with SAS/Genetics (SAS Institute). Correlation between the serum activity of PON1 and BMD was determined by simple regression analysis. Unless indicated otherwise, a $P$ value of < 0.05 was considered statistically significant.

## Results

The distribution of combined genotypes of three SNPs is shown in Table 1. Linkage disequilibrium was apparent between the 584A → G (Gln192Arg) and 172T → A (Leu55Met) SNPs of PON1 [D', pairwise linkage disequilibrium coefficient ($D/D$max), 0.4254; $r$, standardized linkage disequilibrium coefficient, 0.1673; $P$ < 0.0001], between the PON1 584A → G and PON2 959G → C (Cys311Ser) SNPs ($D'$, 0.2112; $r$, 0.1470; $P$ < 0.0001), and between the PON1 172A → T and PON2 959G → C SNPs ($D'$, 0.2491; $r$, 0.1421; $P$ < 0.0001).

Characteristics of study subjects according to genotypes for the 584A → G SNP or 172T → A SNP of PON1 or for the 959G → C SNP of PON2 are shown in Table 2. The distributions of genotypes were in Hardy-Weinberg equilibrium for premenopausal or postmenopausal women or for men in three SNPs. There was no

difference in age or body mass index for premenopausal or postmenopausal women or for men among genotypes of each SNP.

The relation of the 584A → G SNP of PON1 to BMD was examined (Table 3). To examine the possible influence of menopause on the relation between genotype and BMD, we analyzed BMD and other characteristics for premenopausal and postmenopausal women independently. Given the multiple comparisons of genotypes with BMD, we considered a $P$ value of < 0.01 to be significant for such associations. For premenopausal women, BMD was not associated with 584A → G genotype with or without adjustment for age. In contrast, for postmenopausal women, BMD for the femoral neck was significantly lower in those with the GG genotype than in those with the GA or AA genotype. After adjustment for age, BMD for the femoral neck was also significantly lower in postmenopausal women with the GG genotype than in those with the GA or AA genotype or the GA genotype. The difference in adjusted BMD between the GG genotype and the GA or AA genotype (expressed as a percentage of the corresponding larger value) in postmenopausal women was

**Table 1** Distribution of combined genotypes of polymorphisms of PON1 and PON2 among study subjects ($n = 2{,}196$). Data are numbers of subjects (%)

| PON1 584A → G | PON1 172T → A | | | PON2 959G → C |
|---|---|---|---|---|
| | TT | TA | AA | |
| GG | 811 (36.93) | 0 (0) | 0 (0) | CC |
| | 141 (6.42) | 0 (0) | 0 (0) | CG |
| | 13 (0.59) | 0 (0) | 0 (0) | GG |
| GA | 470 (21.40) | 59 (2.69) | 0 (0) | CC |
| | 292 (13.30) | 133 (6.06) | 1 (0.05) | CG |
| | 26 (1.18) | 15 (0.68) | 1 (0.05) | GG |
| AA | 64 (2.91) | 18 (0.82) | 2 (0.09) | CC |
| | 57 (2.60) | 36 (1.64) | 8 (0.36) | CG |
| | 19 (0.87) | 24 (1.09) | 6 (0.27) | GG |

**Table 2** Characteristics of study subjects according to the 584A → G (Gln192Arg) or 172T → A (Leu55Met) genotype of PON1 or the 959G → C (Cys311Ser) genotype of PON2. Data are means ± SE. *BMI* body mass index

