CLINICAL STUDY

A gene-to-gene interaction between aromatase and estrogen receptors influences bone mineral density

José A Riancho¹, María T Zarrabeitia², Carmen Valero¹, Carolina Sañudo¹, Verónica Mijares² and Jesús González-Macías¹

¹Department of Internal Medicine and ²Unit of Legal Medicine, Hospital U.M. Valdecilla, University of Cantabria, 39008 Santander, Spain

(Correspondence should be addressed to J A Riancho; Email: rianchoj@unican.es)

Abstract

Objective: The aromatization of androgenic precursors is the main source of estrogens in postmenopausal women. We tested the hypothesis that allelic variants of the genes coding for aromatase and estrogen receptors (ER) could interact to determine the estrogenic signals on the bone tissue and, consequently, bone mineral density (BMD).

Design: Cross-sectional study including 331 postmenopausal women.

Methods: BMD was measured by dual energy x-ray absorptiometry. A CG polymorphism of the aromatase gene as well as three polymorphisms of ER α (a TA repeat in the promoter region, a C T single nucleotide polymorphism (SNP) in intron 1 and an AG SNP in exon 8) and a CA repeat polymorphism of ER β were studied.

Results: Age, body weight and the aromatase genotype were associated with BMD. Allelic variants of ER β and the exon 8 of ER α did not show a significant association with BMD. The polymorphisms located on the promoter and intron 1 of ER α interacted strongly with aromatase. Thus, in women TT homozygous for the ER α gene, there was a marked influence of aromatase genotypes on BMD: spine BMD was 0.724 ± 0.027 g/cm² in women with CC aromatase alleles and 0.926 ± 0.032 g/cm² in those with GG alleles ($P < 0.001$). Hip BMD in women with CC and GG aromatase genotypes was 0.722 ± 0.020 and 0.842 ± 0.026 g/cm² respectively ($P = 0.002$). On the contrary, there were no aromatase-related differences in BMD in women with CT/CC alleles of ER α . Similarly, aromatase-related differences in BMD were found in women with short alleles at the promoter region of ER α , but not in those with long alleles. Both ER α polymorphisms were in strong linkage disequilibrium ($P < 0.001$).

Conclusion: These results suggest that the interaction between polymorphisms of genes involved in estrogen synthesis and estrogen signaling exerts an important influence on BMD in postmenopausal women, thus helping to explain, in part, its heritable component. Nevertheless, further studies are warranted to confirm this gene-to-gene interaction in other populations.

European Journal of Endocrinology 155 53–59

Introduction

Heritable factors are thought to explain up to 50–80% of the variation of bone mineral density (BMD) (1, 2). Estrogens play a critical role in bone homeostasis, as revealed by the progressive decline in BMD experienced by postmenopausal women. Thus, genes involved in estrogen metabolism and activity are strong candidates to explain, at least in part, the genetic influence on BMD. In postmenopausal women, most estrogens are derived from the conversion of androgenic precursors in the peripheral tissues – a reaction catalyzed by aromatase, the product of *CYP19* gene (3, 4). We and other researchers have recently shown that common polymorphisms of the aromatase gene are associated with BMD and estradiol serum levels in postmenopausal women (5–8). On the

other hand, the possible association of polymorphisms of the estrogen receptor (ER) with BMD and fractures has been widely studied, with conflicting results (reviewed in (9)). Two types of ER, named α (or type 1) and β (or type 2), have been described (10, 11). Both are expressed in bone, but α receptors appear to be the most abundant (9). Estrogen signaling in bone cells is a consequence of the binding of estrogenic ligands, either synthesized locally or circulating, to specific receptors. Therefore, we hypothesized that genetic variants of the estrogen-synthesizing enzyme aromatase and ER could interact to determine the estrogen signaling on the bone tissue and, consequently, BMD. To test this hypothesis, we have analyzed some common polymorphisms in the genes coding for aromatase and ER, and studied their relationship with BMD in postmenopausal women.

Materials and methods

Study population

Three-hundred and thirty-one postmenopausal women of Spanish ancestry were studied. They were recruited among volunteers and patients with primary postmenopausal osteoporosis. All women were interviewed by one of the investigators. Those with a present or past history of diseases known to affect skeletal homeostasis, or taking drugs which are able to interfere with bone metabolism (for example, glucocorticoids, antiepileptics, estrogens, or thiazides) were excluded. The study was approved by the Institutional Committee on Ethics in Clinical Research.

