Journal of Steroid Biochemistry & Molecular Biology 127 (2011) 413–417

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

The ESR2 *Alu*I gene polymorphism is associated with bone mineral density in postmenopausal women

Monica Currò[a,1], Herbert Marini[a,1], Angela Alibrandi[e], Nadia Ferlazzo[a], Salvatore Condello[a], Francesca Polito[a], Elena B. Adamo[a], Marco Atteritano[c], Rosario D'Anna[d], Domenica Altavilla[b], Alessandra Bitto[b], Francesco Squadrito[b], Riccardo Ientile[a], Daniela Caccamo[a,*]

[a] Department of Biochemical, Physiological and Nutritional Sciences, University of Messina, Via C. Valeria, Policlinico "G. Martino", 98124 Messina, Italy
[b] Department of Clinical and Experimental Medicine and Pharmacology, University of Messina, Via C. Valeria, Policlinico "G. Martino", Messina, Italy
[c] Department of Internal Medicine, University of Messina, Via C. Valeria, Policlinico "G. Martino", Messina, Italy
[d] Department of Obstetrical and Gynecological Sciences, University of Messina, Via C. Valeria, Policlinico "G. Martino", Messina, Italy
[e] Department of Economical, Financial, Social, Environmental, Statistical and Territorial Sciences (S.E.FI.S.A.S.T.), University of Messina, Messina, Italy

ARTICLE INFO

Article history:
Received 3 April 2011
Received in revised form 13 May 2011
Accepted 31 May 2011

Keywords:
Osteoporosis
Postmenopausal women
BMD
Bone turnover markers
Estrogen receptor beta
ESR2 gene variants

ABSTRACT

Multiple factors may contribute to the pathogenesis of postmenopausal osteoporosis including environmental, life-style and genetic factors. Common variants in ESR2 gene encoding for ER-beta, highly expressed in bone tissue, have recently been proposed as candidates for affecting bone phenotype at the population level, particularly in postmenopausal women.

In this study, we examined the genetic background at ESR2 *Alu*I (rs4986938, 1730G>A) locus in 89 osteopenic, postmenopausal women (age range 49–56 years) together with BMD at lumbar spine and femoral neck sites as well as variations in plasma levels of bone metabolism and turnover markers.

Genotyping for ESR2 G1730A polymorphism showed that the frequency of A mutated allele accounted for 0.4 in our cohort of postmenopausal women; moreover, the GA1730 heterozygous individuals were the most represented (50.6%) compared with GG (37.8%) and AA homozygous ones (14.6%). A regression analysis showed that lumbar spine BMD values were significantly associated with both ESR2 AA1730 genotype ($p = 0.044$) and time since the onset of menopause ($p = 0.031$), while no significant association was detected between biochemical markers and genetic background. Interestingly, 85% of patients with AA1730 genotype presented the smallest lumbar spine BMD values.

These findings first indicate a worsening effect of ESR2 *Alu*I polymorphism on lumbar spine BMD reduction in postmenopause, suggesting that the detection of this ESR2 variant should be recommended in postmenopausal women, particularly in populations with a high prevalence of ESR2 AA1730 homozygous genotype.

1. Introduction

Low levels of estrogen are common among menopausal women and further raise the risk of decrease in bone mineral density (BMD), osteoporosis, bone fractures and subsequent complications. Postmenopausal osteoporosis which is considered a public health issue threatening a large part of the population above 50 years of age, generally occurs asymptomatically and, consequently, the afflicted individuals will only be diagnosed after the occurrence of fractures [1]. Indeed, during menopausal bone loss, bone resorption activity mediated by osteoclasts outweighs bone regeneration by osteoblasts, resulting in an overall weakening of the bone, particularly the trabecular one, in typical sites as lumbar spine or femoral neck. Multiple factors may contribute to the pathogenesis of postmenopausal osteoporosis including environmental, lifestyle and genetic factors [1,2]. Twin and family studies showed that genetic components can influence the variance in peak bone mass up to 60–80%, and that an important role is also played by the interaction between genes and environmental or lifestyle factors [3,4]. Several candidate genes have been proposed as risk markers of postmenopausal osteoporosis, such as vitamin D receptor, collagen type I A1, and interleukin-6, that are involved in the regulation of BMD [5]. However, to date, our ability to evaluate the risk for BMD reduction and/or postmenopausal osteoporotic fractures based on genetic screening is still far to be complete.

