ELECTRONIC LETTER

Associations of catalase gene polymorphisms with bone mineral density and bone turnover markers in postmenopausal women

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Background: Oxidative stress has been recently suggested to play a part in the development of osteoporosis. Catalase is a major antioxidant enzyme that detoxifies hydrogen peroxide by converting it into water and oxygen, thereby preventing cellular injury by oxidative stress.

Aims: To examine the associations between the catalase gene (CAT) polymorphisms and bone mineral density (BMD) and bone turnover markers in postmenopausal Korean women.

Methods: All exons, their boundaries and the promoter region (approximately 1.5 kb) were directly sequenced in 24 individuals. Among 18 variants identified by a direct sequence method, four polymorphisms were selected and genotyped in all study participants (n=560). BMD at the lumbar spine and proximal femur was measured using dual-energy x ray absorptiometry. Serum osteocalcin concentrations and bone-specific alkaline phosphatase activity were determined by an immunoradiometric assay and an immunoassay, respectively.

Results: The mean (standard deviation) age of the participants was 59.4 (7.2) years. Multivariate analysis showed an association of the +22348C→T polymorphism with BMD at the lumbar spine (p=0.01 in the dominant model) and at femur neck (p=0.05 in the dominant model), and with serum osteocalcin level (p=0.008 in the dominant model). Haplotype analyses showed that HT4 (-20T, +144C, +22348T, +33078A) was significantly associated with higher BMD at various sites (p<0.001–0.03) and with lower serum osteocalcin levels (p=0.01 in the codominant model).

Conclusions: These findings indicate that the +22348C→T polymorphism and HT4 of CAT may be useful genetic markers for bone metabolism.

Oxidative stress is defined as a disturbance in the balance between the production of reactive oxygen species (ROS) and antioxidant defenses, including enzymatic and non-enzymatic systems.¹ Perturbation of this balance may lead to accumulation of ROS, such as hydrogen peroxide. These ROS can be further converted to more potent oxidants, such as hydroxyl radicals, leading to oxidative damage to lipids, protein and DNA, which, in turn, can result in cell death.^{2,3} Recently, oxidative stress has been also suggested to participate in the development of osteoporosis. Some in vitro and animal studies have suggested that oxidative stress decreases bone formation by modulating the differentiation and survival of osteoblasts⁴ and stimulating bone resorption.^{5,6} However, only a few of the clinical studies have shown an important role of oxidative stress in the development of osteoporosis.^{7,8}

Catalase is a major antioxidant enzyme that detoxifies hydrogen peroxide by converting it to water and oxygen,

thereby preventing cellular injury.⁹ Individuals with reduced catalase activity have an increased incidence of oxidative stress-related diseases, such as atherosclerosis,¹⁰ diabetes,^{11,12} dyslipidaemia¹² and neurodegenerative disease,¹³ and catalase over-expression was shown to diminish or retard atherosclerosis in transgenic mice.^{14,15} In addition, catalase administration was shown to prevent oophorectomy-induced bone loss,¹⁶ suggesting that catalase activity may be involved in the development of oxidative stress-related diseases, including postmenopausal osteoporosis.

Recently, the associations of the *catalase* gene (CAT) polymorphisms with hypertension,¹⁷ diabetes,¹⁸ Alzheimer's disease¹⁹ and vitiligo²⁰ have been investigated. However, to our knowledge, its genetic effects on the determination of bone mass and bone turnover rate have not been studied thus far, despite a presumptively important role of catalase in bone metabolism. In this study, we performed extensive screening of the gene by direct sequencing to detect polymorphisms, and we analysed their associations with bone mineral density (BMD) and biochemical bone turnover markers in postmenopausal women.

