CLIMACTERIC 2012;15:587–593

Estrogen-related genes and postmenopausal osteoporosis risk

N. Mendoza, F. Quereda, J. Presa†, A. Salamanca, R. Sánchez-Borrego‡, F. Vázquez** and T. Martínez Astorquiza††*

Department of Obstetrics and Gynecology, University of Granada; *Hospital Universitario San Juan, Alicante; †Hospital Princesa, Jaén; ‡Clínica Diatros, Barcelona; **Clínica GEOGA, Lugo; ††Hospital Cruces, Bilbao, Spain

Key words: OSTEOPOROSIS, POLYMORPHISM, ESTROGEN-RELATED GENES

ABSTRACT

Background To date, more than 150 candidate genes related to osteoporosis have been described, but osteoporosis has increasingly been considered a polygenic disease modulated by environmental factors. It is thought that osteoporosis predisposition, pathology, and treatment response depend on the interaction between different genes or between genes and environmental factors.

Objective The aim of this study was to evaluate the relationship between the presence of single nucleotide polymorphisms (SNPs) in the estrogen metabolic pathway and the development of osteoporosis and to determine whether this relationship is monogenic or whether interactions between genes exist.

Materials and methods A multicentric study with 1980 postmenopausal Spanish women in five Spanish communities was conducted. The women completed a specific questionnaire that inquired about risk factors for osteoporosis. Data on participants' bone mineral density were obtained with dual-energy X-ray densitometers, and genetic data were obtained from frozen peripheral blood.

Results The digenic protection combinations indicated involvement of the wild-type genotype (WT) of the 3′UTR marker for the *CYP19A1* gene, the IVS4 marker of the same gene, and the *BMP15* and *FSHR* genes. Among patients who carried two or more of the genotypes considered 'risky', the triple combination among markers of the *ESR2* and *NRIP1* genes with any of the two mutations of the analyzed markers of the *BMP15* gene gave a mean *T*-score value of -2.32 ± 0.91 ($p = 0.02$).

Conclusion Variants of the new candidate genes (*NRIP* and *BMP15*) can predispose patients to osteoporosis.

INTRODUCTION

Knowledge of risk factors associated with the development of osteoporosis is vital in the prevention strategy of this disease and its physical and economic consequences. However, diagnostic modalities and factors identified in the epidemiology of osteoporosis have not succeeded in identifying all patients who are in danger of presenting with a fracture due to fragility.

The correct and early identification of women at risk of suffering an osteoporotic fracture would be a decisive advance in minimizing the physical and economic consequences of this infirmity. Genetic studies have become a focus for achieving this goal. The heritability of this condition with respect to both disease development and of the degree of bone mass lost is greater than 75%; thus, investigations in the field of genomics that attempt to find new markers of osteoporosis and fracture risk are warranted. The basic underlying idea in all genetic studies thus far is that normal phenotypic characteristics, clinical features, illnesses, and responses to treatment are due to an interaction between or a combination of environmental factors that act on a particular individual's genetic background. In fact, two human genomes are different in an average of one in every 1000 nucleotide positions, thus permitting the identification of approximately 10 million markers or single nucleotide polymorphisms (SNPs) throughout our genome.

The identification of the gene or genes implicated in the development of osteoporosis is difficult due to the multifactorial character of the illness as well as the heterogeneity of different populations. To date, more than 150 candidate genes related to osteoporosis have been described, with the most studied being the vitamin D receptor, estrogen receptors,

Correspondence: Professor N. Mendoza, Maestro Montero 21, 18004 Granada, Spain

ORIGINAL ARTICLE

DOI: 10.3109/13697137.2012.656160

Received 30-06-2011
Revised 15-11-2011
Accepted 04-01-2012

RANKL or LRP5. An important part of these genes have been identified by Genome-wide association studies, as much related with bone mineral density (BMD) as with ultrasound properties of bone, skeletal geometry and bone turnover[1–6]. Moreover, previous publications have not demonstrated the consistency expected and refute the hypothesis that a single genetic marker can serve to detect at-risk patients.

However, as implicated genes continue to be discovered, we begin to consider osteoporosis as a polygenic disease modulated by environmental factors, such that predisposition, pathogenesis and response to treatment all depend on the interaction between different genes or between genes and environmental factors.

