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Original Article

Genetic Variations of *ESR1* Gene are Associated with Bone Mineral Density Traits in Korean Women

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Bone mineral density (BMD) is used in the clinical diagnosis of osteoporosis and the assessment of fracture risk. Osteoporosis, characterized mainly by decreased BMD, is a highly heritable complex disorder and a major public health concern to hundreds of millions of elderly persons worldwide. However, the specific genetic variants determining risk for low bone density are still largely unknown. Here, we performed association analysis to elucidate the possible relations of genetic polymorphisms in *ESR1* gene with low bone density. By examining genotype data of a total of 1813 women in the Korean Association REsource (KARE) study, we discovered the *ESR1* gene polymorphisms are associated with decreased BMD and osteoporosis. The results on the BD-RT (bone density estimated by T-score at distal radius), three SNPs (rs2248586, rs9371557, and rs1569788) within the *ESR1* gene were significantly associated with bone density. The results on the BD-TT (bone density estimated by T-score at midshaft tibia), five SNPs (rs9371552, rs2248586, rs712221, rs7772475, and rs3798577) were significantly associated with bone density. The SNP rs2248586 within the *ESR1* gene had commonly significance in both BD-RT (β=-0.151, dominant *P*=0.049) and BD-TT (β=-0.156, dominant *P*=0.039). In the SNP rs2248586, their β-values in BD-RT and/or BD-TT showed consistent trends with the odds ratios (ORs) of osteoporosis. In summary, we found statistically significant SNPs in *ESR1* gene that are associated with both decreased BMD and osteoporosis traits. Therefore, our findings suggest *ESR1* gene could be related to pathogenesis of osteoporosis.

Key Words: Bone Mineral Density, Osteoporosis, *ESR1*, SNP, Association

INTRODUCTION

Morbidity and mortality associated with osteoporosis and osteoporotic fractures will increase substantially as population age and be a major health threat to hundreds of millions of elderly individuals worldwide (Wiktorowicz et al., 2001).

Bone mineral density (BMD) is referring to the amount of matter per square centimeter of bones, and used in clinical practice as an indirect indicator of osteoporosis and in the assessment of fracture risk (Kanis et al., 1997). BMD is measured by densitometry, often performed in the radiology or nuclear medicine departments of hospitals. The measurement is painless and non-invasive and involves minimal radiation exposure. Bone density measurements are used to screen women for osteoporosis risk and to identify those who might benefit from measures to improve bone strength (Cole, 2008).

Several efforts have been dedicated to understanding whether variants in genes known to influence bone physiology also influence risk for decreased BMD and possible fractures - called candidate genes (Williams and Spector, 2007). Whether these genes do indeed influence propensity to osteoporosis and fracture has remained uncertain because many candidate gene studies lacked

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sufficient sample sizes and a replication group with which to validate findings (Albagha and Ralston, 2006).

Given the strong genetic contribution to BMD, genome-wide association studies (GWAS) have already been successful in identifying 10 loci associated at a genome-wide significance (GWS) level with BMD (Styrkarsdorrir *et al*., 2008; Styrkarsdorrir et al., 2009). Recently Estrada et al. had reported the 56 bone mineral density loci and 14 loci associated with risk of fracture by genome-wide meta-analysis (Estrada et al., 2012). The human *ESR1* gene (OMIM133430) is located in chromosome 6q25.1 region and encodes an estrogen receptor, a ligand-activated transcription factor composed of several domains important for hormone binding, DNA binding, and activation of transcription. The protein localizes to the nucleus where it may form a homodimer or a heterodimer with estrogen receptor 2 (Parker et al., 1993).

There are 2 known estrogen receptors: estrogen receptor α (ESR1) and estrogen receptor β (ESR2). Estrogen is known to have long-term effects on the bone turnover. These effects are mediated through estrogen-activated transcription factors, ESR1 (encoded by the *ESR1* gene). Functional ESR1 is activated by binding to estrogen response elements located near the promoter sequence of many genes.

Considering the important role of estrogens in bone turnover and their protective effect on BMD, numerous studies have targeted *ESR1* gene to evaluate its association with BMD. However, the results are inconsistent and the effects of these two polymorphisms on BMD and fracture risk remain ambiguous (Ioannidis et al., 2002; Ioannidis et al., 2004; van Meurs et al., 2003; Yamada et al., 2002).

In this study, to further understand whether *ESR1* gene is associated not only with BMD but also with osteoporosis, we have extensively examined the SNPs in *ESR1* gene as the genetic factors in the Korean population. Notably, this study provides insight into the relation with *ESR1* gene and osteoporosis.

MATERIALS AND METHODS

Subjects and clinical characteristics

Subjects in the Korean population in the Korean Association REsource (KARE) study were described in more detail by other study (Cho et al., 2009). Briefly 10,038 persons in the Ansung-Ansan prospective community cohorts were recruited. A two-community cohort study in South Korea was initiated beginning in 2001 as part of a major project for the Korean Health and Genome Study (KHGS) in Korea National Health and Genome Study (KNIH).