PARTICIPANTS AND METHODS

Participants

The study population was composed of apparently healthy postmenopausal Korean women (n = 560) who had visited the Asan Medical Center, Seoul, Korea, as described previously.²¹ Briefly, menopause was defined as the absence of menstruation for at least 6 months and was confirmed by measurement of the serum follicle-stimulating hormone levels. Women who were prematurely menopausal (aged <40 years) were excluded. Those who had taken drugs that might affect bone metabolism for >6 months or within the previous 12 months were also excluded. In addition, women were excluded if they had any disease that might affect bone metabolism. The mean (standard deviation (SD) age was 59.4 (7.2) years, and the mean number of years since menopause (YSM) was 10.4 (SD 8.2, range 1–35) years.

BMD measurement

Areal BMD (g/cm²) at the lumbar spine (L2–L4) and femoral neck was measured using dual-energy x ray absorptiometry (Lunar, Expert XL, Madison, Wisconsin, USA) in 431 women. In the remaining 129 women, BMD was measured using the Hologic equipment (Hologic, QDR 4500-A, Waltham, Massachusetts, USA). The precisions for the Lunar and Hologic equipment, presented as the coefficient of variation,

Abbreviations: BMD, bone mineral density; CAT, catalase gene; ROS, reactive oxygen species; SNP, single-nucleotide polymorphism; YSM, years since menopause

were 0.82% and 0.85% for the lumbar spine and 1.12% and 1.20% for the femoral neck, respectively. These values were obtained by scanning 17 volunteers who were not part of the study; each volunteer underwent five scans on the same day, getting on and off the table between examinations. To derive cross-calibration equations between the two systems, the BMD values were measured by the two machines in 109 healthy Korean women (mean age 55 SD 11, range 31–75 years), and cross-calibration equations were calculated as follows²²:

L2–L4 BMD (g/cm²):

Lunar = 1.1287×Hologic–0.0027

Femoral neck BMD (g/cm²):

Lunar = 1.1556×Hologic–0.0182

We also obtained other BMD values at other proximal femoral sites, all taken after January 2001. The Hologic machine did not measure BMD at the femoral shaft. BMD values at the femoral shaft and at other proximal femur sites (total femur, trochanter and Ward’s triangle) were available for 331 and 460 participants, respectively. Associations between these BMD values and CAT genetic variations were determined using statistical adjustments with the machine as a covariate, because the cross-calibration data were not available at these sites in Korean women so far.

Measurement of biochemical bone turnover markers

For determinations of bone-specific turnover markers, fasting blood samples were obtained between 08:00 and 10:00. Blood samples were centrifuged and stored at –80°C. Serum osteocalcin concentration was determined using an immuno-radiometric assay kit (OSTEO-RIACT, CIS bio international, Saclay, France), with interassay and intra-assay coefficient of variations of 2.8% and 5.2%, respectively. Serum bone-specific alkaline phosphatase was determined using the Metra BAP immunoassay (Quidel, San Diego, California, USA), with interassay and intra-assay coefficient of variations of 4.4% and 3.6%, respectively.

Detection of radiological vertebral compression fracture

Lateral thoracolumbar (T4–L4) radiographs were obtained in 520 participants. A vertebral fracture was defined quantitatively as a loss of ≥15% in the anterior, posterior or middle height of ≥1 vertebral sites in patients without a history of major trauma such as traffic accidents.

Sequencing analysis of the CAT gene

Genomic DNA was extracted from peripheral blood leucocytes using a commercial kit (Wizard Genomic DNA purification kit, Promega, Madison, Wisconsin, USA). We sequenced all exons, their boundaries and the promoter region (about 1.5 kb) using the ABI PRISM 370 DNA analyser (Applied Biosystems, Foster City, California, USA) to identify single-nucleotide polymorphisms (SNPs) in 24 Korean DNA samples. Sixteen primer sets for the amplification and sequencing analysis were designed on the basis of the GenBank sequences (CAT mRNA NM_001752; contig: NT_009237). Sequence variants were verified by chromatograms. Table A available online at <http://jmg.bmj.com/supplemental> provides more information regarding the primers used in this study.