The principal objective of this study was to evaluate the relationship between the presence of genetic SNPs in the estrogen metabolic pathway and the development of osteoporosis in our population and to thereby determine whether this relationship is monogenic or whether interactions exist between genes. The secondary objectives were to analyze the existence of other hygienic-dietary risk factors or medical-social characteristics related to postmenopausal osteoporosis and to distinguish such factors from those noted in other epidemiological studies.

MATERIALS AND METHODS

The study population consisted of 1980 postmenopausal Spanish women (post-hysterectomy or after 1 year without menstruation) who consulted a gynecologist for advice about bone health. All participants received written information about the objective of the study and gave written consent to participate.

The clinical data were obtained using a structured questionnaire that was developed in consensus by the clinical investigators involved in the study. This questionnaire collects specific information about known risk factors for osteoporosis (Table 1) as well as other factors considered in our study. This project was approved by the ethics committees of each hospital involved in the study.

Patients who were premenopausal, who did not complete the questionnaire completely, who were receiving any type of treatment for osteoporosis at the time of the study, or for whom bone absorptiometry or genetic information was not available were excluded.

BMD data were obtained from participants with dual-energy X-ray densitometers using a Hologic QDR 1000/W instrument in 72.6% of cases and a Norland densitometer in the remainder of cases. To solve the problem of obtaining data through two different measurement systems, the value of the *T*-score was used for statistical calculations, with *T*-scores greater than −1.0 standard deviation (SD) considered normal, values between −1.0 SD and −2.5 SD considered indicative of osteopenia, and values less than −2.5 SD considered indicative of osteoporosis.

BMD data (g/cm^2) were also obtained using the total value of the lumbar spine (L2–L4) as a reference for the measurement of spongy BMD and using the value of the femoral neck as a reference for the cortical BMD of the femur.

Genetic analysis

The extraction of genomic DNA from frozen peripheral blood was performed in a MagNa Pure LC system (Roche Diagnostics) using the extraction MagNa Pure LC DNA Isolation Kit (Roche Diagnostics) according to the manufacturer's instructions. For the polymerase chain reactions (PCRs), aliquots of DNA were prepared at a concentration of 10 ng/µl, and the remaining stock was stored at −20°C.

Amplification of the DNA chain was performed by PCR using specific primers designed with the Oligo and GeneFisher programs (available at http://www.hgmp.mrc.ac.uk).

Genotyping was conducted using the LightCycler™ (Roche Diagnostics) and Pyrosequencing™ techniques for six SNPs in five genes from the estrogen pathway (*FSHR*, *ESR1*, *ESR2*, *NRIP1* and *CYP19A1*) and for four other SNPs in the *BMP15* gene.

Statistical analysis

A descriptive study and a bivariate analysis of the data were performed using the statistical program SPSS 15.0.

For dichotomous variables, the χ^2 test was corrected through the Yates test when the percentage of cells with an expected frequency less than 5 was 20% or less. If these conditions were not met, then the correction was made using the Fisher test. Qualitative variables with more than two categories were compared with the χ^2 test, and correction by grouping categories was attempted if more than 20% of cells had an expected frequency less than five.

When the independent variables were numerical, their normality was first tested with the Kolmogorov–Smirnov contrast; then, the Student's *t*-test or the Mann–Whitney *U*-test was subsequently applied, depending on whether the variable was distributed normally or not, respectively.

In the interaction study, the results are shown for equality of means testing in all groups using the ANOVA test and Kruskal–Wallis *post-hoc* test to study the equality of the distributions for the distinct genotype pairs. The identification of groups of pairs of genotypes in conflict was performed using a Bonferroni analysis. Values of $p < 0.05$ were considered statistically significant.

We conducted a unilocus study developed through a Kruskal–Wallis test of the equality of distributions in which the null hypothesis tested was that the behavior of the groups was identical.

Two different approaches were used to analyze the possible influences of gene interactions on bone mass. For the identification of gene–gene interactions in the lumbar spine study, we employed different modules of the SPSS program to perform a χ^2 analysis with one degree of freedom (gl) in each of the possible combinations for each stratum and later discarded

the interactions that were not statistically significant (according to the Breslow–Day test or test of homogeneity of the odds ratio), as these would indicate that the interaction was not due to the effect of only one stratum, which is described as the 'drag phenomenon'.

