CYP19 and *ESR1* gene polymorphisms: response of the bone mineral density in post-menopausal women to hormonal replacement therapy

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Summary

***Objectives*. Sex steroids are important regulators of bone physiology and play an essential role in the maintenance of bone health throughout the life. Hormonal replacement therapy (HRT) is a treatment commonly used to relieve symptoms and some undesirable consequences of menopause such as osteoporosis. Osteoporosis, characterized by the loss of bone mass and deterioration of microarchitecture with a consequent higher risk of fragility fractures, is under genetic influence. A tetranucleotide (TTTA)n microsatellite repeat polymorphism, at intron 4 of the *CYP19* (aromatase) gene, has been previously associated with higher lumbar spine bone mineral density (LS-BMD) and lower risk of spine fracture in postmenopausal women. Moreover, the ERα encoded by the *ESR1* gene is another important candidate for the regulation of bone mass of menopause. Moreover prospective analysis from >18.000 subjects at the GENOMOS study indicated that XX homozygotes genotype had a reduced risk of fracture independently from BMD.**

In the present study, we investigated in postmenopausal Italian women, at baseline and after 1 year of HRT, whether *ESR1* and *CYP19* gene polymorphisms could affect BMD through different statistical models.

***Methods*. This study has been performed on 100 postmenopausal Italian women, from a larger group of 250. The study group was administred HRT and LS-BMD was measured at baseline and after 1 year of therapy. Genetic analysis evaluating *ESR1* and *CYP19* gene polymorphisms was performed.**

***Results*. Generalized Linear Models (GLMs) test showed that women with normal LS-BMD at the baseline had a major statistically significant BMD increase of 0.1426 gr/cm^2 (p= 0.0001) with respect to the osteoporotic patients. In addition, subjects with genotype 1 and 2 of *CYP19* gene had a lower modification in LS-BMD after 1 year of HRT (0.0837 gr/cm^2 and 0,076 g/cm^2; p=0.0470 and 0,0547 respectively) when compared to genotype 3. No influences of the aromatase genotypes were observed in the variable *difference* using both Anova and GLMs test. Regarding the *ESR1* gene polymorphism, the LS-BMD after 1 year of HRT was influenced by the diagnosis at the baseline and height and ERα genotypes were able to influence *difference* with statistical significant results with both test.**

***Conclusions*. In the present study, we have demonstrated that *CYP19* gene polymorphism is able to influence the effect of 1 year HRT on LS-BMD with no influence on pre-/ and post-/HRT LS-BMD differences. Although *ESR1* gene polymorphism is not able to influence the LS-BMD after 1 year HRT, it influences the observed modifications during the year of therapy. These data underlie the complexity of the genetics of the bone mass and its importance in influencing the response to HRT.**

KEY WORDS: CYP19 gene; ESR1 gene; polymorphism; Hormonal Replace Therapy (HRT).

Introduction

Sex steroids are important regulators of bone physiology and play an essential role in the maintenance of the bone health through the life. Negative effects of hormone deficiency on bone turnover can be observed at any age in both sexes (1). In recent years, our understanding on the role of estrogens in both females and males has made great progress, with a considerable emphasis being focused on the regulation of the extragonadal estrogen biosynthesis (1). An increasing evidence that extragonadal production of C(18) steroids from C(19) precursors, is important in physiological control of skeletal metabolism and in the pathogenesis of bone disorders (1). The enzyme aromatase has been found in several human tissues and cells including bone, where it locally catalyzes the conversion of the C(19) steroids precursors into estrogens (2, 3). The observation of a marked bone phenotype in men with mutation of either the *ESR1* or *CYP19* gene (4, 5) has led to the conclusion that the local bone estrogen production and effect plays an important role in the maintenance of bone mineralization and in the prevention of osteoporosis in men and in women (1). In bone, the local

aromatase expression represents the major source of estradiol (E_2) responsible for the maintenance of bone mineralization, while the circulating 17βE_2 levels reflect the sum of the estrogen synthesis at various sites (6).

It is well known that in the pathogenesis of osteoporosis, multiple genetic and environmental factors are involved, the former principally consisting of genetic variations, relatively common in the general population (7). Genes involved in the estrogen metabolism (such as the *CYP19* gene) and in the estrogenic response (the *ESR1* gene) are possible contributors to the pathophysiological processes associated with osteoporosis (8, 9).