Genotyping with fluorescence polarisation detection

For genotyping of polymorphic sites, amplifying primers and probes were designed for TaqMan.²³ Primer Express (Applied Biosystems) was used to design both the polymerase chain

Table 1 Linkage disequilibrium among catalase gene polymorphisms identified in 24 unrelated Korean women

ID ¹	r ²														
	–844G→A	–262C→T	–89A→T	–20T→C	+144C→T	+151G→A	+10119G→A	+11970G→A	+14161C→T	+15128T→T	+15189A→G	+15228T→C	+17091A→G	+22348C→T	+22587A→G
–844G→A	–	0.027	1	1	1	1	0.631	1	1	1	0.631	0.611	1	1	1
–262C→T	0.027	–	1	1	1	1	1	1	1	1	1	1	1	1	1
–89A→T	1	1	–	1	1	1	1	1	1	1	1	1	1	1	1
–20T→C	1	1	1	–	1	1	1	1	1	1	1	1	1	1	1
+144C→T	0.159	0.159	0.159	0.159	–	0.553	1	1	1	1	1	1	1	1	1
+151G→A	0.842	0.842	0.842	0.842	0.11	–	0.563	1	1	1	1	1	1	1	1
+10119G→A	0.285	0.285	0.285	0.285	0.596	0.191	–	0.023	1	1	1	1	1	1	1
+11970G→A	0.017	0.017	0.017	0.017	0.039	0.014	0.026	0	1	1	1	1	1	1	1
+14161C→T	0.026	0.026	0.026	0.026	0.042	0.016	0.026	0.02	0	1	1	1	1	1	1
+15128T→G	0.027	0.027	0.027	0.027	0.012	0.032	0.02	0	0.026	0.02	–	1	1	1	1
+15189A→T	0.023	0.023	0.023	0.023	0.596	0.191	1	0.023	0.042	0.012	0.596	1	1	1	1
+15228T→C	0.159	0.159	0.159	0.159	1	0.11	0.596	0.039	0.042	0.023	1	1	1	1	1
+17091A→G	0.027	0.027	0.027	0.027	0.039	0.014	0.023	0	1	0.039	0.023	–	1	1	1
+22348C→T	0.285	0.285	0.285	0.285	0.596	0.191	1	0.023	0.026	0.02	1	0.596	0.023	–	1
+22587A→G	0.285	0.285	0.285	0.285	0.596	0.191	1	0.023	0.026	0.02	1	0.596	0.023	1	–
+32187T→C	0.159	0.159	0.159	0.159	1	0.11	0.596	0.039	0.042	0.012	0.596	1	0.039	0.596	1
+33078A→G	0.569	0.569	0.569	0.569	0.233	0.512	0.401	0.01	0.011	0.051	0.401	0.233	0.01	0.401	–
+33241T→C	0.029	0.029	0.029	0.029	0.042	0.014	0.024	0	1	0	0.024	0.042	1	0.024	0.01

Table 2 Clinical profiles and multiple regression analyses of bone mineral density in postmenopausal Korean women (n = 560)

Clinical profiles		Lumbar spine BMD (g/cm ²)			Femoral neck BMD (g/cm ²)		
Variables	Mean (SD)	Regression coefficient	SE	p Value	Regression coefficient	SE	p Value
Age (years)	59.4 (7.2)	-0.004	0.002	0.01	-0.003	0.001	0.005
Weight (kg)	56.5 (7.4)	0.006	0.001	<0.001	0.003	0.001	<0.001
Height (cm)	154.8 (5.3)	0.003	0.001	0.013	0.002	0.001	0.09
YSM (years)	10.4 (8.2)	-0.003	0.002	0.02	-0.005	0.001	<0.001
Adjusted R ² = 0.254				Adjusted R ² = 0.342			

BMD, bone mineral density; YSM, years since menopause.