Since estrogens have important effects on bone mass and bone remodeling, estrogen receptor genes (ESRs) have been tested for

* Corresponding author. Tel.: +39 0902213386; fax: +39 0902213382.
E-mail address: dcaccamo@unime.it (D. Caccamo).
[1] These authors contributed equally to this article.

0960-0760/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jsbmb.2011.05.008

association with osteoporosis and fracture risk. In the last decade, most independent research groups investigating the relationships between BMD and ESRs in population-based studies have been focused on polymorphisms in the estrogen receptor α (ESR1) gene that is robustly expressed in reproductive tissues. The results obtained even if compelling for ESR1 involvement in osteoporosis or fracture, were not conclusive, likely because of confounding factors, i.e. the ethnic- and gender-distribution of ESR1 polymorphisms [6–8].

More recently, after the identification of the estrogen receptor β gene (ESR2) and its gene product ER-β, which is more abundant than the ER-α one in trabecular bone, several studies have also evaluated polymorphisms of ESR2 as risk markers for osteoporosis [9–13]. However, a literature review suggests that the clinical predictability of ESR2 polymorphisms is largely dependent on the analysis of homogenous population, since, similarly to association studies on ESR1 polymorphisms, ESR2 polymorphism distribution has been shown to be highly variable according to race/ethnicity and gender. Moreover, the effects of ESR2 genetic variants on BMD have been mostly investigated in cross-sectional studies, examining different age ranges with dissimilar estrogen status.

In light of this background, to improve the understanding of estrogenic signaling in postmenopausal women, we investigated the influence of ESR2 *Alu*I gene polymorphism (*rs4986938, G/A1730*) on changes in BMD as well as bone turnover markers in a cohort of postmenopausal women.

2. Materials and methods

2.1. Patients

The patients presented in this study were part of a cohort of Sicilian postmenopausal women who were referred for clinical evaluation the Center for Osteoporosis in the Department of Internal Medicine and the Center for Menopause in the Department of Obstetrical and Gynecological Sciences, University of Messina (Messina, Italy), as previously described [14]. The study design is consistent with the principles of the Declaration of Helsinki.

Briefly, participants were 89 women (aged 49–56 years) who had been postmenopausal for at least 12 months, were in good general health, not had a menstrual period in the preceding year, had not undergone surgically induced menopause, and had a follicle-stimulating hormone level greater than 50 IU/L and a serum 17β-estradiol level of 100 pmol/L or less (≤27 pg/mL). A complete family history was obtained for all subjects, that underwent physical examination, laboratory analysis of biochemical and hematological parameters, and BMD measurement at the lumbar spine (LS) and femoral neck (FN). Exclusion criteria were clinical or laboratory evidence of confounding systemic diseases, such as cardiovascular, hepatic, or renal disorders; coagulopathy; use of oral or transdermal estrogen, progestin, androgens, selective estrogen receptor modulators, or other steroids; use of biphosphonates, cholesterol-lowering therapy, or cardiovascular medications (including anti-hypertensive drugs) in the preceding 6 months; smoking habit of more than 2 cigarettes daily; treatment in the preceding year with any drug that could have affected the skeleton; family history of estrogen-dependent cancer; BMD at the femoral neck greater than 0.795 g/cm^2 (which corresponds to a *T*-score of −1.0 SD).

2.2. BMD measurement

BMD at the lumbar spine (LS BMD; g/cm^2) and at the femoral neck (FN BMD; g/cm^2) was determined by dual-energy X-ray absorptiometry (DEXA) (Hologic QDR 4500W, Technologic, Turin, Italy). The instrument was calibrated daily according to the manufacturer's instructions. Reproducibility was calculated as a coefficient of variation obtained by weekly measurements of a standard phantom on the instrument and by repeated measurements obtained in 3 patients of different ages. The coefficient of variation of our instrument is 0.5% with the standard phantom; in vivo, we calculated a coefficient of variation of 1.1% for the lumbar spine and 1.5% for the femoral neck.

2.3. Biochemical assays

Bone metabolism markers were analyzed as previously described [14]. Briefly, serum calcium and phosphorus, and urinary creatinine were measured by using automated routine procedures. Urinary excretion of pyridinium crosslinks (pyridinoline, PYR, and deoxypyridinoline, D-PYR) as well as 25-hydroxyvitamin D3 (VITD3) was measured by using high-performance liquid chromatography (Bio-Rad Laboratories, Milano, Italy). Serum levels of parathyroid hormone (PTH), bone-specific alkaline phosphatase (bALP) as well soluble receptor activated nuclear factor-kappa B ligand (sRANKL) and osteoprotegerin (OPG) were determined by immunometric assays following manufacturer's instructions (Pantec, Turin, Italy).