RESULTS

In 1695 women, genetic data and data on the BMD of the lumbar spine were obtained; in 913 of the women, the BMD of the hip was also measured. The results indicate that BMD is decreased in both the lumbar spine and the hip of patients with lower weights and heights ($p < 0.05$) (Table 1). In terms of age at menarche and age at menopause, differences were observed between the three groups in that menarche was delayed and menopause was earlier among osteoporotic women compared to osteopenic and normal women ($p < 0.05$). This result is consistent with the fact that, in the group of patients with osteoporosis, there were significantly more cases of early menopause (less than 40 years old). For the findings in the hip, no parameter reached statistical significance due to the limited sample size.

In terms of modifiable factors (hygienic-dietary) related to development of osteoporosis in the women in our study, the BMD of the lumbar spine showed a direct relationship with the consumption of tobacco ($p < 0.05$) and an inverse relationship with moderate consumption of alcohol ($p < 0.05$). In our study, neither physical exercise habits nor fat distribution corresponded with a clear BMD profile.

With respect to the unilocus study, no significant differences were observed between genotypes and groups according to BMD level in the lumbar spine or the femur. Thus, there is not sufficient empirical evidence to say that the BMD level depends on the unilocus analysis of any of the studied genotypes.

The digenic interactions are summarized in Table 2 (significant values are in bold type). Among the different digenic combinations, we observed that the protective combinations appear to be the wild-type genotype of the 3′UTR marker of the *CYP19A1* gene, the wild-type genotype (II) of the IVS4 marker of the same gene, and the wild-type genotypes of the *BMP15* and *FSHR* genes. Conversely, it seems that risky combinations are the mutated genotypes (CC) of the 3′UTR marker of the *CYP19A1* gene, the mutated genotype (DD) of the IVS4 marker of the same gene, and the mutated genotypes of *BMP15* and *FSHR* genes.

Given the clinical importance of the femur, analysis was conducted on genetic interactions relevant to this region. First, BMD values were quantified from the *T*-score of each genotype to isolate the most extreme variants. This process allowed us to compare the patients who carried two or more genotypes considered 'risky' (because they give a low *T*-score) to a control group of women who did not carry those variants. In the group with 'risky' genotypes, 6.4% of patients had an absorptiometric diagnosis of osteoporosis in the entire femur, with a mean *T*-score of -0.769 ± 1.1 and a mean BMD of 0.824 ± 0.3 g/cm^2. The most interesting combinations involved the mutated markers of the *ESR2*, *NRIP1*, *CYP19A1* (marker IVS4) and *BMP15* (markers 905A > G and −9C > G) genes. Specifically, the triple combination among these markers of the *ESR2* and *NRIP1* genes with any of the two mutations of the analyzed markers of the *BMP15* gene gave a mean *T*-score value of -2.32 ± 0.91 ($p = 0.02$). Two-thirds of the carriers had an absorptiometric diagnosis of osteoporosis of the hip ($p = 0.03$) (see Table 3).

In addition to these analyses of risk markers, we also compared the results in women with a 'protective' genotype

Table 1 Epidemiological characteristics of normal, osteopenic and osteoporotic women. Data are given as mean ± standard deviation or *n* (%)