reaction primers and the MGB TaqMan probes. One allelic probe was labelled with the FAM dye and the other with the fluorescent VIC dye. Polymerase chain reaction was performed using the TaqMan Universal Master mix without uracil-N-glycosylase (Applied Biosystems), with polymerase chain reaction primer concentrations of 900 nM and TaqMan MGB-probe concentrations of 200 nM. Reactions were performed in a 384-well format in a total reaction volume of 5 µl using 20 ng of genomic DNA. The plates then were placed in a thermal cycler (PE 9700, Applied Biosystems) and heated at 50°C for 2 min and at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The TaqMan assay plates were transferred to a Prism 7900HT instrument (Applied Biosystems) where the fluorescence intensity in each well of the plate was read. Fluorescence data files from each plate were analysed using automated software (SDS V.2.1; Applied Biosystems). Table B available online at <http://jmg.bmjournals.com/supplemental> gives more information regarding the primers used.

Statistics

χ^2 tests were used to determine whether individual variants were in equilibrium at each locus in the population (Hardy-Weinberg equilibrium). We also examined Lewontin's D' ($|D'|$) and the linkage disequilibrium coefficient (r^2) between all pairs of biallelic loci. Genotypes were given codes of 0, 1 and 2 for the codominant model; 0, 1 and 1 for the dominant model; and 0, 0 and 1 for the recessive model. Haplotypes of each individual were inferred using the PHASE algorithm developed by Stephens *et al.*²⁴ which uses a Bayesian approach incorporating a priori expectations of haplotypic structure based on population genetics and coalescent theory. Phase probabilities of all polymorphic sites for haplotypes were also calculated for each individual using this software. Individuals with phase probabilities of <97% were excluded from the analysis. The genetic effects of inferred haplotypes were analysed in the same way as the polymorphisms. Multiple regression analyses of BMD and biochemical bone turnover markers were performed using age, YSM, weight and height as covariates. The genotype and haplotype distributions between the participants with and

without vertebral fractures were also analysed with a logistic regression model controlling for age, YSM, weight and height.

RESULTS

We identified 18 SNPs through direct sequencing three in the promoter region, four in the exons, nine in the introns and two in the 3'-flanking region (see supplementary fig A available online at <http://jmg.bmjournals.com/supplemental>). Pairwise comparisons of the 18 SNPs in 24 unrelated Korean women showed 11 sets of absolute linkage disequilibrium ($|D'| = 1$ and $r^2 = 1$; table 1). We selected 4 of the 18 SNPs on the basis of the linkage disequilibrium and their frequencies, including one of the SNPs with absolute linkage disequilibrium; SNPs with <0.05 allele frequency were omitted from further analyses.

Table 2 shows clinical profiles of participants. As expected, the age, YSM, weight and height were correlated significantly with BMD at the lumbar spine and femoral neck. In these participants, genotype distributions of all loci were in Hardy-Weinberg equilibrium (table 3), and none of the SNPs was significantly associated with age, weight, height and YSM (data not shown).

Of the four polymorphic sites, we did not find an association of the -20T→C in the 5' untranslated region, the +144C→T at intron 1, or the +33078 A→G with BMD and biochemical bone turnover markers (table 4). However, we found a significant association between the +22348C→T in exon 9 and BMD at the lumbar spine after adjusting for confounding variables ($p = 0.02$ and 0.01, in the codominant and dominant models, respectively). Women with the T allele (homozygotic TT and heterozygotic CT) had higher BMD at the lumbar spine compared with those without this allele. Femoral neck BMD was also associated with the polymorphism ($p = 0.05$ in the dominant model). However, the genotype was not significantly associated with any BMD values at the total femur, trochanter, femoral shaft and Ward's triangle (data not shown). Additionally, the +22348C→T was significantly associated with serum osteocalcin levels (table 4). Women with the T allele, which was shown to be associated with a higher BMD at the lumbar spine, had mean (SD) osteocalcin levels lower than those without the T allele (28.8 (11.3) v 32.1