2.4. ESR2 genotyping

Genomic DNA was extracted from white cells of freshly drawn peripheral blood samples using Gentra Puregene Blood DNA Extraction kit (Qiagen, Milan, Italy) according to manufacturer's instructions.

The ESR2 G1730A polymorphism (rs4986938) was analyzed using a Pre-Designed TaqMan® SNP Genotyping Assays (assay ID: C_11462726_10) on a 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA). The reactions were set up in a 96-well plate and were carried out in a final volume of 10 μL containing 1× TaqMan® Genotyping Master Mix, 1× TaqMan-specific assay, and 10 ng genomic DNA.

To exclude selection bias in our postmenopausal population, ESR2 genotyping was carried out also in a population of 90 volunteer Sicilian blood donors who attended the local Blood Bank & Transfusion Centre.

2.5. Statistical analysis

The numerical data are expressed as mean ± standard deviations (S.D.) and the categorical variables as number and percentage.

Deviation from Hardy–Weinberg equilibrium for ESR2 genotypes was calculated by the chi-square test. A non-parametric analysis was carried out as examined variables did not present normal distribution according to Kolmogorov–Smirnov test. Spearman correlation test was applied to assess the existence of any significant interdependence between numerical parameters. The between-groups variability was analyzed by both Kruskall–Wallis and Mann–Whitney tests.

Univariate logistic regression models were estimated to first verify the possible dependence of BMD and biochemical parameters on either genetic background or menopausal months. In a second time, a multiple logistic regression analysis was performed to assess the dependence of BMD and biochemical parameters on both genotype and menopausal months.

Finally, in order to further assess the influence of genotype on BMD values in our postmenopausal women, we estimated the odds ratio, the chi square test and the relative significance.

A p-value ≤ 0.05 was considered statistically significant for all the analyses. Statistical analyses were performed using SPSS 11.0 for Window package.

Table 1
General anthropometric and biochemical features of the selected postmenopausal population ($n = 89$ subjects).

General features	Mean ± SD	Reference range
Age	54.4 ± 3.26	–
Menopausal age	49.2 ± 2.9	–
Menopausal months	60 ± 39.5	–
LS BMD (g/cm^2)	0.854 ± 0.108	–
FN BMD (g/cm^2)	0.661 ± 0.054	–
Calcium (mg/dL)	9.60 ± 0.44	9–11
Phosphorus (mg/dL)	3.65 ± 0.53	3.5–4.5
Vit D (ng/mL)	30.12 ± 11.61	20–120
PTH (pg/dL)	50.10 ± 18.79	12–100
bALP (μg/L)	10.25 ± 2.15	8.5–17.9
Pyr/creatinine (pmol/μmol creatinine)	93.63 ± 33.92	26–91
D-Pyr (pmol/μmol creatinine)	22.25 ± 6.65	3–21
OPG (pmol/L)	4.79 ± 0.6	2.5–4.5
sRANKL (pmol/L)	0.339 ± 0.085	0.35–0.39
sRANKL/OPG	0.072 ± 0.021	–

A power analysis was used to guide in the choice of sample size; it allowed us to calculate the minimum sample size required to accept the outcome of a statistical test, with the confidence level $\alpha = 0.050$. So, we established that the power level we wished to achieve was 0.80 and consequently we estimated the sample size (89 patients) needed to ensure this specific power level.

3. Results

3.1. General features and genetic background of postmenopausal women

The anthropometric features and biochemical parameters of the recruited postmenopausal women are summarized in Table 1. Plasma mean levels of biochemical markers for bone metabolism, such as calcium, phosphorus, PTH and 25-OH-vitamin D3, were within the normal range (Table 1). However, DEXA analysis revealed a mild osteopenia (*T*-score between −1 and −1.5 SD) in all patients examined according to a reference value for FN BMD lower than 0.795 g/cm^2 (−1.0 SD). Moreover, evaluation of LS BMD values also showed a mild osteopenia according to LS BMD reference value of 0.925 g/cm^2 (−1.0 SD).

The assessment of bone turnover classical markers, including bALP (bone formation) and Pyr/DPyr (bone resorption), showed that all values were in the normal range (Table 1).