The *CYP19* gene, a member of the cythocrome P450 enzymes family, is located on chromosome 15q21.1 (10). A tetranucleotide (TTTA)n microsatellite repeat polymorphism is present in intron 4 and we have previously observed that a higher number of repeats was associated with higher lumbar spine bone mineral density (LS-BMD) and lower risk of spine fracture (6).

The ERα encoded by the *ESR1* gene, is located on chromosome 6q25.1 and represents an important functional candidate gene for the regulation of bone health. Studies of *ESR1* alleles in relation to BMD have yielded inconsistent results (11). Recently, a prospective analysis from >18.000 subjects at the GENOMOS study indicated that the XX homozygous genotype segregate with a reduced risk of fragility fracture, independently from BMD (12).

In the present study, we have investigated in a population of Italian postmenopausal women whether *ESR1* and *CYP19* gene polymorphisms could affect BMD at baseline and after 1 year of Hormonal Replacement Therapy (HRT) through different statistical models.

Materials and methods

Participants

Patients eligible for the study were 250 Italian postmenopausal women with an age range of 47-67 years (mean 57.5±8.7). An homogeneous subgroup of 100 subjects, receiving sequential combination of 17β-E_2 (17β-E_2 TTS 50μg/day) and Normegestrol acetate (5mg/day, 12 days/month), was selected for the analysis. Women included in the study had the last menstrual bleeding at least 6 months before the study entry, in combination with the assessment of postmenopausal serum FSH (FSH more than 2 SD over the premenopausal mean value). For all the subjects a detailed medical history was obtained and dietary calcium intake was assessed by a sequential self-questionnaire including calcium-rich foods (Table 1). Subjects with previous or current cancer or thromboembolic disease were excluded from the study. On the basis of their weight, the women were divided in four categories: weight under 50, between 55/65, 65/75, and 75/90 Kg.

Body mass index was calculated as the weight in Kg divided by the square of the height in meters (BMI, Kg/m^2). An informed written consent was obtained from each participant and the Institutional Review Board of the "Azienda Ospedaliera Universitaria Careggi" of Florence approved the study.

Bone Mineral Density

Areal BMD (g/cm^2) for LS (L1-L4) was determined at the baseline and after 1 year HRT by dual energy X-ray absorptiometry (DXA) using the Hologic 4500 instrument (Hologic, Waltham, MA, USA) with short-term *in vivo* coefficients of variation of 0.9%. Body weight and height were recorded at each BMD measurement.

On the basis of LS-BMD, subjects were classified in 3 groups indicated with the letter D (D1: normal, D2: osteopenic, D3: osteoporotic), according to the WHO's criteria (24).

Genetic analysis

Genomic DNA was extracted from EDTA peripheral blood samples collected in ethyl-enediamine tetraacetate by a standard phenol/chloroform extraction procedure.

a) *CYP19* gene [(TTTA)n repeats]

The DNA region containing the polymorphic (TTTA)n repeats at 1174-base pair at the human *CYP19* gene was amplified by PCR as previously described (4). TTTA repeats for the aromatase gene were evaluated by sequence analysis using the ABI Prism 310 (Perkin Elmer, Monza, Italy). The frequency of each genotype was observed in agreement with the Hardy-Weinberg equilibrium. We identified two alleles, allele 1 and allele 2 and the related genotypes were divided in three categories: genotype 1: subjects with allele 1 and 2 with repeats <8; genotype 2: subjects with allele 1 repeats <8 and allele 2 repeats >10; genotype 3: subjects with allele 1 and allele2 with repeats >10 (Table 1).

b) *ESR1* gene (*ER*α)

For the *ER*α gene polymorphism, the PCR product was digested with Pvu II and Xba I restriction endonuclease and electrophoresed in 2.0% agarose gel. The capital P and X and the lower-case p and x represent respectively the absence and the presence of the restriction sites in the *ER*α (11). Four genotypes were identified: genotype E (Pp; Xx); genotype O (PP; XX); genotype o (pp; xx); and genotype d the residual cases (PP-Xx; PP-xx; Pp-XX; Pp-xx; pp-Xx; pp-XX) (Table 1).

Statistical analysis

The statistical analysis was performed through two statistical methods : Analysis of variance (ANOVA), and Generalized Linear Models (GLMs) (13, 14). For each polymorphism we considered two dependent variables: 1) the modification of the LS-BMD from the baseline at the end of 1 year HRT on the basis of the genotypes; and 2) the one called *difference* indicating the difference of LS-BMD between baseline and after 1 year therapy mean level.