Clinical and densitometric measurements

Height was measured using a wall-mounted stadiometer. Weight was measured with an upright balance scale. Both measurements were rounded to the nearest integer. BMD was determined by anterior–posterior dual energy x-ray absorptiometry scans at the lumbar spine (L2–L4) and the total hip region was determined using a Hologic QDR densitometer (Hologic; Waltham, MA, USA). Calcium intake from dairy products was estimated by a food frequency questionnaire (12). Present recreational physical activity was graded semiquantitatively by the number of weekly sessions (at least 45 min long) of recreational weight-bearing activity (13), and the subjects were classified into three groups (I, none; II, 1–3 sessions; III, more than 3 sessions). Alcohol intake was defined as a daily intake of more than 10 g. Subjects were regarded as smokers if they had a present or past history of smoking at least five cigarettes/day for a minimum of 5 years.

Genetic analysis

DNA was obtained from peripheral blood using a commercial kit, according to manufacturer's instructions (Qiagen, Hilden, Germany). Tandem repeat polymorphisms were studied by PCR with fluorochrome-labeled primers and subsequent analysis of fragment size by capillary electrophoresis (ABI310, Applied Biosystems, Foster City, CA, USA) (14). Genotyping of single nucleotide polymorphisms was performed by a procedure based on the exonuclease activity of Taq DNA-polymerase, using allele-specific Taqman probes labeled with VIC and FAM. Primers and probes were designed by the manufacturer with Primer Express software (Applied Biosystems; sequence details available upon request). Amplification reactions were performed in a 5 µl final volume in optical 96-well plates, following manufacturer's instructions with some modifications (annealing time was increased to 30 s and the number of PCR cycles to 48). After amplification in an ABI9700 thermal cycler (Applied Biosystems), the

fluorescence was read in an ABI7000 sequence detector (Applied Biosystems). About 5% of the results were ambiguous and samples had to be re-typed. Random samples were analyzed twice to check for consistency of results, which was perfect in 99.5% of the samples. In some cases, single nucleotide polymorphisms (SNP) were also analyzed by electrophoresis in agarose gels after digestion with the restriction enzyme *PvuII* (see below). The following polymorphisms were studied:

- CYP19.** This gene codes for aromatase, a critical enzyme in the peripheral synthesis of estrogens by catalyzing the aromatization of C19 androgens to C18 estrogens. A CG polymorphism situated in the 5'UTR was analyzed as previously reported. We have previously shown that this SNP is associated to BMD in postmenopausal women (5,8).
- ESR1 (ER α).** Three loci were studied: A TA repeat polymorphism in the promoter region (15); a CT SNP situated in intron 1, frequently determined by other investigators with the endonuclease *PvuII* (16–18), and an AG SNP in exon 8 (19). Identical results were obtained in random samples that had the allelic variants of intron 1 analyzed with Taqman probes and with *PvuII*.
- ESR2 (ER β).** A CA repeat polymorphism located in intron 6, about 3600 bp from the termination of exon 6, was studied by using previously reported primers (20).

Statistical analyses

SNP-related genotypes were coded as 1 (homozygous), 2 (heterozygous) or 3 (homozygous). The allelic variants of microsatellites were grouped into short (S) and long (L) alleles, according to the median number of repeats (ER α , 18; ER β , 22). Hardy–Weinberg equilibrium was tested with HWSIM software (<http://krunch.med.yale.edu/hwsim/hwsim.doc>). The association of genotypes with BMD was tested by analysis of covariance. All analyses were two-tailed and *P* values less than 0.05 were regarded as statistically significant. The SPSS software was used (SPSS Inc., Chicago, IL, USA).

Table 1 Characteristics of women.

	Mean (s.d.)	Range
Age, yr	70 (7)	45–86
Height, cm	154 (6)	138–172
Weight, kg	65 (10)	40–93
Body mass index, kg/m ²	27.6 (4.0)	18.3–41.9
Age at menarche, yr	14 (2)	9–19
Age at menopause, yr	49 (5)	35–59
Years since the menopause	22 (8)	1–49
Calcium intake, mg/day	682 (372)	0–1980

Table 2 Genotype frequencies of single nucleotide polymorphisms.