To deeply investigate the role of local factors conditioning bone remodeling rate, i.e. the balance between osteoblast and osteoclast activation, the concentrations of OPG and RANKL were also evaluated.

Results from this analysis, based on sRANKL/OPG ratio assessment, revealed OPG exceeding sRANKL (Table 1) that indicates an imbalance toward inhibition of osteoclastogenesis.

Genotyping for ESR2 G1730A polymorphism in postmenopausal, mildly osteopenic women showed that the genotype distributions (Table 2) were found to be in Hardy–Weinberg equilibrium ($\chi^2 = 0.263$; $p = 0.6$), suggesting that the enrolled subjects represented a homogeneous genetic background.

The evaluation of allele frequency showed that the A mutated allele accounted for 0.4 in the selected population, with a higher prevalence in heterozygous state (GA1730 genotype) than homozygous (AA1730 genotype) (50.56% vs 14.6%). Moreover, the analysis of genotype distribution showed that GA1730 heterozygous individuals were the most represented among our postmenopausal women compared with GG1730 homozygous ones (50.56% vs 34.8%) (Table 2).

ESR2 AluI genotype frequencies of postmenopausal women were found not significantly different ($p > 0.05$) from those of Sicilian general population. Indeed, the A mutated allele frequency among the 90 blood donors included for comparison was 0.393, with the GA1730 heterozygous genotype accounting for 48.9% and the AA1730 homozygous one for 14.9%. The GG1730 homozygotes were 36.2%.

3.2. Analysis of ESR2 genetic background effects in postmenopausal women

Statistical analysis did not show any significant interdependence between the examined variables in our postmenopausal women except between LS BMD and FN BMD ($p < 0.001$).

A comparison of between-groups variability in BMD and bone turnover markers in the recruited subjects, grouped according to their ESR2 G1730A genotype, showed that carriers of AA1730 mutated genotype exhibited the lowest mean values of LS BMD as well as serum concentrations of calcium, phosphorus, 25-OH-vitamin D3, PTH, bALP and sRANKL/OPG. Conversely, the same subjects showed the highest concentrations of PYR/D-PYR (Table 3). However, these differences between the groups were not statistically significant according to either Kruskal–Wallis test or Mann–Whitney test ($p > 0.05$).

An univariate regression analysis showed that, among the anthropometric and biochemical parameters examined in our postmenopausal women, LS BMD was the only variable which was significantly associated with either ESR2 *Alu*I variant or menopausal months. In particular, LS BMD values were negatively associated with either AA1730 genotype ($p = 0.044$) compared with subjects having GA or GG genotypes, or a longer time since the onset of menopause ($p = 0.031$). BMD values adjusted for menopausal months were, therefore, used in all further analyses. Interestingly, 85% of patients with homozygous genotype AA1730 presented the smallest lumbar spine BMD values.

A multiple regression analysis using ESR2 genotype and menopausal months as covariates confirmed the strong association of LS BMD with ESR2 AA1730 genotype ($p = 0.013$) and menopausal months ($p = 0.004$).

Finally, the genotype relative risk for a statistically significant decrease in LS BMD values was evaluated using as threshold reference the mean value of 0.854 g/cm^2 calculated in our postmenopausal population. The relative risk in the AA group vs GG group was 4.53 (95% CI: 0.857–23.928; chi-square = 2.341; $p = 0.05$). Although the confidence interval was not small, there was a borderline significant difference between wild-type and carrier groups for ESR2 G1730A polymorphism.

4. Discussion and conclusions

In the present study, we investigated whether the *Alu*I variant (rs4986938, G/A1730) of the ESR2 gene, coding for ER-β and located

Table 2
Genetic background of the selected postmenopausal population at ESR2 *Alu*I gene locus.

SNP	dbSNP	Location within gene	Allele frequency	Genotype frequency (%)	Subjects ($n = 89$)
ESR2 *Alu*I 1730 G>A	rs 4986938	38 bp 3′ of STP A>G (3′UTR)	G allele 0.610 A allele 0.399	GG 34.8 GA 50.6 AA 14.6	GG $n = 31$ GA $n = 45$ AA $n = 13$

Table 3
Comparison of general features in the selected postmenopausal population divided in subgroups according to different ESR2 AluI (G1730A) genotypes.