Among the women participants (n=4,659), 855 subjects who had been treated with any kind of drugs and 234 subjects who did not participate in measurement of bone density were excluded, and the only women above 50 years, 1813 subjects, were finally investigated in this study. And we divide into osteoporosis cases (575 women) and control (463 women) to analyze the association between the SNPs in *ESR1* and osteoporosis status. The age and cohort were included in the model as covariates.

For quantitative bone mineral density trait analysis, 1813 women with 50 above aged subjects (mean age 59.33 ± 5.62 years) were investigated. For the case-control analysis of osteoporosis, the subjects whose bone density T scores of either distal radius or midshaft tibia were less than -2.5 SD were allocated as the case (N=575) and the subjects whose bone densities T-scores for both distal radius and midshaft tibia were more than -1 SD were allocated as the control (N=463), according to the general diagnostic categories to be established for adult women (Kanis et al., 1994). Clinical characteristics of the subjects are summarized in Table 1.

This study was approved by the institutional review board of the Korean National Institute of Health (KNIH). Written informed consent was obtained from all subjects.

Measurement of bone mineral density

Bone density is a proxy measurement for bone strength, which is the resistance to fracture, widely used to screen for osteoporosis. Bone density was estimated by T-score by dividing the difference of measured SOS (speed of sound) from mean SOS in healthy young adult population by the standard deviation of SOS in young adult population. Bone SOS was measured quantitative ultrasound at distal radius or mid-shaft tibia using the Omnisense 7000P QUS (Sunlight Medical Ltd, Tel-Aviv, Israel) in the subjects of the KARE study.

Table 1. Basic characteristics of in the KARE women (age ≥ 50) subjects

Characteristics	Quantitative analysis for bone density	Case-control analysis for osteoporosis		
		Controls	Cases	*P* value*
No.	1813	463	575	
Age (year)	59.33 ± 5.62	56.25 ± 5.23	61.50 ± 4.92	< 0.0001
BMI (kg/m^2)	24.85 ± 3.24	24.49 ± 2.92	25.11 ± 3.38	0.0016
Distal Radius T score	-0.53 ± 1.54	0.70 ± 1.16	-1.45 ± 1.56	< 0.0001
Midshaft Tibia T score	-1.55 ± 1.51	0.14 ± 0.86	-3.11 ± 1.02	< 0.0001

Abbreviation: BMI; Body Mass Index, Osteoporosis was defined as any bone density T score of -2.5 SD or below and non-osteoporosis was defined as both bone densities T-score of -1 SD over. *Significant differences in characteristics between the controls and cases were determined by the two-tailed Student's *t*-test.

Genotyping and selection of *ESR1* gene SNPs

The detailed genotyping, quality control processes and quantitative traits including BD-RT and BD-TT were described in the previous report (Cho et al., 2009). Briefly, most DNA samples were isolated from the peripheral blood of participants and genotyped using the Affymetrix Genome-Wide Human SNP array 5.0 (Affymetrix, Inc., Santa Clara, CA, USA). The accuracy of the genotyping was calculated by Bayesian Robust Linear Modeling using the Mahalanobis Distance (BRLMM) algorithm (Rabbee et al., 2006). Samples that had genotyping accuracies were lower than 98%, high missing genotype call rates (≥4%), high heterozygosity (>30%), or gender biases were excluded.

The SNPs in *ESR1* that we analyzed were selected from the KARE data, based on their positions within the gene boundary (5 kb upstream and downstream of the first and last exons, respectively) (Table 2). The positions of the SNPs were validated in the NCBI database (http://www.ncbi.nlm.nih.gov). The clinical information and genotype data that we used were graciously provided by the Center for Genome Science, KNIH, Korea Center for Disease Control (KCDC).

Statistical analysis

Most statistical analyses were performed using PLINK version 1.07 (http://pngu.mgh.harvard.edu/~purcell/plink) and PASW Statistics version 17.0 (SPSS Inc., Chicago, IL, USA). Linear regression was used to analyze the bone density as quantitative traits, controlling for cohort and age as covariates. The 60 selected SNPs were also analyzed in osteoporosis case-control studies using logistic regression analysis.

The association tests were based on an additive, dominant, and recessive genetic model, and *P*-values were not adjusted for multiple tests. Statistical significance was determined at a two-tailed value of $P < 0.05$.

Haploview version 4.2 (Whitehead Institute for Biomedical Research, Cambridge, MA, USA) was used to examine the structure of the linkage disequilibrium (LD) block (Barrett et al., 2005) using the KARE genotype data for *ESR1* gene (Fig. 1). The LD coefficient r^2 was examined between all pairs of biallelic loci (Hedrick et al., 1987).

RESULTS

Association analysis with SNPs in *ESR1* gene and bone density

We informed the *ESR1* gene and its 60 SNPs (Table 2). And, the basic characteristics of study subjects were shown to Table 1. The mean age of the 1813 study women was 59.33 years. The mean BD-RT of the 1813 women was -0.53 ± 1.54, and the mean BD-TT was -1.55 ± 1.51 (Table 1). And, the mean and variance of BD-RT and BD-TT were all statistically different between case-control groups by Student's T-test in Table 1.