	Normal	*Osteopenia*	*Osteoporosis*	*p Value*
BMD of lumbar spine	(*n* = 479)	(*n* = 766)	(*n* = 450)	
Age (years)	62 ± 8.14	62 ± 8.12	62 ± 8.10	NS
Weight (kg)	69.68 ± 11.44	65.89 ± 10.41	63.40 ± 9.87	< 0.05
Height (cm)	158.56 ± 6.37	157.18 ± 6.08	155.74 ± 5.87	< 0.05
Age at menarche (years)	12.61 ± 1.50	12.74 ± 1.56	12.87 ± 1.7	< 0.05
Age at natural menopause (years)	48.05 ± 5.26	47.40 ± 5.59	46.84 ± 5.7	< 0.05
Early menopause	23 (6.7%)	40 (7.3%)	36 (11.59%)	< 0.05
Parity	2.84 ± 1.89	2.65 ± 1.74	2.77 ± 1.83	NS
BMD of femoral neck	(*n* = 456)	(*n* = 398)	(*n* = 59)	
Age (years)	61 ± 6.7	65 ± 8.5	66 ± 9.4	< 0.05
Weight (kg)	70.08 ± 11.04	64.57 ± 9.29	58.16 ± 7.83	< 0.05
Height (cm)	157.43 ± 6.1	156.5 ± 6.09	154.7 ± 6.9	< 0.05
Age at menarche (years)	12.71 ± 1.54	12.81 ± 1.55	13.12 ± 1.93	NS
Age at natural menopause (years)	47.64 ± 5.29	47.33 ± 5.68	45.33 ± 5.80	< 0.05
Early menopause	56 (12.58%)	54 (13.74%)	13 (22.41%)	NS
Parity	2.27 ± 1.37	2.40 ± 1.55	2.05 ± 1.50	NS

BMD, bone mineral density; NS, not significant

Table 2 Study of digenic interactions in the lumbar spine. Significant values are in bold type

Locus/ genotype	*ESR1*			*ESR2*			*CYP19A1 (3′UTR marker)*			*CYP19A1 (IVS4 marker)*			*NRIP1*			*BMP15 (-9C>G)*			*BMP15 (905A>G)*		
	CC	*CT*	*TT*	*AA*	*AG*	*GG*	*TT*	*TC*	*CC*	*II*	*ID*	*DD*	*AA*	*AG*	*GG*	*CC*	*CG*	*GG*	*AA*	*AG*	*GG*
FSHR																					
AA	0.29	0.91	0.34	0.80	0.35	0.85	0.32	0.11	**0.01**	0.12	0.22	0.14	0.11	0.69	0.63	0.52	0.89	0.28	0.06	0.70	0.56
AS	0.29	0.91	0.34	0.80	0.35	0.85	0.24	0.19	**0.01**	0.08	0.34	0.10	0.08	0.52	0.80	0.37	0.73	0.25	0.04	**0.04**	0.41
SS	0.70	0.98	0.87	0.65	0.96	0.53	0.52	0.97	0.16	0.24	0.045	0.07	0.89	0.30	0.61	0.64	0.07	0.25	**0.03**	0.08	0.58
ESR1																					
CC	....	....	....	0.67	0.73	0.70	0.45	0.10	0.52	0.41	0.46	0.41	0.22	0.44	0.13	0.90	0.31	0.44	0.76	0.21	0.83
TC	....	....	....	0.71	0.88	0.27	0.32	0.29	0.62	0.37	0.10	0.80	0.61	0.28	0.91	0.68	0.60	0.51	0.74	0.47	0.86
TT	....	....	....	0.64	0.29	0.38	0.14	0.27	0.13	0.30	0.092	0.31	0.33	0.53	0.64	0.70	0.19	0.70	0.20	0.34	0.84
ESR2																					
AA	....	....	....	....	....	....	0.10	0.65	0.31	0.38	0.74	0.49	0.28	0.93	0.32	0.86	0.77	0.44	0.44	0.54	0.42
GA	....	....	....	....	....	....	0.16	0.52	0.15	0.48	0.55	0.27	0.42	0.52	0.73	0.79	0.46	0.78	0.25	0.29	0.52
GG	....	....	....	....	....	....	0.24	0.74	0.29	0.37	0.96	0.21	0.39	0.46	0.39	0.85	0.40	0.50	0.36	0.77	0.39
CYP19A1 (3′UTR marker)																					
TT	....	....	....	....	....	....	....	....	....	....	....	....	0.88	**0.04**	**0.04**	0.43	0.83	0.48	0.29	0.74	0.30
TC	....	....	....	....	....	....	....	....	....	....	....	....	0.85	0.57	0.62	**0.04**	0.11	0.88	0.69	0.18	0.19
CC	....	....	....	....	....	....	....	....	....	....	....	....	0.22	0.39	0.19	0.09	0.16	0.61	0.29	0.20	0.68
CYP19A1 (IVS4 marker)																					
II	....	....	....	....	....	....	....	....	....	....	....	....	0.46	0.44	0.32	**0.02**	0.25	0.80	0.53	0.49	0.25
ID	....	....	....	....	....	....	....	....	....	....	....	....	0.89	0.06	0.16	0.06	0.45	0.47	0.54	0.22	0.38
DD	....	....	....	....	....	....	....	....	....	....	....	....	0.42	0.59	0.26	0.72	0.39	**0.03**	0.36	0.52	0.79
NRIP1																					
AA	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	0.22	0.17	0.36	0.60	0.93	0.81
GA	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	0.54	0.85	0.96	0.54	0.33	0.52
GG	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	0.34	0.28	0.59	0.51	0.64	0.38