Locus	Genotypes	Frequency
Aromatase	CC	0.36
	CG	0.47
	GG	0.17
ER α -exon 8	AA	0.05
	AG	0.26
	GG	0.69
ER α -intron 1	TT	0.28
	CT	0.47
	CC	0.25

Results

The characteristics of the study population are shown in Table 1. Only 5% of women ingested more than 10 g of alcohol per day; 3% were current smokers and 5% were past smokers.

Genotype frequencies of SNPs are shown in Table 2. There was no evidence for departure from Hardy-Weinberg equilibrium. The allelic frequency distribution of microsatellites is shown in Fig. 1. As found in many studies, age and body-weight were significantly related to BMD (not shown). In age- and weight-adjusted models, aromatase was the only gene showing genotype-related differences in BMD (Table 3), thus confirming the results of a previous report in a smaller group of women (8). Therefore, we next studied possible gene-gene interactions by analyzing the association of the various genotypes with BMD in covariance models including age, body-weight and the aromatase genotype. That analysis revealed an interaction between the allelic variants of the aromatase and ER α genes. At the lumbar spine, there was a significant interaction between the aromatase genotype and ER variants at the promoter region and intron 1. At the hip, the interaction between aromatase and ER α genes was statistically significant when the promoter variants of ER α were considered, and marginally significant with intron 1 variants (Table 4). As it is shown in Fig. 2, there was a marked influence of aromatase genotypes on BMD in women being TT homozygous for the ER α

gene. The adjusted spine BMD was 0.724 ± 0.027 g/cm² in women with CC aromatase alleles and 0.926 ± 0.032 g/cm² in those with GG alleles ($P < 0.001$ after Bonferroni's adjustment). The difference between those extreme genotypes was equivalent to 1.3 s.d. of the subgroup BMD. The hip adjusted BMD was also associated to aromatase genotype. In women with CC and GG aromatase genotypes, hip BMD was 0.722 ± 0.020 and 0.842 ± 0.026 g/cm² respectively ($P = 0.002$ after Bonferroni's adjustment), equivalent to 0.9 s.d. On the contrary, aromatase genotype was not associated with BMD in heterozygous women with CT alleles (spine, $P = 0.9$; hip, $P = 0.2$) or in CC homozygous women (spine, $P = 0.9$; hip, $P = 0.9$) (Fig. 2). Similarly, aromatase-related differences in BMD were found in women with two short alleles at the promoter region of ER α (SS genotype), but not in those with long alleles (SL or LL genotypes) (Fig. 3). Both ER α polymorphisms were in linkage disequilibrium ($P < 0.001$): women with long TA tracts at the promoter tended to have C alleles at intron 1, whereas short TA tracts were associated with T alleles. Women with different ER α and aromatase genotypes did not show significant differences in body-weight, height, age at menopause, age at menarche, years since the menopause, calcium intake, tobacco or alcohol consumption or physical activity (not shown).

Discussion

In this study, we have not been able to show a significant relationship between common polymorphisms in the genes coding for ER β or exon 8 of the ER α and BMD in postmenopausal women. Some investigators have reported an association of the CA polymorphism in ER β and BMD in postmenopausal women (20, 21) and in a mixed group of pre- and postmenopausal women from the Framingham cohort (22). However, other investigators did not find ER β -related differences in BMD in postmenopausal women (23). We and other researchers have shown that ER β influences BMD in young subjects and in premenopausal women (14, 24,

Figure 1 Allelic frequency distribution of microsatellites of estrogen receptor α (ER α) and estrogen receptor β (ER β).

Table 3 Relationship between genotypes and BMD (*P* values in age- and weight-adjusted models).

Locus	Spine BMD	Hip BMD
Aromatase	0.04	0.03
Estrogen receptor α		
Promoter	0.16	0.97
Intron 1	0.53	0.35
Exon 8	0.99	0.99
Estrogen receptor β	0.57	0.40

Table 4 Gene-to-gene interactions on bone mineral density. *P* values for the interactions between aromatase genotypes and other genes in age- and weight-adjusted models.