Linear regression analysis was used to associate genotypes with bone density traits, controlling for age and cohort as covariates. The results of associations on the seven SNPs of *ESR1* gene in women were listed in Table 3.

The results on the BD-RT, three SNPs (rs2248586, rs9371557 and rs1569788) were significantly associated with

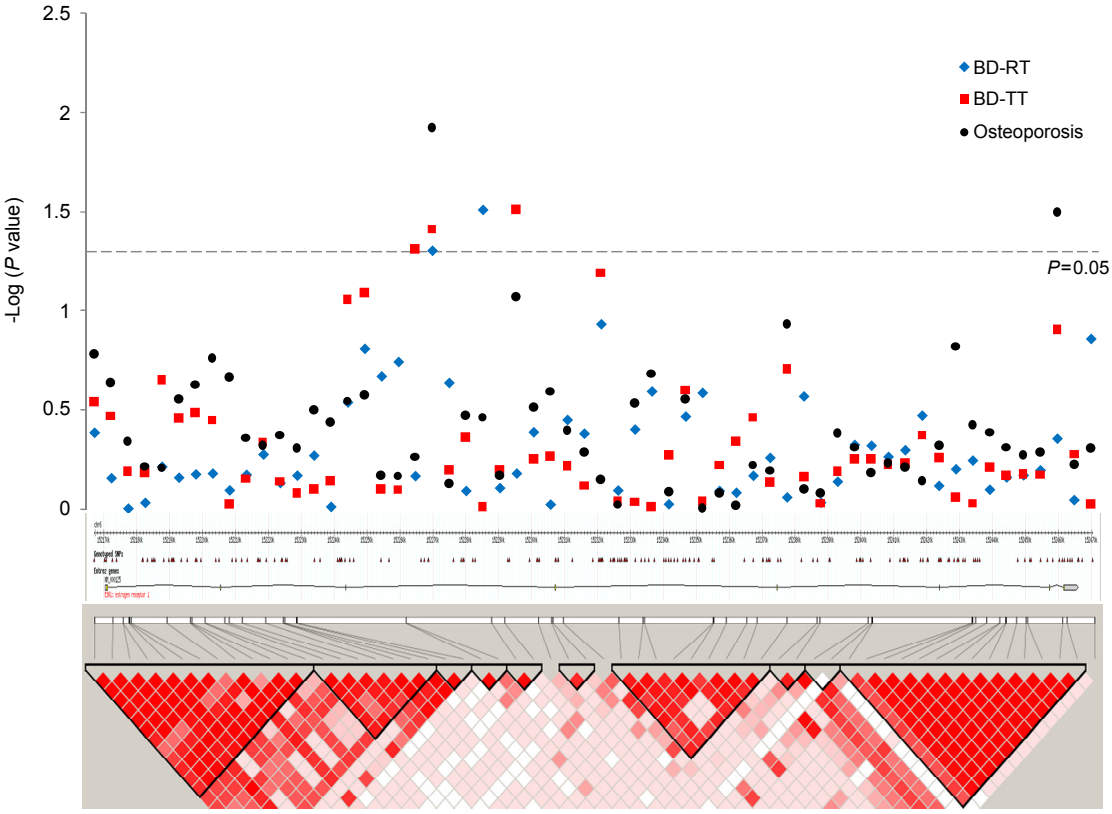

Fig. 1. The plot of *P*-values of the studied 60 SNPs, the gene structure and linkage disequilibrium blocks in the *ESR1* gene. The top panel shows the plots of *P*-values of the studied 60 SNPs in the *ESR1* gene in the bone mineral density traits analysis (BD-RT, bone density estimated by T-score at distal radius; BD-TT, bone density estimated by T-score at midshaft tibia) and the logistic case-control analysis of the KARE women (Additive genetic model). Standard significant *P*-value threshold (*P*=0.05) are indicated by the dotted lines. The middle panel shows the physical position of *ESR1* on chromosome 6. The bottom panel shows a Haploview of LD (r^2) based on genotyping data from 1813 KARE women and are generated by using the Haploview program.

bone density of distal radius in the 50 above aged women (Table 3). The SNP rs2248586 had significance (β=-0.151, dominant *P*=0.049), the SNP rs9371557 had significance (β=0.148, dominant *P*=0.031), and the SNP rs1569788 was also significantly associated (β=0.253, recessive *P*= 5.4×10^{-3}).