combination (those with high *T*-scores); however, none of these women showed better bone density values than a control group of women who did not carry any of those genotypes.

DISCUSSION

The present study aimed to deepen our knowledge of the genetic variants related to bone metabolism to try to add them to the list of risk factors associated with osteoporosis. The detailed description of the group of women who participated in the study allows us to identify the distribution of known osteoporosis risk factors (weight, age at menopause, concomitant illness) in our study and to describe the existence of other triggering or protective parameters that have been poorly studied to date.

One of the limitations of this work is its cross-sectional design, which does not allow us to establish a strong causal relationship between the values found and the BMD measurement, which should be confirmed with longitudinal studies. One strength of the study is the large number of participants, which gives it high power in drawing conclusions. In our country, few studies have analyzed the characteristics of patients evaluated by gynecologists in which the principal motivation for the consultation was to inquire about the state of their bone health. The severity of BMD loss corresponded to a higher intensity of detected risk factors: lower BMDs and greater frequencies of osteoporosis were observed with lower weight and height, older age at menarche, younger age at menopause, less dairy consumption, and greater consumption of tobacco.

Regular physical exercise is considered to be a protective factor for the development of femur fractures[7,8]. In our work, however, we did not find this association. We did not have sufficient data available on the habits of our postmenopausal women and whether the percentage we observed deviated from the real Spanish population, which leads us to think that perhaps other investigators have overestimated what is defined as 'exercise'.

Over the last years, genome-wide association studies have identified the SNPs associated with osteoporosis, BMD and other determinants of fracture risk. Some of these SNPs were mapped close to or within the estradiol pathway genes[9]. It is

Table 3 Multilocus 'risk' combinations in the hip. Significant values are indicated in bold type

		T-score			*BMD* (g/cm^2)			%	
Combination	*n*	*Mean*	*SD*	*p**	*Mean*	*SD*	*p**	*osteoporosis*	*p***
ESR2 (GA)–*CYP19A1* 3′UTR (CC)–*NRIP1* (AA)	12	−1.01	0.88	0.47	0.73	0.36	0.28	8.3	1
ESR2 (GA)–*CYP19A1* 3′UTR (CC)–*BMP15* (-9C>G) (GG)	4	−1.52	0.76	0.18	0.77	0.06	0.69	25	0.6
ESR2 (GA)–*CYP19A1* 3′UTR (CC)–*BMP15* (905A>G)(GG)	9	−1.17	0.79	0.29	0.81	0.08	0.89	11.1	1
ESR2 (GA)–*CYP19A1* VS4 (DD)–*NRIP1* (AA)	6	−0.85	0.59	0.86	0.64	0.33	0.10	0	1
ESR2 (GA)–*CYP19A1* IVS4 (DD)–*BMP15* (-9C>G) (GG)	2	−1.05	0.35	0.72	0.81	0.03	0.95	0	1
ESR2 (GA)–*CYP19A1* IVS4 (DD–*BMP15* (905A>G)(GG)	6	−0.89	0.70	0.78	0.84	0.09	0.85	0	1
ESR2 (GA)–*NRIP1* (AA)–*BMP15* (-9C>G) (GG)	3	**−2.32**	0.91	**0.02**	0.74	0.05	0.58	**66.7**	**0.03**
ESR2 (GA)–*NRIP1* (AA)–*BMP15* (905A>G)(GG)	3	**−2.32**	0.91	**0.02**	0.74	0.05	0.58	**66.7**	**0.03**
ESR2 (GA)–*CYP19A1* 3′UTR (CC)–*NRIP1* (AA)–*BMP15* (-9C>G) (GG)	1	−2.6		0.11	0.689		0.61	100	0.07
ESR2 (GA)–*CYP19A1* 3′UTR (CC)–*NRIP1* (AA)–*BMP15* (905A>G)(GG)	1	−2.6		0.11	0.689		0.61	100	0.07