A summary of the dependent variables and statistical methods has been reported in Table 2.

Results

The frequencies of each genotype for *CYP19* and *ESR1* genes were observed in agreement with the Hardy-Weinberg equilibrium.

a) *CYP19* gene: (TTTA)n repeats and LS-BMD

In the first preliminary statistical analysis, the ANOVA model has been applied considering the changes of LS-BMD after 1 year of HRT as the dependent variable. We studied the effect of the three genotypes on this modification. No statistically significant differences were observed (data not shown).

In a second step of the analysis we used the GLMs model. Women with normal LS-BMD (D1) at the baseline had an increase of 0.1426 gr/cm^2 (p= 0.0001), statistically significant higher with respect to the osteoporotic patients (D3) (Table

Table 1 - Clinical characteristic of study population chosen for the study.

Subjects	**100**
Age	mean: 57.5±8.7 years
Weight (Kg) <=50 50-65 65-75 75-90	Number (%) 17 (17.35%) 46 (46.94%) 23 (22.45) 13 (13.27)
Height (cm) 149/160 160/166 >=166	Number (%) 41 (41.84) 40 (40.82) 17 (17.35)
BMI (Kg/m^2)	
Therapy	17β-Estradiol (17β E2 TTS 50μg/day) and Normegestrol acetate 5mg/day (12 days/month)
Calcium-intake (mg/day)	650

Table 2 - Summary of statistical methods and analysis.

Statistical methods

Dependent Variable	**ANOVA**	**GLMs**
LS-BMD	**ERα** LS-BMD after 1 year HRT was influenced by D and height (Table 5) **(TTTA)n repeats** Non significant results	**ERα** Analysis confirmed the ANOVA results (data not shown) **(TTTA)n repeats** Model results shown in table 2 Orthogonal c. in table 4
Difference	**ERα** ERα genotypes were able to influence difference (Table 6) **(TTTA)n repeats** Non significant results	**ERα** Women with dia1 had a difference lower with respect D 3 (Table 7A, B) **(TTTA)n repeats** Non significant results

3). Considering the *CYP19* gene genotypes, we observed that genotype 1 and 2 had a lower modification in LS-BMD after HRT (0.0837 gr/cm^2 and 0,076 g/cm^2; p=0.0470 and 0,0547 respectively) in comparison with the genotype 3. Figure 1 represents the LS-BMD change after 1 year HRT with respect to the aromatase genotype.

The statistical model used with link function equal to the identity, is the following:

$$E[Y_{\text{—bmd-after 1 yr. HRT}}] = \beta_0 + \beta_{1\ D\_1} D + \beta_{2\ D\_2} D + \beta_{3\ D\_3} D + \beta_{1\ bmi} BMI + \beta_{1\ allele\_1} ALLELE + \beta_{2\ allele\_2} ALLELE + \beta_{3\ allele\_3} ALLELE + \beta_{1\ weight\_<=50} WEIGHT + \beta_{2\ weight\_50-l65} WEIGHT + + \beta_{3\ weight\_65-l75} WEIGHT + \beta_{4\ weight\_75-l90} WEIGHT + \varepsilon$$

TTTA repeats genotype 1 denotes the category no.1: repeats <8; genotype 2 denotes the category no.2: one allele with <8 repeats and one with >10; genotype 3 denotes the category no.3: repeats >10.