| Characteristic | 584A → G (Gln192Arg) | | | 172T → A (Leu55Met) | | | 959G → C (Cys311Ser) | | |
|---|---|---|---|---|---|---|---|---|---|
| | GG | GA | AA | TT | TA | AA | CC | CG | GG |
| **Premenopausal women** | | | | | | | | | |
| Number (%) | 107 (38.6) | 136 (49.1) | 34 (12.3) | 239 (86.0) | 35 (12.6) | 4 (1.4) | 173 (62.2) | 92 (33.1) | 13 (4.7) |
| Age (years) | 46.1 ± 0.4 | 46.2 ± 0.4 | 46.6 ± 0.8 | 46.0 ± 0.3 | 47.1 ± 0.8 | 48.3 ± 2.3 | 46.2 ± 0.4 | 46.2 ± 0.5 | 45.7 ± 1.3 |
| BMI (kg/m²) | 22.9 ± 0.3 | 22.7 ± 0.3 | 22.7 ± 0.6 | 22.8 ± 0.2 | 22.5 ± 0.5 | 22.2 ± 1.6 | 22.9 ± 0.2 | 22.6 ± 0.3 | 22.8 ± 0.9 |
| **Postmenopausal women** | | | | | | | | | |
| Number (%) | 369 (45.6) | 353 (43.6) | 88 (10.9) | 702 (86.2) | 107 (13.1) | 5 (0.6) | 551 (67.5) | 227 (27.8) | 38 (4.7) |
| Age (years) | 64.1 ± 0.4 | 63.8 ± 0.5 | 64.1 ± 0.9 | 64.1 ± 0.3 | 63.4 ± 0.8 | 63.4 ± 3.8 | 64.1 ± 0.4 | 63.5 ± 0.6 | 63.9 ± 1.4 |
| BMI (kg/m²) | 22.8 ± 0.2 | 23.0 ± 0.2 | 23.5 ± 0.4 | 23.0 ± 0.1 | 22.7 ± 0.3 | 23.9 ± 1.5 | 22.9 ± 0.1 | 23.2 ± 0.2 | 23.3 ± 0.5 |
| **Men** | | | | | | | | | |
| Number (%) | 493 (44.3) | 504 (45.3) | 115 (10.3) | 965 (86.3) | 144 (12.9) | 9 (0.8) | 720 (64.0) | 351 (31.2) | 54 (4.8) |
| Age (years) | 59.2 ± 0.5 | 59.2 ± 0.5 | 58.7 ± 1.0 | 59.0 ± 0.4 | 60.0 ± 0.9 | 59.4 ± 3.6 | 59.1 ± 0.4 | 59.2 ± 0.6 | 59.2 ± 1.5 |
| BMI (kg/m²) | 22.9 ± 0.2 | 23.1 ± 0.2 | 22.6 ± 0.3 | 23.0 ± 0.1 | 22.8 ± 0.2 | 24.0 ± 1.2 | 23.1 ± 0.1 | 22.7 ± 0.2 | 23.0 ± 0.4 |

3.1% for the femoral neck. For men, BMD for the lumbar spine or femoral neck did not differ among 584A → G genotypes with or without adjustment for age (data not shown).

The relation between the 172T → A SNP of *PON1* and BMD is shown in Table 4. For premenopausal women, BMD was not associated with 172T → A genotype with or without adjustment for age. For postmenopausal women, BMD for the lumbar spine or femoral neck was significantly lower in those with the *TT* genotype than in those with the *TA* or *AA* genotype or the *TA* genotype, with or without adjustment for age. The differences in adjusted BMD between the *TT* genotype and the *TA* or *AA* genotype in postmenopausal women were 5.3% for the lumbar spine and 4.0% for the femoral neck. For men, BMD did not differ among 172T → A genotypes with or without adjustment for age (data not shown).

The relation of the 959G → C SNP of *PON2* with BMD is shown in Table 5. BMD did not differ among 959G → C genotypes for premenopausal women with or without adjustment for age. For postmenopausal women, BMD for the femoral neck was significantly lower in those with the *CC* genotype than in those with the *CG* or *GG* genotype with or without adjustment for age. The difference in adjusted BMD between the *CC* genotype and the *CG* or *GG* genotype in postmenopausal women was 2.9% for the femoral neck. BMD did not differ significantly among 959G → C genotypes in men with or without adjustment for age (data not shown).