BMD, bone mineral density; SD, standard deviation
*, Student's *t*-test; **, χ^2 test. *p* relative to a control group of women without these genotypes

widely accepted that estradiol plays an important role in the acquisition and maintenance of bone mass as well as its extension to multiple targets and its pathophysiological implications for other diseases.

ESR1 and some of its SNPs have been linked to BMD, bone remodelling, and fractures due to fragility in different populations, with somewhat contradictory results[10–12]. Our data show that the TC SNP is the most common, although we did not find a significant relationship between the different alleles and the development of the disease. The results obtained by Geng and colleagues[13] regarding the association of postmenopausal osteoporosis and SNPs in *ESR2* in an Asian population have not been confirmed in our population.

In a study conducted in the United States with 1301 women from various ethnic groups[14], it was observed that the relationships between distinct SNPs of *ESR1* and *2* and BMD vary as a function of the ethnic group considered. In this population, which in theory would have common environmental factors, it was the genetic heritage of each ethnicity that determined the existence or lack of an association. This result could explain why we have not found an association between the SNPs of these or other studied genes in our fairly genetically uniform population while they were found in other populations.

Although a relationship has been described between the SNPs of the aromatase gene and osteoporosis in various studies[15], the individual analysis of our data showed no relationship between osteoporosis and either the IVS4 or the 3′UTR markers for that gene.

While the links between the SNPs of the *BMP15* gene and bone mass have not been studied, such associations do have a significant association with a reduction in the number of fertile years and, as a consequence, with the time exposed to estrogens[16]. Although these findings have been verified by other authors, the evidence surrounding the role of *BMP15* SNPs as a cause of early menopause is called into question by the publication of those SNPs in other populations[17].

For osteoporosis and other estrogen-related diseases, publications exist that describe various genetic interactions, not only among genes in the estrogenic pathway but also among other osteoporosis candidate genes, such as those that code for the vitamin D receptor (VDR) or insulin growth factor type 1 (IGF-1)[18–20].

In a previous study with some of our patients, we proposed a non-additive, non-multiplicative oligenic model including *ESR2* (GA) genotype modulated by *NRIP1* (AA) or *ESR1* (TT) genotypes involved in osteoporosis[21]. In the current study, we differentiated between hip and lumbar osteoporosis and we followed by observing the *ESR2–NRIP1* interaction in the hip. The most involved gene in vertebral osteoporosis is the *CYP19A1*.

In a recent study, Zhao and colleagues showed that the interaction of osteoporosis susceptibility SNPs, such as *ESR1, MHC, LRP4* and *jagged1* might influence the age at menopause or the maximal height[22]. We analyzed the genes related to the estrogen signalling pathway and found that the multigenic interaction formed by *ESR2* (AA), *BMP15* 905A>G (TC) and *NRIP1* (AA) has the lower age at natural menopause[23]. It is interesting to point out that the markers in *ESR2* and *BMP15*, especially the *ESR2* (GA) that we have found in the current study, are different, so the relation of this interaction with hip osteoporosis is independent of the relation with the age at menopause.

The difficulty in studies of genetic interactions for the prediction of common illnesses lies in the need for a large study population that permits comparisons among genetic variants, and hence the current shortage of this type of publication on osteoporosis. Moreover, this research is evolving toward investigating more complex forms of multi-gene interactions, necessitating a corresponding multiplication in the size of study populations. Recently, the issue of which statistical method can best quantify the implications of these interactions has been raised[24]. The strong point of our work, without a doubt, is the size of our sample, which is what permitted us to perform this type of analysis.

The main limitation of this work, and the limitation in general of all studies of genetic interaction, is probably the

necessity for a much higher statistical significance than that which is usually established.