General features	ESR2 *Alu*I Genotype		
	GG1730	GA1730	AA1730
Menopausal months	56.5 ± 38.8	57.8 ± 38.9	78.46 ± 43.14
LS BMD (g/cm^2)	0.859 ± 0.093	0.866 ± 0.12	0.798 ± 0.079
FN BMD (g/cm^2)	0.659 ± 0.052	0.66 ± 0.051	0.666 ± 0.069
Calcium (mg/dL)	9.61 ± 0.44	9.62 ± 0.47	9.51 ± 0.31
Phosphorus (mg/dL)	3.72 ± 0.57	3.63 ± 0.45	3.6 ± 0.67
Vit D (UI/L)	28.6 ± 9.46	32.44 ± 13.28	25.69 ± 8.32
PTH (mg/dL)	49.2 ± 16.4	51.16 ± 21.27	48.56 ± 15.66
bALP (μg/L)	10.36 ± 1.91	10.24 ± 2.39	10.02 ± 1.85
Pyr/creatinine (pmol/μmol creatinine)	97.25 ± 38.16	89.38 ± 24.08	99.66 ± 50.27
D-Pyr (pmol/μmol creatinine)	22.78 ± 6.29	21.34 ± 6.38	24.11 ± 8.25
OPG (pmol/L)	4.72 ± 0.48	4.8 ± 0.69	4.94 ± 0.54
sRANKL (pmol/L)	0.352 ± 0.076	0.334 ± 0.093	0.325 ± 0.080
sRANKL/OPG	0.076 ± 0.019	0.071 ± 0.023	0.065 ± 0.011

in the chromosome 14q region that has been linked to hip BMD [15], is associated with variation in bone mass in postmenopausal Caucasian women living in Sicily (Italy).

Recent experimental studies utilizing ESR2 knockout mice remarked the important involvement of ER-β in bone formation [16]. Indeed, ER-β is robustly expressed by developing human bone, especially the cancellous bone compartment that is the most subjected to loss following gonadal hormone deprivation [17]. Accordingly, there is a greater than 9-fold increase in ER-β expression in cultured human osteoblasts during bone mineralization, whereas ER-α levels remain unchanged during this process [18].

The human ER-β synthesis shows variability in a gender, age and cell-type dependent manner. Specifically, it has been suggested that the worsening effects of ESR2 polymorphisms on female bone mass become more evident at lower estrogen concentrations, as occurring in postmenopausal women, than at higher estrogen concentrations, as seen in premenopausal women.

To date, several ESR2 polymorphisms have been extensively tested for their association with BMD and changes in bone turnover markers. However, only few studies reported a positive association between ER-β gene variants and BMD or fracture risk [9,10,13,19–21], while other results were inconclusive [22,23] or showed a dissimilar effect at different ESR2 loci [24]. These discrepancies were probably due to different sample size, cross-sectional study approaches as well as ethnic difference in populations examined, and other variables affecting phenotype. For example, in the SWAN study enrolling 1,301 premenopausal and early perimenopausal women living in US (295 Afro-American, 693 Caucasian, 151 Chinese and 162 Japanese), specific associations of BMD with ESR2 genotypes, at different loci than the one (rs4986938) investigated in this study, varied according to race/ethnicity [7].

The present results show that ESR2 *Alu*I polymorphism (rs4986938, G/A1730) was associated with BMD in a cohort of Sicilian, osteopenic and postmenopausal women. In particular, a significant, negative correlation was observed between the mutated homozygous genotype AA1730 and lumbar spine BMD values in comparison to subject with GA or GG genotypes. Interestingly, 85% of patients with homozygous genotype AA1730 presented the smallest lumbar spine BMD values, thus suggesting that this genetic variant may likely contribute for bone loss worsening in postmenopausal women.

Moreover, our data first demonstrated that the negative effect of AA1730 genotype is reinforced by longer times since the onset of menopausal status, accordingly with a progressive reduction in estrogen synthesis. No association was observed between this genotype and biochemical markers of human bone homeostasis, including calcium, phosphorus, 25-OH-vitamin D3, and bALP in agreement with previous reports [13,23]. Notably, it has been shown that the ESR2 *Alu*I polymorphism is not associated with changes in plasma levels of specific markers of bone remodeling, i.e. osteocalcin, hydroxyproline, C-telopeptide of type I collagen and N-terminal propeptide of type I procollagen [13,23]. We here examined the relation of this polymorphism with different markers, such as RANKL, osteoprotegerin, as well as pyridinium cross-links (pyridinoline, deoxypyridinoline). However, we did not find any significant association between these variables.

To date, very few population-based studies have examined the correlation of the ESR2 *Alu*I polymorphism with bone traits. Although the sample size in the present study is relatively small compared with previous investigations [13,20,22–24], our analysis had, at least in our opinion, several strengths.