We examined the relation of haplotypes of the 584A → G, 172T → A, and 959G → C SNPs to BMD (Table 6). Given the small number of subjects with the *GAC* (one woman and no men) and *GAG* (no women and one man) haplotypes, these groups were excluded from the analysis. For postmenopausal women, BMD

**Table 3** Bone mineral density (BMD) of women (n = 1,087) according to the 584A → G (Gln192Arg) genotype of *PON1*. Data are means ± SE

| Characteristic | Premenopausal (n = 277) | | | | Postmenopausal (n = 810) | | | |
|---|---|---|---|---|---|---|---|---|
| | *GG* | *GA* | *AA* | *GA + AA* | *GG* | *GA* | *AA* | *GA + AA* |
| L2–L4 | 1.035 ± 0.012 | 1.026 ± 0.011 | 0.988 ± 0.021 | 1.018 ± 0.010 | 0.797 ± 0.008† | 0.816 ± 0.008 | 0.830 ± 0.016 | 0.819 ± 0.007 |
| Adjusted L2–L4[a] | 1.034 ± 0.012 | 1.026 ± 0.010 | 0.990 ± 0.021 | 1.019 ± 0.009 | 0.798 ± 0.007† | 0.814 ± 0.007 | 0.831 ± 0.015 | 0.818 ± 0.007 |
| Femoral neck | 0.777 ± 0.010 | 0.776 ± 0.008 | 0.745 ± 0.017 | 0.770 ± 0.008 | 0.632 ± 0.005‡|| | 0.655 ± 0.006 | 0.649 ± 0.011 | 0.654 ± 0.005 |
| Adjusted femoral neck[a] | 0.776 ± 0.009 | 0.776 ± 0.008 | 0.747 ± 0.016 | 0.770 ± 0.007 | 0.633 ± 0.005§†† | 0.654 ± 0.005 | 0.650 ± 0.009 | 0.653 ± 0.004 |

[a]BMD with adjustment for age
†$P < 0.05$
‡$P < 0.005$

§$P < 0.001$ versus *GA + AA*
||$P < 0.01$
††$P < 0.005$ versus *GA*

**Table 4** Bone mineral density (BMD) of women (n = 1,092) according to the 172T → A (Leu55Met) genotype of *PON1*. Data are means ± SE

| Characteristic | Premenopausal (n = 278) | | | | Postmenopausal (n = 814) | | | |
|---|---|---|---|---|---|---|---|---|
| | *TT* | *TA* | *AA* | *TA + AA* | *TT* | *TA* | *AA* | *TA + AA* |
| L2–L4 | 1.030 ± 0.008 | 0.996 ± 0.021 | 0.940 ± 0.062 | 0.990 ± 0.020 | 0.803 ± 0.006†§ | 0.852 ± 0.015 | 0.849 ± 0.068 | 0.852 ± 0.014 |
| Adjusted L2–L4[a] | 1.029 ± 0.008 | 1.002 ± 0.020 | 0.954 ± 0.060 | 0.997 ± 0.019 | 0.803 ± 0.005‡§ | 0.848 ± 0.013 | 0.845 ± 0.061 | 0.848 ± 0.013 |
| Femoral neck | 0.775 ± 0.006 | 0.754 ± 0.017 | 0.709 ± 0.050 | 0.749 ± 0.016 | 0.639 ± 0.004†|| | 0.672 ± 0.010 | 0.628 ± 0.047 | 0.670 ± 0.010 |
| Adjusted femoral neck[a] | 0.774 ± 0.006 | 0.758 ± 0.016 | 0.720 ± 0.048 | 0.755 ± 0.015 | 0.640 ± 0.003†§ | 0.669 ± 0.009 | 0.625 ± 0.039 | 0.667 ± 0.008 |