The results on the BD-TT, five SNPs (rs9371552, rs2248586, rs712221, rs7772475 and rs3798577) were significantly associated with bone density of midshaft tibia (Table 3). The SNP rs9371552 had significance (β=0.117, additive *P*=0.019; β=0.133, dominant *P*=0.049), and the SNP rs2248586 had significance (β=-0.142, additive *P*= 0.037; β=-0.156, dominant *P*=0.039). The SNP rs712221 had only significance (β=-0.154, dominant *P*=0.031), and the SNP rs7772475 was significantly associated (β=0.178, additive *P*=0.027; β=1.105, recessive *P*=0.014). And the last SNP rs3798577 was significantly associated (β=-0.266, recessive *P*=3.8×10^{-3}). The SNP (rs2248586) had commonly significance in both BD-RT and BD-TT.

Association analysis with SNPs in *ESR1* gene and osteoporosis

The most important risk factors for osteoporosis are advanced age, caused by a rapid reduction of bone density is related with estrogen deficiency following menopause in women. Therefore, we focused on the subjects with 50 above aged women. And, genetic variations of the present study were reanalyzed in the osteoporosis case and control subgroups (Table 4).

Genetic variations of *ESR1* in 50 above aged women were associated with bone density. The three SNPs (rs2248586, rs4870061, rs750686) of *ESR1* gene were associated with

Table 2. Information about the SNPs in the *ESR1* gene analyzed in this study

No.	SNP	Location (bp)	Minor allele	MAF	Genotype missing rate	HWE-p	Function
1	rs2982571	152054432	A	0.119	0.0002	0.4402	INTRONIC
2	rs851985	152062083	G	0.113	0.0006	0.0398	INTRONIC
3	rs851983	152066108	G	0.108	0.0069	0.1475	INTRONIC
4	rs17081604	152066213	T	0.024	0.0000	0.5256	INTRONIC
5	rs9383591	152068705	G	0.379	0.0002	0.6186	INTRONIC
6	rs851981	152068767	T	0.112	0.0000	0.4185	INTRONIC
7	rs851980	152069648	G	0.111	0.0011	0.3350	INTRONIC
8	rs7772579	152084195	C	0.173	0.0000	0.3313	INTRONIC
9	rs2982565	152093547	A	0.068	0.0004	0.8171	INTRONIC
10	rs6899458	152093736	A	0.195	0.0002	0.7085	INTRONIC
11	rs2982561	152094345	T	0.166	0.0013	0.4268	INTRONIC
12	rs3020348	152099607	T	0.243	0.0002	0.0167	INTRONIC
13	rs2982554	152099703	G	0.243	0.0000	0.0151	INTRONIC
14	rs3020306	152107579	A	0.242	0.0000	0.6897	INTRONIC
15	rs1856057	152109562	C	0.287	0.0000	0.5664	INTRONIC
16	rs9322330	152115011	A	0.276	0.0000	0.8835	INTRONIC
17	rs9371551	152124339	T	0.297	0.0086	0.6219	INTRONIC
18	rs6916835	152131717	G	0.294	0.0015	0.8598	INTRONIC
19	rs6939257	152131738	T	0.294	0.0000	0.9156	INTRONIC
20	rs9371552	152132228	T	0.362	0.0000	0.1628	INTRONIC
21	rs2248586	152137025	C	0.137	0.0002	0.8526	INTRONIC
22	rs2347759	152137153	G	0.297	0.0047	0.5500	INTRONIC
23	rs3844508	152181735	C	0.288	0.0097	0.8296	INTRONIC
24	rs9371557	152181902	C	0.299	0.0000	0.8612	INTRONIC
25	rs9340817	152216799	T	0.256	0.0032	0.2987	INTRONIC
26	rs712221	152221934	T	0.437	0.0009	0.3406	INTRONIC
27	rs1709183	152235689	T	0.395	0.0000	0.7591	INTRONIC
28	rs11155819	152241052	G	0.170	0.0000	0.5332	INTRONIC
29	rs9322335	152241822	G	0.400	0.0009	0.6036	INTRONIC
30	rs11155820	152245903	G	0.054	0.0408	0.3756	INTRONIC
31	rs7772475	152268615	A	0.102	0.0011	0.0307	INTRONIC
32	rs9397453	152278572	T	0.101	0.0479	0.8041	INTRONIC
33	rs4870061	152279161	T	0.473	0.0006	0.8832	INTRONIC
34	rs1801132	152307215	G	0.481	0.0000	0.8373	SYNONYMOUS_CODING
35	rs9397459	152307352	A	0.094	0.0058	0.0469	INTRONIC
36	rs3020314	152312365	T	0.200	0.0002	0.1299	INTRONIC
37	rs3020394	152320906	A	0.373	0.0116	1.0000	INTRONIC
38	rs1884051	152324972	T	0.476	0.0000	0.3040	INTRONIC
39	rs9383951	152337306	G	0.091	0.0002	0.2489	INTRONIC
40	rs2144025	152349399	G	0.432	0.0002	0.4737	INTRONIC
41	rs12664544	152350666	G	0.319	0.0024	0.9462	INTRONIC
42	rs1569788	152370309	T	0.442	0.0099	0.9524	INTRONIC