On the one hand, our work reinforces the hypothesis that the *CYP19A1* gene is one of the most important 'candidate genes' in studies of the genetics of osteoporosis and should to be included in any future gene interaction analysis project. On the other hand, the results show that the genes *FSHR, BMP15, CYP19A1*, and *NRIP* and the interactions among them seem to be most related to osteoporosis predisposition.

In this analysis, we also assessed whether the combination of more than two mutant genotypes increased the risk of developing osteoporosis. The log reduction of the sample does not permit these cases to reach statistical significance (nor is it achieved in the interactions between the genotypes we have called 'protective'). Nonetheless, some 'risky' combinations do reach statistical significance, in particular, the one formed by the mutant genotypes of the *ESR2* and *NRIP1* genes with either of the two mutations of the analyzed markers of the *BMP15* gene, which gave a mean *T*-score value of −2.3 and an elevated proportion (two-thirds) of women diagnosed with osteoporosis of the hip ($p = 0.02$).

The most interesting finding is the exclusion of the markers of the *CYP19A1* gene in the risk for osteoporosis of the hip and the involvement of the *ESR2* gene. This result is exactly the opposite of the situation observed in vertebral osteoporosis. The two new candidate genes that we have analyzed in our study (*BMP15* and *NRIP*) are related to both processes. Perhaps the distinction is between two distinct forms (vertebral and cortical) within the same disease (osteoporosis), in which estrogen involvement is equally important but the pathophysiological mechanism is different: the process that affects trabecular bone (vertebral) is focused on an alteration in the level of aromatase, while in cortical bone (femur), this process is secondary to an alteration in the estrogen receptor.

In conclusion, these results reinforce two hypotheses raised in relation to osteoporosis. First, we have data that some genetic SNPs in the estrogen pathway are implicated in the development of osteoporosis. This result is also the first reference to the idea that the variations in our candidate genes (*NRIP* and *BMP15*) could predispose patients to osteoporosis as they do for other gynecological processes[25,26]. Second, we have provided new arguments to accentuate the complex and multifactorial nature of osteoporosis, not only in terms of the relationship between genetic and environmental factors but also with respect to the interaction between different genetic factors. Genetic interaction can allow us to predict a patient's risk of suffering from osteoporosis. We also suspect that osteoporosis has different pathophysiological mechanisms depending on the bone affected.

Although we are in the early phases of studying osteoporosis genetically, we hope that genetic research enters fully into the clinical approach to osteoporosis. Detailing the genetic map of SNPs or mutations that influence BMD or the risk of suffering from osteoporosis will allow for better future knowledge of its pathogenesis and allow for the identification of individuals who are at high risk for suffering fractures due to fragility. With this knowledge, it would be possible to develop individualized prevention programs.

Conflict of interest The authors report no conflict of interest. The authors alone are responsible for the content and writing of this paper.

Source of funding Nil.

References

1. Duncan EL, Danoy P, Kemp JP *et al.* Genome-wide association study using extreme truncate selection identifies novel genes affecting bone mineral density and fracture risk. *PLoS Genet* 2011;7:e1001372
2. Tan L, Liu R, Lei S, *et al.* A genome-wide association analysis implicates SOX6 as a candidate gene for wrist bone mass. *Sci China Life Sci* 2010;53:1065–72
3. Hsu YH, Zillikens MC, Wilson SG, *et al.* An integration of genome-wide association study and gene expression profiling to prioritize the discovery of novel susceptibility loci for osteoporosis-related traits. *PLoS Genet* 2010;6:e1000977
4. Zhao LJ, Liu XG, Liu YZ, *et al.* Genome-wide association study for femoral neck bone geometry. *J Bone Miner Res* 2010;25:320–9
5. Kung AW, Xiao SM, Cherny S, *et al.* Association of JAG1 with bone mineral density and osteoporotic fractures: a genome-wide association study and follow-up replication studies. *Am J Hum Genet* 2010;86:229–39
6. Guo Y, Tan LJ, Lei SF, *et al.* Genome-wide association study identifies ALDH7A1 as a novel susceptibility gene for osteoporosis. *PLoS Genet* 2010;6:e1000806
7. Berard A, Bravo G, Gautier P. Meta-analysis of the effect of physical activity on the prevention of bone loss in postmenopausal women. *Osteoporos Int* 1997;7:331–7
8. Feskanich D, Willett W, Colditz G. Walking and leisure-time activity and risk of hip fracture in postmenopausal women. *JAMA* 2002;288:2300–6
9. Karasik D, Dupuis J, Cho K, *et al.* Refined QTLs of osteoporosis-related traits by linkage analysis with genome-wide SNPs: Framingham SHARe. *Bone* 2010;46:1114–21
10. Gómez R, Magaña JJ, Cisneros B, *et al.* Association of the estrogen receptor alpha gene polymorphisms with osteoporosis in the Mexican population. *Clin Genet* 2007;72:574–81
11. Langdahl BL, Løkke E, Carstens M, Stenkjaer LL, Eriksen EF. A TA repeat polymorphism in the estrogen receptor gene is associated with osteoporotic fractures but polymorphisms in the first exon and intron are not. *J Bone Miner Res* 2000;15:2222–30
12. Becherini L, Gennari L, Masi L, *et al.* Evidence of a linkage disequilibrium between polymorphisms in the human estrogen receptor alpha gene and their relationship to bone mass variation