Table 3 Frequencies of the catalase gene polymorphisms in postmenopausal Korean women

Loci	Position	rs#	Total genotyped women (n)	Genotype (participant number)			HWE*	
							χ^2	p Value
-20T→C	5'-UTR	rs17881315	547	TT (259)	CT (232)	CC (56)	T (0.686)	C (0.314)
+144C→T	Intron 1	rs17886119	549	CC (287)	CT (227)	TT (35)	C (0.730)	T (0.270)
+22348C→T	Exon 9	rs17880449	553	CC (186)	CT (261)	TT (106)	C (0.572)	T (0.428)
+33078A→G	3'-UTR	rs17879188	549	AA (284)	AG (227)	GG (38)	A (0.724)	G (0.276)

HWE, Hardy-Weinberg equilibrium; UTR, untranslated region.

*p Values of deviation from HWE.

Table 4 Regression analysis of bone mineral density at the lumbar spine and femoral neck, and serum levels of bone turnover markers according to catalase gene polymorphisms in postmenopausal Korean women

Loci	C/C ^a	C/R ^a	R/R ^a	P _a	P _b	P _c
Lumbar spine BMD (g/cm²)						
-20 T→C	0.88 (0.17)	0.86 (0.17)	0.87 (0.18)	0.53	0.50	0.79
+144 C→T	0.87 (0.18)	0.88 (0.17)	0.88 (0.15)	0.83	0.89	0.79
+22348C→T	0.85 (0.17)	0.88 (0.18)	0.88 (0.15)	0.02	0.01	0.29
+33078 A→G	0.88 (0.17)	0.86 (0.17)	0.83 (0.19)	0.30	0.50	0.22
Femoral neck BMD (g/cm²)						
-20 T→C	0.73 (0.12)	0.71 (0.13)	0.73 (0.13)	0.68	0.43	0.70
+144 C→T	0.72 (0.13)	0.72 (0.13)	0.73 (0.11)	0.98	0.76	0.49
+22348C→T	0.71 (0.13)	0.73 (0.12)	0.73 (0.12)	0.07	0.05	0.35
+33078 A→G	0.73 (0.12)	0.72 (0.13)	0.71 (0.13)	0.37	0.44	0.51
Serum osteocalcin concentration (ng/ml)						
-20 T→C	29.2 (11.5)	30.2 (12.0)	32.4 (12.7)	0.09	0.16	0.16
+144 C→T	30.4 (12.4)	29.4 (11.8)	30.5 (9.0)	0.58	0.43	0.80
+22348C→T	32.1 (12.7)	28.9 (11.8)	28.5 (10.1)	0.02	0.008	0.23
+33078 A→G	29.7 (11.8)	30.5 (13.0)	32.3 (13.8)	0.23	0.31	0.35
BSAP activity (U/l)						
-20 T→C	30.5 (10.9)	32.4 (10.0)	33.0 (9.2)	0.11	0.08	0.55
+144 C→T	32.1 (10.0)	30.9 (10.9)	33.5 (9.1)	0.72	0.41	0.40
+22348C→T	32.2 (9.7)	32.1 (11.1)	29.5 (9.3)	0.14	0.51	0.06
+33078 A→G	31.7 (10.7)	32.8 (10.3)	35.2 (10.8)	0.09	0.10	0.25

BMD, bone mineral density; BSAP, bone-specific alkaline phosphatase.

C/C, C/R and R/R represent homozygotes for the common allele, heterozygotes and homozygotes for the rarer allele, respectively.

P_a, P_b and P_c are p values of codominant, dominant and recessive models for multiple regression analysis, respectively, controlling for age, years since menopause, weight and height.^aValues are mean (SD).

(12.7) ng/ml, $p = 0.008$). None of the SNPs, however, was significantly associated with serum levels of bone-specific alkaline phosphatase.