Locus	Spine BMD	Hip BMD
Estrogen receptor α		
Promoter	0.040	0.017
Intron 1	0.004	0.046
Exon 8	0.26	0.74
Estrogen receptor β	0.48	0.60

25). Thus, ER β polymorphisms appear to influence the peak bone mass more consistently than the bone loss, taking place after the menopause. The AG polymorphism in ER α has been rarely studied. Ongphiphadhanakul *et al.* found a reduced BMD in postmenopausal women from Thailand bearing the A allele (19). However, we found no evidence of such an

association in the present study. The AG SNP is a synonymous one which does not alter the amino acid sequence. However, it is in linkage disequilibrium with other polymorphisms of possible functional significance at the 3'-UTR (26). As the degree of linkage disequilibrium is rather variable not only through the different genome regions, but also among different populations (27, 28), the different genetic backgrounds may explain the discrepancy between our results and those previously reported in the Thai population. The results of the present study confirm with those obtained with a smaller group of women, showing an association between the SNP at the 5' region of the aromatase gene and BMD. Although the mechanism of action has not been elucidated, allelic variants seem to influence aromatase activity, as they are associated with some differences in circulating estrogen levels (8). As the SNP is located at the UTR of the gene, such a difference must be related to differences in gene transcription or mRNA stability rather than in the protein enzymatic activity. Since the seminal paper by Kobayashi *et al.* (18), there have been many studies about the relationship between the allelic variants of the intron 1 of ER α and BMD. The results have been conflicting. Nevertheless, a recent meta-analysis suggests that the locus variation is indeed associated with fracture risk (29). Studies about the association between the TA polymorphism at the promoter region of ER α and BMD have also shown conflicting data (see ref (9) for review). In the present

Figure 2 Interaction between the aromatase gene and the polymorphism of intron 1 of the ER α on bone mineral density (BMD). Top: In women with TT alleles at intron 1 of ER α , the aromatase genotypes were associated with BMD differences both at the lumbar spine (left) and the hip (right). Bottom: However, in women with CT/CC alleles of ER α , the aromatase genotypes were not associated with BMD. *P*-values correspond to the *F*-test of the overall comparison of aromatase genotypes in age- and weight-adjusted models.

Figure 3 Interaction between the aromatase gene and the polymorphism of the promoter region of the ER on BMD. Top: In women with two short alleles (SL) of ER, the aromatase genotypes were associated with BMD differences at the lumbar spine (left) and the hip (right). Bottom: However, in women bearing long alleles (SL/LL) of ER, there were no aromatase-related differences in BMD. *P*-values correspond to the *F*-test of the overall comparison of aromatase genotypes in age- and weight-adjusted models.

study, we did not find a statistically significant association between the allelic variants of the proximal regions of ER (promoter and intron 1) and BMD. However, our results show that those variants exert a marked influence on the association between the aromatase genotype and BMD. Indeed, the effect of aromatase gene was marked in women with ER α homozygous for T alleles, but not in those bearing C alleles. We wonder whether such interaction may help to explain the conflicting results of previous studies. As the aromatization of androgenic precursors is the main source of estrogens in postmenopausal women, these results could be explained by variations in the response to estrogenic ligands in women with different ER α genotypes. This would seem plausible from a pathophysiological point of view, but direct proof is still lacking. We are not aware of other studies about this kind of interaction, that is, between genes influencing endogenous estrogen availability and response. Nevertheless, the hypothesis is consistent with a number of investigations showing that the ER α genotype influences the response to exogenous estrogenic compounds. It has been reported that the TA microsatellite, and several SNPs in intron 1 and other regions of the ER, modulate the skeletal response to hormone replacement therapy both in Asian and European women (30–33). The AG SNP in intron 1 has also been reported to modulate the BMD response to raloxifene in women on chronic