Table 2. Continued

No.	SNP	Location (bp)	Minor allele	MAF	Genotype missing rate	HWE-p	Function
43	rs9340955	152371894	A	0.011	0.0090	1.0000	INTRONIC
44	rs9340958	152372366	T	0.075	0.0354	0.5892	INTRONIC
45	rs9341004	152411941	C	0.309	0.0277	0.0003	INTRONIC
46	rs3020368	152412883	A	0.079	0.0017	0.4807	INTRONIC
47	rs6930355	152413084	G	0.336	0.0017	0.1223	INTRONIC
48	rs6557192	152414235	A	0.335	0.0011	0.0523	INTRONIC
49	rs1884152	152418873	T	0.335	0.0002	0.0565	INTRONIC
50	rs2273206	152424004	A	0.335	0.0206	0.0031	INTRONIC
51	rs2273207	152424018	C	0.314	0.0002	0.0448	INTRONIC
52	rs2207396	152424075	T	0.205	0.0101	0.7517	INTRONIC
53	rs3798571	152426493	C	0.343	0.0004	0.0293	INTRONIC
54	rs3778080	152426929	C	0.313	0.0019	0.0346	INTRONIC
55	rs3798573	152431055	C	0.332	0.0000	0.0642	INTRONIC
56	rs9479191	152434853	C	0.332	0.0019	0.0316	INTRONIC
57	rs3778089	152435454	A	0.332	0.0004	0.0552	INTRONIC
58	rs750686	152449819	C	0.450	0.0032	0.0753	INTRONIC
59	rs9322359	152451715	A	0.317	0.0163	0.1090	INTRONIC
60	rs3798577	152462823	G	0.387	0.0004	0.8288	3PRIME_UTR

osteoporosis status. The significant SNP were rs2248586 (OR=1.41, CI: 1.06~1.87, additive P=0.018), rs4870061 (OR=0.70, CI: 0.50~0.97, recessive P=0.032), and rs-750686 (OR=0.71, CI: 0.52~0.97, dominant P=0.032) (Table 4).

Especially, rs2248586 showed their β-values in bone densities (BD-RT and BD-TT) consistent trends with the odds ratios (ORs) of osteoporosis. Consequently, these results suggested that the individuals with the minor alleles of the rs2248586 SNP in the *ESR1* gene may be more susceptible to the development of osteoporosis in the Korean 50 above aged women.

DISCUSSION

In this study, we had investigated the genetic variations of *ESR1* gene are associated with BMD and osteoporosis in the 1813 Korean women (Table 1). The association study showed that the 3 SNPs were significant for the BD-RT, and 5 SNPs were significant for the BD-TT. The SNP rs2248586 was found to be significant in two kinds of bone densities, and their effect directions were coincident (Table 3). Furthermore the SNP rs2248586 had interrelated with decreased BMD and positive OR of osteoporosis (Table 3, 4). This significant SNP had negative beta value in BMD, and those means that the carrier of minor allele had low BMD, and also susceptibility for osteoporosis. Therefore this SNP would be contributed low value of BMD, and contribute to pathogenesis of osteoporosis. Limitations of this study included the lack of detailed covered SNPs in the *ESR1* gene, but this report has the value of association study for *ESR1* and BMD traits.

By *in silico* analysis of the 9 significant SNPs in the TRANSFAC database (http://www.cbrc.jp/research/db/TFSEARCH.html), we found that the minor allele of rs750686 contained ATF2 and SREBP1 transcription factor binding site (89.0 and 85.2 scoring point, repeatedly). ATF2, Activating transcription factor-2, was linked to NFκB and MAPK signaling pathway (Zayzafoon et al., 2002), and SREBP1, sterol regulatory element binding transcription factor 1, was related to hyperlipidemia in low-density lipoprotein receptor-deficient mice (Karasawa et al., 2011).

Therefore, two transcription factors could be connected with bone metabolism. The polymorphism in the *ESR1* gene may affect the expression or the function of *ESR1* gene. In women, BMD and *ESR1* gene polymorphisms seem to play a determinant role in osteoporotic pathology.

The association of BMD with *ESR1* genotypes has been identified in several studies (Kobayashi et al., 1996; Mahonen et al., 1997; Willing et al., 1998). Authors of those reports emphasize that the genotype that predicts low or high BMD may be population specific. Indeed, Japanese women with the PP genotype had lower BMD while in American and Finnish females decreased BMD was associated with the pp genotype.