After haplotypes were constructed using the four SNPs, four haplotypes were selected (table 5). Among these haplotypes, we found a significant association between HT4 and BMD values at the various sites, such as lumbar spine, total femur, trochanter and Ward's triangle ($p = 0.001$ – 0.03 ; table 6). The participants with HT4 had higher BMD than those without. Further, the levels of bone turnover markers, especially serum osteocalcin concentration, were lower in the participants with HT4. Any other haplotypes (HT1–HT3) were not associated with the values of BMD and bone turnover markers (data not shown).

Vertebral fractures were noted in 91 women. Those with fractures were significantly older (mean (SD) 65.5 (6.6) ν 57.9 (6.6) years, $p < 0.001$) and had experienced more YSM (18.3 (9.6) ν 8.7 (7.3) years, $p < 0.001$) than those without. Despite the significant associations of the +22348C→T polymorphism and HT4 haplotype with lumbar BMD and serum osteocalcin concentration, we did not observe significant associations between the frequency of vertebral fracture and this polymorphism or haplotype after adjusting for confounding variables (see supplementary table C available online at <http://jmg.bmjournals.com/supplemental>).

DISCUSSION

Although multiple environmental factors are involved in the pathogenesis of osteoporosis, genetic factors are also largely responsible for bone mass, accounting for about 50–85% of the variance in BMD on the basis of twin and family studies.^{25–27} In this study, we focused on the role of *CAT* polymorphisms in the determinations of bone mass and bone turnover rate. Although we could not establish that the genetic polymorphisms have a critical role for the development of osteoporotic fractures, we observed that one SNP (+22348C→T) and one haplotype (HT4) were significantly associated with higher BMD values at the lumbar spine or proximal femur sites, as well as with lower serum osteocalcin concentrations. To our knowledge, this report is the first to suggest that the *CAT* gene may be a genetic determinant of bone mass and bone turnover marker, and may therefore have an important role in bone metabolism. In addition, our results suggest that antioxidant capacity and oxidative stress may be important for bone metabolism in humans.

Some reports have suggested that the +22348C→T is a susceptible locus for a family with hypocatalasaemia in Hungary,²⁸ type 1 diabetes¹⁸ and vitiligo.²⁰ However, the +22348C→T nucleotide substitution in exon 9 does not cause an amino acid change (asp389asp), suggesting that the

Table 5 Haplotypes and their frequencies for the catalase gene in postmenopausal Korean women

Haplotype	-20T→C	+144C→T	+22348C→T	+33078A→G	Frequencies
HT1	C	C	C	G	0.289
HT2	T	T	T	A	0.264
HT3	T	C	C	A	0.252
HT4	T	C	T	A	0.157
Others					0.038

Only those with frequencies > 0.1 are shown.

Table 6 Regression analysis of bone mineral density at the various sites and serum levels of bone turnover markers according to haplotype 4 of the catalase gene in postmenopausal Korean women

Sites	HT4−/HT4−*	HT4+/HT4−*	HT4+/HT4+*	p _a	p _b	p _c
BMD values (g/cm²)						
Lumbar spine	0.86 (0.17) (n = 391)	0.90 (0.18) (n = 139)	0.90 (0.15) (n = 17)	0.004	0.004	0.11
Femoral neck	0.69 (0.13) (n = 391)	0.73 (0.13) (n = 139)	0.72 (0.13) (n = 17)	0.11	0.09	0.63
Total femur	0.77 (0.13) (n = 312)	0.79 (0.13) (n = 120)	0.79 (0.13) (n = 14)	0.02	0.02	0.25
Femoral trochanter	0.57 (0.12) (n = 312)	0.58 (0.11) (n = 120)	0.60 (0.12) (n = 14)	0.05	0.16	0.03
Femoral shaft	0.97 (0.17) (n = 216)	1.00 (0.16) (n = 93)	0.99 (0.17) (n = 9)	0.07	0.06	0.51
Ward's area	0.50 (0.14) (n = 312)	0.54 (0.15) (n = 120)	0.51 (0.17) (n = 14)	0.008	0.007	0.34
Serum osteocalcin concentration (ng/ml)						
	31.0 (12.7) (n = 397)	28.6 (11.8) (n = 146)	24.2 (7.4) (n = 17)	0.01	0.03	0.10
BSAP activity (U/l)						
	33.3 (10.5) (n = 397)	29.4 (10.7) (n = 146)	28.5 (7.6) (n = 17)	0.06	0.08	0.21