[a]BMD with adjustment for age
†$P < 0.005$
‡$P < 0.001$ versus *TA + AA*

§$P < 0.005$
||$P < 0.01$ versus *TA*

**Table 5** Bone mineral density (BMD) of women (n = 1,094) according to the 959G → C (Cys311Ser) genotype of *PON2*. Data are means ± SE

| Characteristic | Premenopausal (n = 278) | | | | Postmenopausal (n = 816) | | | |
|---|---|---|---|---|---|---|---|---|
| | *CC* | *CG* | *GG* | *CG + GG* | *CC* | *CG* | *GG* | *CG + GG* |
| L2–L4 | 1.028 ± 0.009 | 1.024 ± 0.013 | 0.983 ± 0.034 | 1.019 ± 0.012 | 0.803 ± 0.006 | 0.825 ± 0.010 | 0.814 ± 0.025 | 0.823 ± 0.009 |
| Adjusted L2–L4[a] | 1.028 ± 0.009 | 1.024 ± 0.013 | 0.979 ± 0.033 | 1.019 ± 0.012 | 0.804 ± 0.006 | 0.822 ± 0.009 | 0.814 ± 0.022 | 0.820 ± 0.008 |
| Femoral neck | 0.769 ± 0.008 | 0.778 ± 0.010 | 0.756 ± 0.028 | 0.775 ± 0.010 | 0.637 ± 0.004†‡ | 0.660 ± 0.007 | 0.655 ± 0.017 | 0.659 ± 0.006 |
| Adjusted femoral neck[a] | 0.770 ± 0.007 | 0.778 ± 0.010 | 0.753 ± 0.027 | 0.775 ± 0.009 | 0.638 ± 0.004†‡ | 0.657 ± 0.006 | 0.655 ± 0.014 | 0.657 ± 0.005 |

[a]BMD with adjustment for age
†$P < 0.005$ versus *CG + GG*
‡$P < 0.05$ versus *CG*

**Table 6** Bone mineral density (BMD) of premenopausal or postmenopausal women or of men according to haplotypes of three polymorphisms. Data are means ± SE

| BMD | Haplotype [584A → G (*PON1*), 172T → A (*PON1*), 959G → C (*PON2*)] | | | | | |
|---|---|---|---|---|---|---|
| | *GTC* | *GTG* | *ATC* | *ATG* | *AAC* | *AAG* |
| Premenopausal women | | | | | | |
| No. of chromosomes (%) | 314 (57.40) | 31 (5.67) | 106 (19.38) | 55 (10.05) | 9 (1.65) | 32 (5.85) |
| L2–L4 | 1.034 ± 0.007 | 1.012 ± 0.022 | 1.014 ± 0.012 | 1.033 ± 0.017 | 0.999 ± 0.041 | 0.986 ± 0.022 |
| Femoral neck | 0.776 ± 0.006 | 0.783 ± 0.018 | 0.766 ± 0.010 | 0.781 ± 0.013 | 0.734 ± 0.033 | 0.751 ± 0.018 |
| Postmenopausal women | | | | | | |
| No. of chromosomes (%) | 1016 (63.18) | 66 (4.10) | 259 (16.11) | 150 (9.33) | 33 (2.05) | 84 (5.22) |
| L2–L4 | 0.802 ± 0.005 | 0.832 ± 0.019 | 0.817 ± 0.009 | 0.802 ± 0.012 | 0.869 ± 0.026 | 0.845 ± 0.017 |
| Femoral neck | 0.638 ± 0.003* | 0.662 ± 0.013 | 0.644 ± 0.007 | 0.658 ± 0.009 | 0.700 ± 0.018 | 0.656 ± 0.011 |
| Men | | | | | | |
| No. of chromosomes (%) | 1366 (62.03) | 110 (5.00) | 339 (15.40) | 227 (10.31) | 46 (2.09) | 113 (5.13) |
| L2–L4 | 0.979 ± 0.004 | 0.970 ± 0.015 | 0.989 ± 0.009 | 0.988 ± 0.011 | 0.993 ± 0.024 | 0.984 ± 0.015 |
| Femoral neck | 0.750 ± 0.003 | 0.747 ± 0.011 | 0.757 ± 0.006 | 0.756 ± 0.008 | 0.765 ± 0.017 | 0.751 ± 0.011 |