There is some convincing evidence on the 19 association between *ESR1* PX haplotype and lower BMD (Colin et al., 2003; van Meurs et al., 2003; Zhang et al., 2003). A Chinese study revealed association between PX haplotype and low spine mineral density while PX carrier had decreased hip

Table 3. The significant associations with bone density estimated by T-score and the SNPs in *ESR1* gene in the KARE women with 50 above aged subjects

Bone density	SNP	Minor allele	MAF	50 above aged women (n=1,813)					
				beta ± s.e.m.	Add *p*	beta ± s.e.m.	Dom *p*	beta ± s.e.m.	Rec *p*
BD-RT									
	rs9371552	T	0.362	0.036 ± 0.05	0.482	0.028 ± 0.07	0.682	0.087 ± 0.11	0.409
	rs2248586	C	0.137	-0.129 ± 0.07	0.062	-0.151 ± 0.08	**0.049**	-0.090 ± 0.25	0.718
	rs9371557	C	0.299	0.101 ± 0.05	0.053	0.148 ± 0.07	**0.031**	0.075 ± 0.12	0.519
	rs712221	T	0.437	-0.014 ± 0.05	0.778	-0.032 ± 0.07	0.661	0.003 ± 0.09	0.970
	rs7772475	A	0.102	-0.107 ± 0.08	0.196	-0.135 ± 0.09	0.117	0.519 ± 0.46	0.258
	rs1569788	T	0.442	0.072 ± 0.05	0.152	-0.012 ± 0.07	0.873	0.253 ± 0.09	$\mathbf{5.4 \times 10^{-3}}$
	rs3798577	G	0.387	0.045 ± 0.05	0.356	0.104 ± 0.07	0.139	-0.019 ± 0.09	0.838
BD-TT									
	rs9371552	T	0.362	0.117 ± 0.05	**0.019**	0.133 ± 0.07	**0.049**	0.189 ± 0.10	0.067
	rs2248586	C	0.137	-0.142 ± 0.07	**0.037**	-0.156 ± 0.08	**0.039**	-0.197 ± 0.25	0.424
	rs9371557	C	0.299	-0.002 ± 0.05	0.967	0.002 ± 0.07	0.968	-0.018 ± 0.11	0.872
	rs712221	T	0.437	-0.050 ± 0.05	0.309	-0.154 ± 0.07	**0.031**	0.078 ± 0.09	0.388
	rs7772475	A	0.102	0.178 ± 0.08	**0.027**	0.156 ± 0.08	0.064	1.105 ± 0.45	**0.014**
	rs1569788	T	0.442	0.075 ± 0.04	0.127	0.093 ± 0.07	0.197	0.104 ± 0.09	0.241
	rs3798577	G	0.387	-0.069 ± 0.05	0.147	0.005 ± 0.07	0.937	-0.266 ± 0.09	$\mathbf{3.8 \times 10^{-3}}$

Statistically significant ($P<0.05$) are indicated in bold. Abbreviations: MAF, Minor allele frequency; BD-RT, bone density estimated by T-score at distal radius; BD-TT, bone density estimated by T-score at midshaft tibia; s.e.m, standard error; Add *p*, Additive genetic model *p* value; Dom *p*, Dominant genetic model *p* value; Rec *p*, Recessive genetic model *p* value.

Table 4. The significant association with Osteoporosis cases and controls and the SNPs in *ESR1* gene in the KARE women with 50 above aged subjects

SNP	Minor allele	MAF	KARE Women Subjects (463 controls, 575 cases)					
			OR (95% CI)	Add *p*	OR (95% CI)	Dom *p*	OR (95% CI)	Rec *p*
rs2248586	C	0.137	1.41 (1.06~1.87)	**0.018**	1.50 (1.09~2.06)	**0.012**	1.24 (0.45~3.37)	0.670
rs4870061	T	0.473	0.82 (0.67~1.00)	0.052	0.85 (0.62~1.16)	0.292	0.70 (0.50~0.97)	**0.032**
rs750686	C	0.450	0.89 (0.73~1.08)	0.233	0.71 (0.52~0.97)	**0.032**	1.06 (0.75~1.49)	0.732

Significant association ($P<0.05$) are indicated in bold and underline. Abbreviations: MAF, Minor allele frequency; OR, Odds ratio; CI, Confidence Interval; Add *p,* Additive genetic model *p* value; Dom *p,* Dominant genetic model *p* value; Rec *p,* Recessive genetic model *p* value.

BMD (Zhang et al., 2003). However, other studies that showed no associations between the polymorphic variants of *ESR1* and BMD (Gennari et al., 1998; Bechereni et al., 2000; Yamada et al., 2002). This conflicting observation may result from a diverse distribution of the polymorphisms among the populations, and the impact of environmental factors such as dietary habits, physical activity and exposure to sunlight.

In view of the above inconsistencies between studies, our results should be interpreted with caution. Osteoporosis is a complex multigenic disease. Moreover, multiple factors and metabolic processes are involved in bone turnover and bone quality. Our study focused on the association between the polymorphisms of the *ESR1* gene and BMD in Korean women. Genome-wide association studies (GWAS), which perform a hypothesis-free search for genetic determinants (McCarthy et al., 2008), have already been successful in identifying 10 loci associated at a genome-wide significance (GWS) level with BMD (Styrkarsdottir et al., 2008; Styrkarsdottir et al., 2009).

From our research, genetic association of the *ESR1* gene has been searched in the genetic association databases (HuGe Navigator: http://hugenavigator.net). They had presented that the *ESR1* gene polymorphisms associated with the neoplasm, hypertension, obesity, cardiovascular disease, diabetes etc including osteoporosis. Francesco *et al*. had reported that ESR1 rs2234693 genotypes correlated with family history of osteoporosis and hip fracture (Francesco et al., 2009). The other article had published that the genetic variants in *ESR1* were associated with osteoporosis and the effects of genetic variants in *ESR1* might be sex dependent (Harslof et al., 2010).