BMD, bone mineral density; BSAP, bone-specific alkaline phosphatase.

p_a, p_b and p_c are p values of codominant, dominant and recessive models for multiple regression analysis, respectively. The values of BMD at the lumbar spine and femoral neck, and those of bone turnover markers, were controlled for age, years since menopause, weight and height as covariates. The BMD values at the total femur, femoral trochanter, femoral shaft and Ward's area, were controlled for age, years since menopause, weight, height and bone densitometry as covariates.

*†Values are mean (SD) (number of participants) BMD and bone turnover markers.

polymorphism is a marker rather than a direct contributor to changes in catalase activity. It is also unlikely that other SNPs almost completely linked with +22348C→T, including the +10119A→G, +15189A→T and +22587A→G polymorphisms, have functional importance, because they are located in introns. Haplotype analyses also showed that the HT4, which included the T allele of +22348C→T, was significantly associated with higher BMD and lower bone turnover markers, and that the association of the HT4 was stronger than that of the +22348C→T polymorphism. The association of HT4 with lumbar BMD was statistically more powerful than the +22348C→T ($p < 0.001$ v $p = 0.01$), and the HT4 was significantly associated with BMD at other femur sites where +22348C→T was not significantly associated with BMD. These findings suggest that the effects of SNPs linked to +22348C→T may have functional relevance, although the precise sites were not shown in this study.

Despite the significant associations of +22348C→T and HT4 with higher BMD and lower serum osteocalcin concentrations, neither was associated with risk of vertebral fracture. This indicates that many fall-related environmental factors other than BMD have important roles in determining risk of fracture. The heritability of fracture itself has been estimated to lie between 25% and 35%,^{29,30} which is much lower than the heritability of the BMD values.^{25–27} Therefore, no association between CAT polymorphisms and vertebral fracture risk cannot exclude a possible role of CAT +22348C→T or HT4 as a genetic marker for bone metabolism.

Our study had several potential limitations. Firstly, the study population comprised of women who visited a university hospital, and may not have been representative of the general population residing in a community, thus possibly resulting in selection bias. Secondly, we did not show the differences in catalase activity or blood ROS content according to the absence

or presence of the +22348C→T and HT4. Therefore, we cannot insist that the genotypes are functionally relevant. Thirdly, it may be argued that the Bonferroni correction should be applied to the p values obtained in our study. If Bonferroni correction were strictly adopted, associated p values could not retain all significances. However, although there is a chance of type I error owing to multiple comparisons, when considering the facts that

- the significant associations were also detected between HT4 and lumbar BMD, even after multiple testing was applied, and
- consistent positive signals at the same sites (+22348C→T and HT4) with related phenotypes (BMD in various bone sites and bone turnover markers), the significance of associations might be acceptable.

In summary, we found that the +22348C→T polymorphism and HT4 of the CAT gene were associated with BMD and bone turnover markers in postmenopausal Korean women. These results suggest that the CAT gene may be a genetic determinant of osteoporosis in postmenopausal women (additional tables D and E are available online at <http://jmg.bmjournals.com/supplemental>).

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Key points

- This is the first report of associations between catalase gene polymorphisms and bone metabolism.
- The +22348C→T polymorphism and HT4 of the *catalase* gene may be useful genetic markers for bone mass and bone turnover marker.
- Additional clinical evidence that antioxidant capacity or oxidative stress may be important for bone metabolism in humans.

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