Indeed, the recruited participants were a homogenous population with regard to age, gender, race, ethnic origin, and menopausal status. Moreover, the ESR2 A1730 allele frequency (0.40) here observed agrees with that reported elsewhere for Italian female population [13] as well as Sicilian general population (this study), and Caucasian women in population-based studies similar to the present one, and several others investigating on different pathological conditions [24,25]. On the other side, the only two investigations reporting a lower A allele frequency and a significantly lower AA1730 genotype distribution were carried out using RFLP with endonuclease *Alu*I as genotyping technique [22,23].

Notably, we report here, for the first time, the association of ESR2 *Alu*I polymorphism with lumbar spine BMD in postmenopausal women. As a matter of fact, previous investigations did not find any significant association of this ESR2 gene variant with BMD [22–24]; alternatively, a significant association was shown with BMD at different sites, such as femoral neck [13]. These discrepancies may be primarily ascribed to the heterogeneity of selected populations encompassing healthy individuals with significantly different ages and hormonal milieu, ranging from fertile to pre- or peri-menopausal status [13,23,24]. In this regard, supporting observations come from the cross-sectional study of Massart et al. [13] reinforcing the hypothesis that ESR2 variants affect BMD in distinct age-sequential windows. The crucial role of hormonal status is also supported by findings on the lack of association of ESR2 *Alu*I polymorphism with BMD in a population of Caucasian men, underlining the gender-related different effects of estrogens [22]. Overall, a summary of published association studies of BMD and ESR2 variant loci [24] shows a more frequent association of the examined polymorphisms with BMD at femoral neck than BMD at lumbar spine in premenopausal women, in agreement with results of BONTURNO study [13], while the same ESR2 variants are positively associated with lumbar spine BMD in both postmenopausal women and men. However, one important feature to consider is that confounding factors may also be represented by different environmental and life-style factors, i.e. nutritional habi-

tus, as suggested by observations on Caucasian women living in US [24].

Although the exact mechanism behind the observed associations with BMD is still not fully understood, it has been hypothesized that the ESR2 *Alu*I polymorphism, consisting in a G/A1730 transition (rs4986938) in the 3′-untraslated region (3′-UTR), could alter mRNA stability and protein levels [25], leading, in turn, to a reduced synthesis of ER-β. This would indicate that this *Alu*I ESR2 gene variant is likely implicated in the distribution and density of ER-β in woman body organs, primarily affecting the interaction estrogen-estrogen receptor on trabecular bone. In this context, the use of natural molecules, i.e. soy isoflavone derivatives, mainly targeting ER-β receptor has already been demonstrated successful in improving both BMD and biochemical bone markers in osteopenic, postmenopausal women [14,26,27].

Available data, providing new insights on molecular mechanisms underlying ER-β activity in human trabecular bone, strongly indicate that, beyond alterations of biochemical markers of bone metabolism, the screening of genetic variants of ESR2 needs to be specifically addressed when programming a multifactorial intervention for osteoporosis prevention in postmenopausal women. In this context, results from the Rotterdam study, evaluating the interaction of ESR2 variants with ESR1 and IGF-1 ones in relation to BMD and risk of osteoporotic fractures in around 4,000 postmenopausal women, remarked the importance of ESR2 polymorphisms alone in determining fracture risk [20].

Given the high socio-economical impact of postmenopausal osteoporotic vertebral fractures [28], the evaluation of ESR2 genetic background at *Alu*I locus (rs4986938) should be included, in our opinion, in a screening panel for osteoporosis risk to allow early intervention and treatment against future bone loss. Moreover, given that the negative effects of ESR2 genetic background are amplified by the progression of menopause, it should be recommended to carry out patient genotyping at perimenopause or early menopause.

In light of our data in order to optimize the patient's response to therapy with synthetic or natural selective agonists/modulators of ER-β receptor, it appears of great interest the detection of ESR2 *Alu*I polymorphism in all postmenopausal women, particularly in Caucasian populations with a high prevalence of ESR2 AA1730 homozygous genotype.