The main limitation of this study was small sample size contrary to GWAS. The observed associations may represent a true trend that might be population-specific. However, further large-scale investigations are required to confirm the evidence of *ESR1* gene linkage to BMD and to clarify whether its polymorphisms may be applicable as genetic markers of osteoporosis prediction among women.

In summary, we investigated the presence of BMD traits-associated SNPs in *ESR1* gene. We also found statistically significant SNPs that are associated with decreased BMD and osteoporosis traits. Therefore, this study suggests *ESR1* gene could be related to pathogenesis of osteoporosis in Korean women.

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REFERENCES

Albagha OM, Ralston SH. Genetics and osteoporosis. Rheum Dis Clin North Am. 2006. 32: 659-680.

Barrett JG, Fry B, Maller J, Daly MJ. Haploview: Analysis and visualization of LD and haplotype maps. Bioinformatics. 2005. 21: 263-265.

Bechereni L, Gennari L, Masi L, Mansani R, Massart F, Morelli A, Falchetti A, Gonnelli S, Fiorelli G, Tanini A, Brandi ML. Evidence of a linkage disequilibrium between polymorphisms in the human estrogen alpha gene and their relationship to bone mass variation in postmenopausal Italian women. Hum Mol Genet. 2000. 9: 2043-2050.

Cho YS, Go MJ, Kim YJ, Heo JY, Oh JH, Ban HJ, Yoon D, Lee MH, Kim DJ, Park M, Cha SH, Kim JW, Han BG, Min H, Ahn Y, Park MS, Han HR, Jang HY, Cho EY, Lee JE, Cho NH, Shin C, Park T, Park JW, Lee JK, Cardon L, Clarke G, McCarthy MI, Lee JY, Oh B, Kim HL. A large-scale genome-wide association study of Asian populations uncovers genetic factors influencing eight quantitative traits. Nat Genet. 2009. 41: 527-534.

Cole RE. Improving clinical decisions for women at risk of osteoporosis: dual-femur bone mineral density testing. J Am Osteopath Assoc. 2008. 108: 289-295.

Colin E, Uitterlinden A, Meurs J, Bergink AP, van de Klift M, Fang Y, Arp PP, Hofman A, van Leeuwen JP, Pols HA. Interaction between vitamin D receptor genotype and estrogen receptor alpha genotype influences vertebral fracture risk. J

Clin Endocrinol Metab. 2003. 88: 3777-3784.

Estrada K, Styrkarsdottir U, Evangelou E, Hsu YH, Duncan EL, Ntzani EE, Oei L, Albagha OM, Rivadeneira F. Genome-wide meta-analysis identifies 56 bone mineral density loci and reveals 14 loci associated with risk of fracture. Nat Genet. 2012. 44: 491-501.

Francesco M, Francesca M, Gerolamo B, Salvatore M, Giovanni L, Antonella P, Sara S, Dino M, Laura M, Maria LB. Age-specific effects of estrogen receptor's polymorphisms on the bone traits in healthy fertile women: the BONTURNO study. Reprod Biol Endocrinol. 2009. 7: 1186-1193.

Gennari L, Becherini L, Masi L, Mansani R, Gonnelli S, Cepollaro C, Martini S, Montagnani A, Lentini G, Becorpi AM, Brandi ML. Vitamin D and estrogen receptor allelic variants in Italian postmenopausal women: evidence of multiple gene contribution to bone mineral density. J Clin Endocrinol Metab. 1998. 83: 939-944.

Harsløf T, Husted LB, Carstens M, Stenkjaer L, Langdahl BL. Genotypes and haplotypes of the estrogen receptor genes, but not the retinoblastoma-interacting zinc finger protein 1 gene, are associated with osteoporosis. Calcif Tissue Int. 2010. 87: 25-35.

Hedrick PW. Gametic disequilibrium measures: Proceed with caution. Genetics. 1987. 117: 331-341.

Ioannidis JP, Stavrou I, Trikalinos TA, Zois C, Brandi ML, Gennari L, Albagha O, Ralston SH, Tsatsoulis A. Association of polymorphisms of the estrogen receptor alpha gene with bone mineral density and fracture risk in women: a metaanalysis. J Bone Miner Res. 2002. 17: 2048-2060.

Ioannidis JP, Ralston SH, Bennett ST, Brandi ML, Grinberg D, Karassa FB, Langdahl B, van Meurs JB, Mosekilde L, Scollen S, Albagha OM, Bustamante M, Carey AH, Dunning AM, Enjuanes A, van Leeuwen JP, Mavilia C, Masi L, McGuigan FE, Nogues X, Pols HA, Reid DM, Schuit SC, Sherlock RE, Uitterlinden AG. GENOMOS Study. Differential genetic effects of ESR1 gene polymorphisms on osteoporosis outcomes. JAMA. 2004. 292: 2105-2114.