hemodialysis (34). As a matter of fact, the influence of the ER genotype on the body response to endogenous and exogenous estrogens appears to be widespread, involving tissues other than the skeleton (35–38). The molecular mechanisms responsible for the functional consequences of these noncoding polymorphisms of the ER α have not been elucidated. It has been suggested that the SNP in intron 1 may modulate gene transcription. The C allele, but not the T allele contains a functional myb binding site and transfection experiments with luciferase reporters suggested an influence on transcription. In comparison with cells transfected with constructs bearing the T allele, those incorporating constructs with the C allele showed a four-fold higher transcription rate (35). Those results would suggest that cells with T alleles express less ER, which could render them more sensitive to a reduced supply of estrogens. Although merely speculative, this mechanism would be consistent with our findings showing that different genotypes of the estrogen-synthesizing aromatase are associated with BMD in women with T alleles, but not in those with C alleles. Since the SNP at intron 1 and the TA polymorphism at the 5' region are in strong linkage disequilibrium, it is hard to know which of them has actually driven the influence on estrogen response. Nevertheless, we found that the differences in BMD related to the aromatase genotype were larger in women selected on the basis of the intron 1 SNP than

in those selected on the basis of the TA polymorphism (0.202 vs 0.149 g/cm²), pointing towards the allelic variants of intron 1 as the stronger candidates to be responsible for the interaction between the aromatase and ER α genes.

In conclusion, our data suggest that genes coding for estrogen synthesis and activity (i.e. aromatase and ER) interact with each other to influence BMD in postmenopausal women. This gene-to-gene interaction is a likely contributor to explain, in part, the genetic influence on bone mass. Nevertheless, further studies in other populations are warranted to confirm the general relevance of this phenomenon. In a more general sense, these results support the concept that residual estrogen bioactivity is an important modulator of bone homeostasis after the menopause.

Acknowledgement

This work was supported by grants FIS 02/0063 and 04/0028.