References

[1] Management of osteoporosis in postmenopausal women: 2010 position statement of The North American Menopause Society, Menopause 17 (2010) 25–54.
[2] C. Winsloe, S. Earl, E.M. Dennison, C. Cooper, N.C. Harvey, Early life factors in the pathogenesis of osteoporosis, Curr. Osteoporos. Rep. 7 (2009) 140–144.
[3] F.M. Williams, T.D. Spector, Recent advances in the genetics of osteoporosis, J. Musculoskelet. Neuronal Interact. 6 (2006) 27–35.
[4] S.H. Ralston, Genetics of osteoporosis, Ann. N.Y. Acad. Sci. 1192 (2010) 181–189.
[5] W.F. Li, S.X. Hou, B. Yu, M.M. Li, C. Férec, J.M. Chen, Genetics of osteoporosis: accelerating pace in gene identification and validation, Hum. Genet. 127 (2010) 249–285.
[6] J.P. Ioannidis, S.H. Ralston, S.T. Bennett, M.L. Brandi, D. Grinberg, F.B. Karassa, B. Langdahl, J.B. van Meurs, L. Mosekilde, S. Scollen, O.M. Albagha, M. Bustamante, A.H. Carey, A.M. Dunning, A. Enjuanes, J.P. van Leeuwen, C. Mavilia, L. Masi, F.E. McGuigan, X. Nogues, H.A. Pols, D.M. Reid, S.C. Schuit, R.E. Sherlock, A.G. Uitterlinden, GENOMOS Study, Differential genetic effects of ESR1 gene polymorphisms on osteoporosis outcomes, JAMA 292 (2004) 2105–2114.
[7] G.A. Greendale, J. Chu, R. Ferrell, J.F. Randolph Jr., J.M. Johnston, M.R. Sowers, The association of bone mineral density with estrogen receptor gene polymorphisms, Am. J. Med. 119 (2006) S79–S86.
[8] M. Durusu Tanriover, G. Bora Tatar, T.D. Uluturk, D. Dayangac Erden, A. Tanriover, A. Kilicarslan, S.G. Oz, H. Erdem Yurter, T. Sozen, G. Sain Guvenm, Evaluation of the effects of vitamin D receptor and estrogen receptor 1 gene polymorphisms on bone mineral density in postmenopausal women, Clin. Rheumatol. 29 (2010) 1285–1293.
[9] S. Ogawa, T. Hosoi, M. Shiraki, H. Orimo, M. Emi, M. Muramatsu, Y. Ouchi, S. Inoue, Association of estrogen receptor β gene polymorphism with bone mineral density, Biochem. Biophys. Res. Commun. 269 (2000) 537–541.
[10] H.H. Lau, A.Y. Ho, K.D. Luk, A.W. Kung, Estrogen receptor β gene polymorphisms are associated with higher bone mineral density in premenopausal, but not postmenopausal southern Chinese women, Bone 31 (2002) 276–281.
[11] A.M. Shearman, D. Karasik, K.M. Gruenthal, S. Demissie, L.A. Cupples, D.E. Housman, D.P. Kiel, Estrogen receptor beta polymorphisms are associated with bone mass in women and men: the Framingham Study, J. Bone Miner. Res. 19 (2004) 773–781.
[12] D.H. Xiong, H. Shen, L.J. Zhao, P. Xiao, T.L. Yang, Y. Guo, W. Wang, Y.F. Guo, Y.J. Liu, R.R. Recker, H.W. Deng, Robust and comprehensive analysis of 20 osteoporosis candidate genes by very high-density single-nucleotide polymorphism screen among 405 white nuclear families identified significant association and gene-gene interaction, J. Bone Miner. Res. 21 (2006) 1678–1695.
[13] F. Massart, F. Marini, G. Bianchi, S. Minisola, G. Luisetto, A. Pirazzoli, S. Salvi, D. Micheli, L. Masi, M.L. Brandi, Age-specific effects of estrogen receptors' polymorphisms on the bone traits in healthy fertile women: the BONTURNO study, Reprod. Biol. Endocrinol. 7 (2009) 32.
[14] H. Marini, L. Minutoli, F. Polito, A. Bitto, D. Altavilla, M. Atteritano, A. Gaudio, S. Mazzaferro, A. Frisina, N. Frisina, C. Lubrano, M. Bonaiuto, R. D'Anna, M.L. Cannata, F. Corrado, E.B. Adamo, S. Wilson, F. Squadrito, Effects of the phytoestrogen genistein on bone metabolism in osteopenic postmenopausal women: a randomized trial, Ann. Intern. Med. 146 (2007) 839–847.