Kanis JA, Melton LJ, 3rd, Christiansen C, Johnston CC, Khaltaev N. The diagnosis of osteoporosis. J Bone Miner Res. 1994. 9: 1137-1141.

Kanis JA, Delmas P, Burckhardt P, Cooper C, Torgerson D. Guidelines for diagnosis and management of osteoporosis. The European Foundation for Osteoporosis and Bone Disease. Osteoporos Int. 1997. 7: 390-406.

Karasawa T, Takahashi A, Saito R, Sekiya M, Igarashi M, Iwasaki H, Miyahara S, Koyasu S, Nakagawa Y, Ishii K, Matsuzaka T, Kobayashi K, Yahagi N, Takekoshi K, Sone H, Yatoh S, Suzuki H, Yamada N, Shimano H. Sterol regulatory element-binding protein-1 determines plasma remnant lipoproteins and accelerates atherosclerosis in low-density lipoprotein receptor-deficient mice. Arterioscler Thromb Vasc Biol. 2011. 31: 1788-1795.

Kobayashi S, Inoue S, Hosoi T, Ouchi Y, Shiraki M, Orimo H. Association of bone mineral density with polymorphism of the estrogen receptor gene. J Bone Miner Res. 1996. 11: 306 -311.

Mahonen A, Turunen AM, Kroger H, Maempaa PH. Estrogen receptor gene polymorphism is associated with bone mineral density in perimenopausal Finnish women. J Bone Miner Res. 1997. 12: T614.

McCarthy MI, Abecasis GR, Cardon LR, Goldstein DB, Little J, Ioannidis JP, Hirschhorn JN. Genome-wide association studies for complex traits: consensus, uncertainty and challenges. Nat Rev Genet. 2008. 9: 356-369.

Parker MG, Arbuckle N, Dauvois S, Danielian P, White R. Structure and function of the estrogen receptor. Ann NY Acad Sci. 1993. 684: 119-126.

Rabbee N, Speed TP. A genotype calling algorithm for Affymetrix SNP array. Bioinformatics. 2006. 22: 7-12.

Styrkarsdottir U, Halldorsson BV, Gretarsdottir S, Gudbjartsson DF, Walters GB, Ingvarsson T, Jonsdottir T, Saemundsdottir J, Center JR, Nguyen TV, Bagger Y, Gulcher JR, Eisman JA, Christiansen C, Sigurdsson G, Kong A, Thorsteinsdottir U, Stefansson K. Multiple genetic loci for bone mineral density and fractures. N Engl J Med. 2008. 358: 2355-2365.

Styrkarsdottir U, Halldorsson BV, Gretarsdottir S, Gudbjartsson DF, Walters GB, Ingvarsson T, Jonsdottir T, Saemundsdottir J, Snorradorrir S, Center JR, Nguyen TV, Alexandersen P, Gulcher JR, Eisman JA, Christiansen C, Sigurdsson G, Kong A, Thorsteinsdottir U, Stefansson K. New sequence variants associated with bone mineral density. Nat Genet. 2009. 41: 15-17.

van Meurs JB, Schuit SC, Weel AE, van der Klift M, Bergink AP, Arp PP, Colin EM, Fang Y, Hofman A, van Duijn CM, van Leeuwen JP, Pols HA, Uitterlinden AG. Association of 5'-estrogen receptor alpha gene polymorphisms with bone mineral density, vertebral bone area and fracture risk. Hum Mol Genet. 2003. 12: 1745-1754.

Wiktorowicz ME, Goeree R, Papaioannou A, Adachi JD, Papadimitropoulos E. Economic implications of hip fracture:

health service use, institutional care and cost in Canada. Osteoporos Int. 2001. 12: 271-278.

Williams FM, Spector TD. The genetics of osteoporosis. Acta Reumatol Port. 2007. 32: 231-240.

Willing M, Sowers M, Aron D, Clark D, Burns T, Bunten C, Crutchfield M, D'Agostino D, Jannausch M. Bone mineral density and its change in white women: estrogen and vitamin D receptor genotypes and their interaction. J Bone Miner Res. 1998. 13: 695-705.

Yamada Y, Ando F, Niino N, Ohta S, Shimokata H. Association of polymorphisms of the estrogen receptor alpha gene with bone mineral density of the femoral neck in elderly Japanese women. J Mol Med. 2002. 80: 452-460.

Zayzafoon M, Botolin S, McCabe LR. P38 and activating transcription factor-2 involvement in osteoblast osmotic response to elevated extracellular glucose. J Biol Chem. 2002. 277: 37212-37218.

Zhang Y, Long J, Liu P, Liu YJ, Shen H, Zhao LJ, Deng HW. Estrogen receptor alpha and vitamin D receptor polymorphisms and bone mineral density: association study of healthy pre- and postmenopausal Chinese women. Biochem Biophys Res Commun. 2003. 308: 777-783.