References

- 1 Peacock M, Turner CH, Econs MJ & Foroud T. Genetics of osteoporosis. *Endocrine Reviews* 2002 **23** 303–326.
- 2 Ralston SH. Genetic control of susceptibility to osteoporosis. *Journal of Clinical Endocrinology and Metabolism* 2002 **87** 2460–2466.
- 3 Simpson ER & Davis SR. Aromatase and the regulation of estrogen biosynthesis—some new perspectives. *Endocrinology* 2001 **142** 4589–4594.
- 4 Kamat A, Hinshelwood MM, Murry BA & Mendelson CR. Mechanisms in tissue-specific regulation of estrogen biosynthesis in humans. *Trends in Endocrinology and Metabolism* 2002 **13** 122–128.
- 5 Riancho JA, Zarrabeitia MT, Valero C, Sañudo C, Hernandez JL, Amado JA, Zarrabeitia A & Gonzalez-Macias J. Aromatase gene and osteoporosis: relationship of ten polymorphic loci with bone mineral density. *Bone* 2005 **36** 917–925.
- 6 Somner J, McLellan S, Cheung J, Mak YT, Frost ML, Knapp KM, Wierzbicki M, Fogelman I, Ralston SH & Hampson GN. Polymorphisms in the P450 c17(17-hydroxylase/17,20-lyase) and P450 c19 (aromatase) genes: association with serum sex steroid concentrations and bone mineral density in postmenopausal women. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 344–351.
- 7 Masi L, Becherini L, Gennari L, Amedei A, Colli E, Falchetti A, Farci M, Silvestri S, Gonnelli S & Brandi ML. Polymorphism of the aromatase gene in postmenopausal Italian women: distribution and correlation with bone mass and fracture risk. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 2263–2269.
- 8 Zarrabeitia MT, Hernandez JL, Valero C, Zarrabeitia AL, Garcia-Unzueta MT, Amado JA, Gonzalez-Macias J & Riancho JA. A common polymorphism in the 5'-untranslated region of the aromatase gene influences bone mass and fracture risk. *European Journal of Endocrinology* 2004 **150** 699–704.
- 9 Gennari L, Merlotti D, De Paola V, Calabro A, Becherini L, Martini G & Nuti R. Estrogen receptor gene polymorphisms and the genetics of osteoporosis: a HuGE review. *American Journal of Epidemiology* 2005 **161** 307–320.
- 10 Herynk MH & Fuqua SAW. Estrogen receptor mutations in human disease. *Endocrine Reviews* 2004 **25** 869–898.
- 11 Koehler KF, Helguero LA, Haldosén LA, Warner M & Gustafsson JA. Reflections on the discovery and significance of estrogen receptor β . *Endocrine Reviews* 2005 **26** 465–478.
- 12 Docio S, Riancho JA, Perez A, Olmos JM, Amado JA & Gonzalez-Macias J. Seasonal deficiency of vitamin D in children: a potential target for osteoporosis-preventing strategies? *Journal of Bone and Mineral Research* 1998 **13** 544–548.
- 13 Katzman DK, Bachrach LK, Carter DR & Marcus R. Clinical and anthropometric correlates of bone mineral acquisition in healthy adolescent girls. *Journal of Clinical Endocrinology and Metabolism* 1991 **73** 1332–1339.
- 14 Valero C, Zarrabeitia MT, Hernandez JL, Zarrabeitia MT, González-Macias J & Riancho JA. Bone mass in young adults: relationship with gender, weight, and genetic factors. *Journal of Internal Medicine* 2005 **258** 554–562.
- 15 Sano M, Inoue S, Hosoi T, Ouchi Y, Emi M, Shiraki M & Orimo H. Association of estrogen receptor dinucleotide repeat polymorphism with osteoporosis. *Biochemical and Biophysical Research Communications* 1995 **217** 378–383.
- 16 Schuit SC, Van Meurs JBJ, Bergink AP, Van der Klift M, Fang Y, Leusink G, Hofman A, van Leeuwen JPTM, Uitterlinden AG & Pols HAP. Height in pre- and postmenopausal women is influenced by estrogen receptor alpha gene polymorphisms. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 303–309.
- 17 Albagha OME, McGuigan FEA, Reid DM & Ralston SH. Estrogen receptor alpha gene polymorphisms and bone mineral density: haplotype analysis in women from the United Kingdom. *Journal of Bone and Mineral Research* 2001 **16** 128–134.
- 18 Kobayashi S, Inoue S, Hosoi T, Ouchi Y, Shiraki M & Orimo H. Association of bone mineral density with polymorphism of the estrogen receptor gene. *Journal of Bone and Mineral Research* 1996 **11** 306–311.
- 19 Ongphiphadhanakul B, Chanprasertyothin S, Payattikul P, Saetung S, Piaseu N, Chailurkit L & Rajatanavin R. Association of a G2014A transition in exon 8 of the estrogen receptor-alpha gene with postmenopausal osteoporosis. *Osteoporosis International* 2001 **12** 1015–1019.
- 20 Ogawa S, Hosoi T, Shiraki M, Orimo H, Emi M, Muramatsu M, Ouchi Y & Inoue S. Association of estrogen receptor β gene polymorphism with bone mineral density. *Biochemical and Biophysical Research Communications* 2000 **269** 537–541.
- 21 Scariano JK, Simplicio SG, Montoya GD, Garry PJ & Baumgartner RN. Estrogen receptor β dinucleotide (CA) repeat polymorphism is significantly associated with bone mineral density in postmenopausal women. *Calcified Tissue International* 2004 **74** 501–508.
- 22 Shearman AM, Karasik D, Gruenthal KM, Demissie S, Cupples LA, Housman DE & Kiel DP. Estrogen receptor β polymorphisms are associated with bone mass in women and men: The Framingham study. *Journal of Bone and Mineral Research* 2004 **19** 773–781.
- 23 Efsthadiadou Z, Koukoulis G, Stakias N, Challa A, Zintzaras E & Tsatsoulis A. Correlation of estrogen receptor beta gene polymorphisms with spinal bone mineral density in peri- and postmenopausal Greek women. *Maturitas* 2006 **53** 380–385.
- 24 Ichikawa S, Koller DL, Peacock M, Johnson ML, Lai D, Hui SL, Johnston CC, Foroud TM & Econs MJ. Polymorphisms in the estrogen receptor beta (ESR2) gene are associated with bone mineral density in Caucasian men and women. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 5921–5927.
- 25 Lau HHL, Ho AYY, Luk KDK & Kung AWC. Estrogen receptor β gene polymorphisms are associated with higher bone mineral density in premenopausal, but not postmenopausal southern Chinese women. *Bone* 2002 **31** 276–281.
- 26 Ongphiphadhanakul B, Chanprasertyothin S, Saetung S & Rajatanavin R. A specific haplotype in the 3'-end of estrogen-receptor alpha gene is associated with low bone mineral density in premenopausal women and increased risk of postmenopausal osteoporosis. *Osteoporosis International* 2005 **16** 1233–1238.
- 27 Ingles SA, Haile RW, Henderson BE, Kolonel LN, Nakaichi G, Shi CY, Yu MC, Ross RK & Coetzee GA. Strength of linkage