*$P < 0.01$ versus *AAC*

**Table 7** Serum activity (nmol min$^{-1}$ ml$^{-1}$) of PON1 in study subjects according to the 584A → G (Gln192Arg) and 172T → A (Leu55Met) genotypes of *PON1* and the 959G → C (Cys311Ser) genotype of *PON2*. Data are means ± SE

| | Total subjects | Women | Men |
|---|---|---|---|
| 584A → G (Gln192Arg) genotype | | | |
| *GG* | 503.7 ± 3.0 ($n = 969$) | 516.5 ± 4.1 ($n = 476$) | 491.2 ± 4.2 ($n = 493$) |
| *GA* | 325.6 ± 2.9 ($n = 993$)* | 333.5 ± 4.0 ($n = 489$)* | 317.9 ± 4.1 ($n = 504$)* |
| *AA* | 139.3 ± 6.1 ($n = 237$)*† | 145.7 ± 8.3 ($n = 122$)*† | 132.2 ± 8.9 ($n = 115$)*† |
| 172T → A (Leu55Met) genotype | | | |
| *TT* | 407.2 ± 3.3 ($n = 1906$) | 413.9 ± 4.7 ($n = 941$) | 400.5 ± 4.5 ($n = 965$) |
| *TA* | 256.5 ± 8.4 ($n = 286$)‡ | 268.7 ± 12.2 ($n = 142$)‡ | 244.5 ± 11.5 ($n = 144$)‡ |
| *AA* | 101.9 ± 32.9 ($n = 18$)‡§ | 111.5 ± 49.1 ($n = 9$)‡|| | 93.3 ± 43.9 ($n = 9$)‡|| |
| 959G → C (Cys311Ser) genotype | | | |
| *CC* | 408.9 ± 3.9 ($n = 1444$) | 415.6 ± 5.6 ($n = 724$) | 402.0 ± 5.5 ($n = 720$) |
| *CG* | 349.1 ± 5.8 ($n = 670$)†† | 361.7 ± 8.4 ($n = 319$)†† | 337.4 ± 7.9 ($n = 351$)†† |
| *GG* | 285.2 ± 15.1 ($n = 105$)††# | 267.3 ± 21.9 ($n = 51$)††# | 302.4 ± 20.7 ($n = 54$)†† |

*$P = 0.0001$ versus *GG*  
†$P = 0.0001$ versus *GA*  
‡$P = 0.0001$ versus *TT*  
§$P = 0.0001$ versus *TA*

||$P < 0.005$ versus *TA*  
††$P = 0.0001$ versus *CC*  
#$P < 0.0005$ versus *CG*

for the femoral neck was significantly lower in those with the *GTC* haplotype than in those with the *AAC* haplotype. For premenopausal women or for men, BMD did not differ among haplotypes.

We examined the relation of three SNPs with the serum activity of PON1 (Table 7). There was a significant association between the serum activity of PON1 and 584A → G, 172T → A, and 959G → C genotypes both for women and for men. With regard to the 584A → G genotype, the activity of PON1 was higher in individuals with the *GG* genotype than in those with the *GA* genotype or those with the *AA* genotype; the activity was also higher in those with the *GA* genotype than in those with the *AA* genotype. For the 172T → A genotype, the activity of PON1 was higher in individuals with the *TT* genotype than in those with the *TA* or those with the *AA* genotypes; the activity was also higher in those with the *TA* genotype than in those with the *AA* genotype. With respect to the 959G → C genotype, the serum activity of PON1 was higher in individuals with the *CC*

genotype than in those with the *CG* or those with the *GG* genotypes; for total subjects and for women, the activity was also higher in those with the *CG* genotype than in those with the *GG* genotype.

Finally, we examined the correlation between the serum activity of PON1 and BMD (Fig. 1). There was no significant relation of the serum activity of PON1 with BMD for the lumbar spine ($r = 0.010$, $P = 0.844$) or for the femoral neck ($r = 0.014$, $P = 0.683$) in postmenopausal women.