[15] M. Peacock, D.L. Koller, S. Hui, C.C. Johnston, T. Foroud, M.J. Econs, Peak bone mineral density at the hip is linked to chromosomes 14q and 15q, Osteoporos. Int. 15 (2004) 489–496.
[16] L. Vico, J.M. Vanacker, Sex hormones and their receptors in bone homeostasis: insights from genetically modified mouse models, Osteoporos. Int. 21 (2010) 365–372.
[17] S. Bord, A. Horner, S. Beavan, J. Compston, Estrogen receptors alpha and beta are differentially expressed in developing human bone, J. Clin. Endocrinol. Metab. 86 (2001) 2309–2314.
[18] J. Arts, G.G. Kuiper, J.M. Janssen, J.A. Gustafsson, C.W. Lowik, H.A. Pols, J.P. van Leeuwen, Differential expression of estrogen receptors alpha and beta mRNA during differentiation of human osteoblast SVHF cells, Endocrinology 138 (1997) 5067–5070.
[19] J.K. Scariano, S.G. Simplicio, G.D. Montoya, P.J. Garry, R.N. Baumgartner, Estrogen receptor β dinucleotide (CA) repeat polymorphism is significantly associated with bone mineral density in postmenopausal women, Calcif. Tissue Int. 74 (2004) 501–508.
[20] F. Rivadeneira, J.B. van Meurs, J. Kant, M.C. Zillikens, L. Stolk, T.J. Beck, P. Arp, S.C. Schuit, A. Hofman, J.J. Houwing-Duistermaat, C.M. van Duijn, J.P. van Leeuwen, H.A. Pols, A.G. Uitterlinden, Estrogen receptor beta (ESR2) polymorphisms in interaction with estrogen receptor alpha (ESR1) and insulin-like growth factor I (IGF1) variants influence the risk of fracture in postmenopausal women, J. Bone Miner. Res. 21 (2006) 1443–1456.
[21] J.T. Wang, Y. Guo, T.L. Yang, X.H. Xu, S.S. Dong, M. Li, T.Q. Li, Y. Chen, H.W. Deng, Polymorphisms in the estrogen receptor genes are associated with hip fractures in Chinese, Bone 43 (2008) 910–914.
[22] S. Khosla, B.L. Riggs, E.J. Atkinson, A.L. Oberg, C. Mavilia, F. Del Monte, L.J. Melton III, M.L. Brandi, Relationship of estrogen receptor genotypes to bone mineral density and to rates of bone loss in men, J. Clin. Endocrinol. Metab. 89 (2004) 1808–1816.
[23] Z. Efstathiadou, G. Koukoulis, N. Stakias, A. Challa, E. Zintzaras, A. Tsatsoulis, Correlation of estrogen receptor beta gene polymorphisms with spinal bone mineral density in peri- and post-menopausal Greek women, Maturitas 53 (2006) 380–385.
[24] S. Ichikawa, D.L. Koller, M. Peacock, M.L. Johnson, D. Lai, S.L. Hui, C.C. Johnston, T.M. Foroud, M.J. Econs, Polymorphisms in the estrogen receptor beta (ESR2) gene are associated with bone mineral density in Caucasian men and women, J. Clin. Endocrinol. Metab. 90 (2005) 5921–5927.
[25] M. Putnik, C. Zhao, J.A. Gustafsson, K. Dahlman-Wright, Effects of two common polymorphisms in the 3′ untranslated regions of estrogen receptor β on mRNA stability and translatability, BMC Genetics 10 (2009) 55.
[26] H. Marini, L. Minutoli, F. Polito, A. Bitto, D. Altavilla, M. Atteritano, A. Gaudio, S. Mazzaferro, A. Frisina, N. Frisina, C. Lubrano, M. Bonaiuto, R. D'Anna, M.L. Cannata, F. Corrado, F. Cancellieri, M. Faraci, R. Marini, E.B. Adamo, S. Wilson, F. Squadrito, OPG and sRANKL serum concentrations in osteopenic, postmenopausal women after 2-year genistein administration, J. Bone Miner. Res. 23 (2008) 715–720.
[27] A. Bitto, F. Polito, F. Squadrito, H. Marini, R. D'Anna, N. Irrera, L. Minutoli, R. Granese, D. Altavilla, Genistein aglycone: a dual mode of action anti-osteoporotic soy isoflavone rebalancing bone turnover towards bone formation, Curr. Med. Chem. 17 (2010) 3007–3018.
[28] N. Harvey, E. Dennison, C. Cooper, Osteoporosis: impact on health and economics, Nat. Rev. Rheumatol. 6 (2010) 99–105.