- disequilibrium between two vitamin D receptor markers in five ethnic groups: implications for association studies. *Cancer Epidemiology, Biomarkers & Prevention* 1997 **6** 93–98.
- 28 Long JR, Zhao LJ, Liu PY, Lu Y, Dvornyk V, Shen H, Liu YJ, Zhang YY, Xiong DH, Xiao P & Deng HW. Patterns of linkage disequilibrium and haplotype distribution in disease candidate genes. *BMC Genetics* 2004 **5** 11.
 - 29 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 genetics effects of ESR1 gene polymorphisms on osteoporosis outcomes. *Journal of American Medical Association* 2004 **292** 2105–2114.
 - 30 Yim CH, Choi JT, Choi HA, Kang YS, Moon IG, Yoon HK, Han IK, Kang DH & Han KO. Association of estrogen receptor alpha gene microsatellite polymorphism with annual changes in bone mineral density in Korean women with hormone replacement therapy. *Journal of Bone and Mineral Metabolism* 2005 **23** 395–400.
 - 31 Ongphiphadhanakul B, Chanprasertyothin S, Payattikul P, Saetung S, Piaseu N, Chailurkit L, Chansirikarn S, Puavilai G & Rajatanavin R. Association of a T262C transition in exon 1 of estrogen-receptor-alpha gene with skeletal responsiveness to estrogen in post-menopausal women. *Journal of Endocrinological Investigation* 2001 **24** 749–755.
 - 32 Salmén T, Heikkinen AM, Mahonen A, Kroger H, Komulainen M, Saarikoski S, Honkanen R & Maenpää PH. The protective effect of hormone-replacement therapy on fracture risk is modulated by estrogen receptor alpha genotype in early postmenopausal women. *Journal of Bone and Mineral Research* 2000 **15** 2479–2486.
 - 33 Yahata T, Quan J, Tamura N, Nagata H, Kurabayashi T & Tanaka K. Association between single nucleotide polymorphisms of estrogen receptor alpha gene and efficacy of HRT on bone mineral density in post-menopausal Japanese women. *Human Reproduction* 2005 **20** 1860–1866.
 - 34 Heilberg IP, Hernandez E, Alonzo E, Valera R, Ferreira LG, Gomes SA, Bellorin-Font E & Weisinger JR. Estrogen receptor (ER) gene polymorphism may predict the bone mineral density response to raloxifene in postmenopausal women on chronic hemodialysis. *Renal Failure* 2005 **27** 155–161.
 - 35 Herrington DM, Howard TD, Brosnihan KB, McDonnell DP, Li X, Hawkins GA, Reboussin DM, Xu J, Zheng SL, Meyers DA & Bleecker ER. Common estrogen receptor polymorphism augments effects of hormone replacement therapy on E-selectin but not C-reactive protein. *Circulation* 2002 **105** 1879–1882.
 - 36 Herrington DM, Howard TD, Hawkins GA, Reboussin DM, Xu J, Zheng SL, Brosnihan KB, Meyers DA & Bleecker ER. Estrogen-receptor polymorphisms and effects of estrogen replacement on high-density lipoprotein cholesterol in women with coronary disease. *New England Journal of Medicine* 2002 **346** 967–974.
 - 37 Onland-Moret NC, van Gils CH, Roest M, Grobbee DE & Peeters PH. The estrogen receptor alpha gene and breast cancer risk (The Netherlands). *Cancer Causes Control* 2005 **16** 1195–1202.
 - 38 Fox CS, Yang Q, Cupples LA, Guo CY, Atwood LD, Murabito JM, Levy D, Mendelsohn ME, Housman DE & Shearman AM. Sex-specific association between estrogen receptor-alpha gene variation and measures of adiposity: the Framingham Heart Study. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 6257–6262.

Received 10 February 2006

Accepted 28 April 2006