## Discussion

We have shown that the 584A → G (Gln192Arg) and 172T → A (Leu55Met) SNPs of *PON1* and the 959G → C (Cys311Ser) SNP of *PON2* are associated with BMD for the lumbar spine or femoral neck in postmenopausal Japanese women, and that the 584*GG*, 172*TT*, and 959*CC* genotypes represent risk factors for

**Fig. 1** Correlation of serum activity of PON1 with bone mineral density (BMD) for the lumbar spine (L2–L4) (*left panel*) or femoral neck (*right panel*) in postmenopausal women (*n* = 816)



Serum activity of PON1 (nmol min$^{-1}$ mL$^{-1}$)
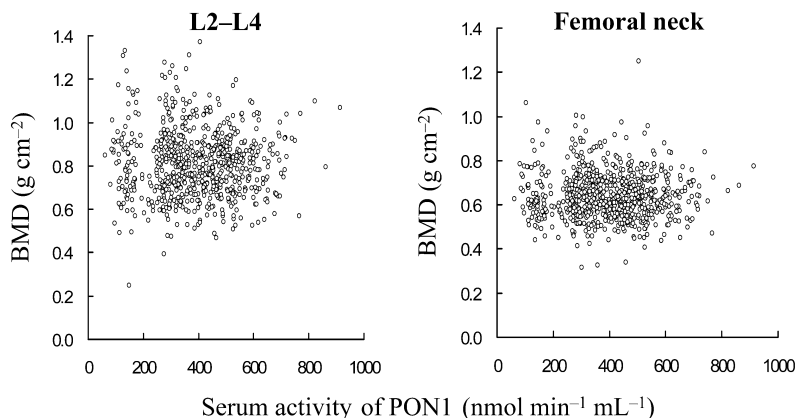
reduced bone mass. Haplotype analysis revealed that the *GTC* haplotype exhibited the lowest BMD and the *AAC* haplotype the highest BMD in postmenopausal women. There was no significant association of polymorphisms of *PON1* or *PON2* with BMD in premenopausal women or in men.

The three SNPs examined in the present study have been previously associated with the activity of PON1 in serum or plasma. We measured the serum activity of PON1 in the present study population to confirm the effects of three SNPs on the activity. Humbert et al. (1993) determined that the 584*G* (192Arg) allele is associated with a higher activity of PON1 in plasma than is the 584*A* (192Gln) allele. Garin et al. (1997) showed that the plasma concentrations and activities of PON1 decreased according to the rank order of 172T → A (Leu55Met) genotypes *TT* > *TA* > *AA*. Our present results are consistent with these previous observations (Garin et al. 1997; Humbert et al. 1993).

Mackness et al. (2000) showed that the 959G → C (Cys311Ser) SNP of *PON2* also affects the serum activity of PON1; among individuals with type 2 diabetes, PON1 activity was highest in those with the 959*GG* genotype. In contrast, our results demonstrate that the *GG* genotype is associated with the lowest PON1 activity and that the *CC* genotype exhibits the highest activity for both women and men.

In the present study, the 584*GG*, 172*TT*, and 959*CC* genotypes, which exhibited the highest serum activity of PON1, were associated with reduced BMD in postmenopausal women. A high serum activity of PON1 may result in a reduced concentration of lipid peroxidation products and might therefore be expected to prevent bone loss (Basu et al. 2001; Garrett et al. 1990; Parhami et al. 1997; Parhami et al. 1999; Parhami et al. 2000). The association between genotypes and BMD in our study is thus opposite to that anticipated from such a mechanism. The molecular mechanisms that underlie the association of SNPs of *PON1* and *PON2* with BMD thus remain unclear. It is possible that the SNPs examined in our study are in linkage disequilibrium with polymorphisms of other nearby genes that are determinants of BMD. Indeed, the interleukin-6 gene (7q21) and calcitonin

receptor gene (7q21.3), both of which have been associated with BMD (Ota et al. 1999; Ota et al. 2001; Taboulet et al. 1998), are located close to *PON1* and *PON2* (7q21-q22). However, our present results suggest that *PON1* and *PON2* are candidate loci for reduced bone mass in postmenopausal Japanese women.

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