in postmenopausal Italian women. *Hum Mol Genet* 2000;9: 2043–50
13. Geng L, Yao Z, Yang H, Luo J, Han L, Lu Q. Association of CA repeat polymorphism in estrogen receptor beta gene with postmenopausal osteoporosis in Chinese. *J Genet Genomics* 2007;34:868–76
14. Greendale GA, Chu J, Ferrell R, Randolph JF Jr, Johnston JM, Sowers MR. The association of bone mineral density with estrogen receptor gene polymorphisms. *Am J Med* 2006;119:S79–86
15. Riancho JA, Zarrabeitia MT, Valero C, *et al*. Aromatase gene and osteoporosis: relationship of ten polymorphic loci with bone mineral density. *Bone* 2005;36:917–25
16. Morón FJ, Mendoza N, Quereda F, *et al*. Pyrosequencing technology for automated detection of the BMP15 A180T variant in Spanish postmenopausal women. *Clin Chem* 2007;53:1162–4
17. Laissue P, Christin-Maitre S, Touraine P, *et al*. Mutations and sequence variants in GDF9 and BMP15 in patients with premature ovarian failure. *Eur J Endocrinol* 2006;154:739–44
18. Zmuda JM, Cauley JA, Danielson ME, Ferrell RE. Vitamin D receptor and aromatase gene interaction and bone mass in older African-American women. *Metabolism* 2003;52:521–3
19. Riancho JA, Zarrabeitia MT, Valero C, *et al*. A gene-to-gene interaction between aromatase and estrogen receptors influences bone mineral density. *Eur J Endocrinol* 2006;155:53–9
20. Rivadeneira F, van Meurs JB, Kant J, *et al*. 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* 2006;21:1443–56
21. Morón FJ, Mendoza N, Vázquez F, *et al*. Multilocus analysis of estrogen-related genes in Spanish postmenopausal women suggests an interactive role of ESR1, ESR2 and NRIP1 genes in the pathogenesis of osteoporosis. *Bone* 2006;39:213–21
22. Zhao L, Cui B, Liu JM, *et al*. Interactions of osteoporosis candidate genes for age at menarche, age at natural menopause, and maximal height in Han Chinese women. *Menopause* 2011;18: 1018–25
23. Mendoza N, Sánchez-Borrego R, Galiano D, *et al*. Multigenic combination of estrogen-related genes is associated with age at natural menopause in a Spanish population. *Menopause Int* 2009;15:150–6
24. Chang HW, Chuang LY, Ho CH, Chang PL, Yang CH. Odds ratio-based genetic algorithms for generating SNP barcodes of genotypes to predict disease susceptibility. *OMICS* 2008;12: 71–81
25. Morón FJ, Mendoza N, Quereda F, *et al*. Pyrosequencing technology for automated detection of the BMP15 A180T variant in Spanish postmenopausal women. *Clin Chem* 2007;53:1162–4
26. González A, Ramírez-Lorca R, Calatayud C, *et al*. Association of genetic markers within the BMP15 gene with anovulation and infertility in women with polycystic ovary syndrome. *Fertil Steril* 2008;90:447–9