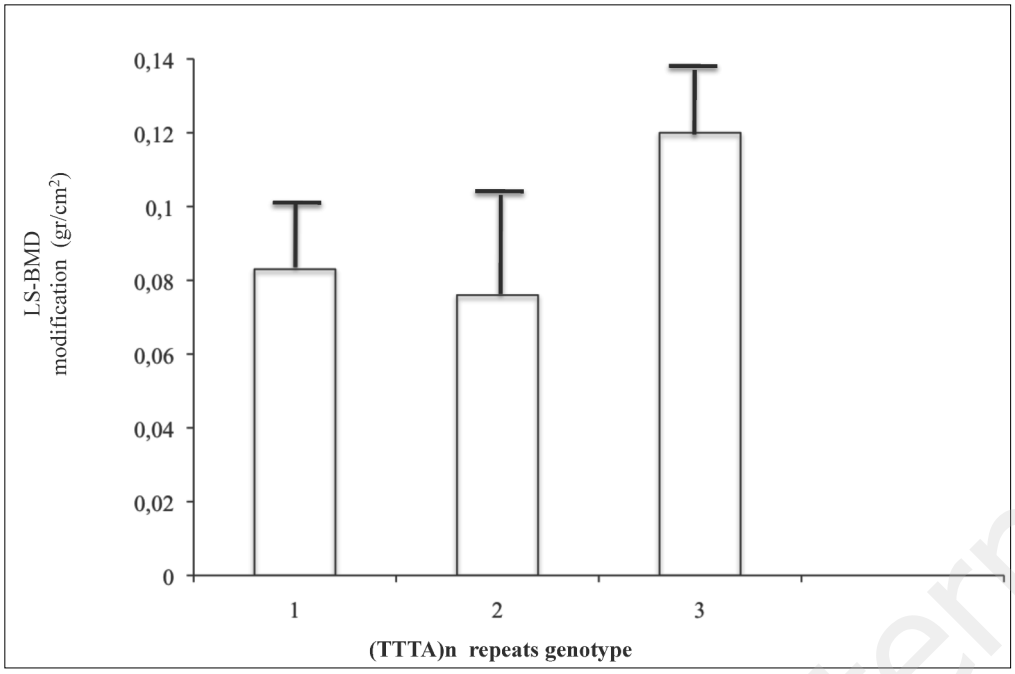

Figure 1 - Modification of the LS-BMD from the baseline at the end of 1 year HRT on the basis of the genotypes using GLMs analysis model.
Genotype 1 and 2 had a lower modification in LS-BMD after HRT (0.0837 gr/cm² and 0,076 g/cm²; p=0.0470 and 0,0547 respectively) in comparison with the genotype 3.
1: subjects with genotype characterized by allele 1 and 2 with repeats <8;
2: subjects with genotype characterized by allele 1 repeats <8 and allele 2 repeats >10;
3: subjects with genotype characterized by allele 1 and allele 2 with repeats >10.

Considering the weight as a variable, women with weight in the 65-75 or 75-90 Kg class had a greater LS-BMD after 1 year of HRT (0,15 gr/cm² and 0,228 gr/cm²; p= 0.0306 and 0.0196, respectively) in comparison with the women with weight less or equal to 50 Kg. However, when we evaluated the change in the LS-BMD according to the BMI, we observed a decrease of 0.017 gr/cm² for the increase of one unit of the BMI (p=0.0290). Table 3 summarized all these results.
The orthogonal analysis (Table 4) reinforced our results confirming a statistically significant change of LS-BMD after therapy on the basis of the genotype. In particular, a significant variability was observed between genotype 1 and 2 versus the genotype 3 (p=0.0316). No statistically significant differences were observed between genotype 1 and 2 (p=0.8466). Regarding the TTTA repeats and *difference* as dependent variable, we did not find significant results applying both ANOVA and GLMs methods (data not shown).
b) *ESR1* gene polymorphism
In the first preliminary statistical analysis, the ANOVA model has been applied considering the modification of LS-BMD after 1 year of HRT as a dependent variable. The independent variables involved were: 1) the clinical bone status of the women at the baseline (D1 or D2 or D3); 2) the *ER*α genotypes; 3) the height; and 4) the interaction effect: *ER*α genotype*height.

Table 3 - Role of the aromatase genotypes on the LS-BMD after one year HRT as dependent variable - GLMs analysis.

Parameter	Df	Difference in LS-BMD (gr/cm²)	Standard Error	Confidence limits Wald 95%	Chi-square	Pr>Chi-square
Intercept	1	1.2507	0.1655	0.9263 1.5752	57.08	<.0001**
D1	1	0.1426	0.0371	0.0698 0.2154	14.75	0.0001**
D2	1	0.0500	0.0434	-0.0351 0.1351	1.32	0.2498
D3	0	0.0000	0.0000	0.0000 0.0000	.	.
genotype 1	1	-0.0837	0.0421	-0.1663 -0.0011	3.95	0.0470*
genotype 2	1	-0.0767	0.0399	-0.1550 0.0016	3.69	0.0547
genotype 3	0	0.0000	0.0000	0.0000 0.0000	.	.
weight 50-65	1	0.0738	0.0460	-0.0164 0.1640	2.57	0.1087
weight 65-75	1	0.1572	0.0727	0.0147 0.2997	4.68	0.0306*
weight 75-90	1	0.2282	0.0977	0.0366 0.4197	5.45	0.0196*
weight <=50	0	0.0000	0.0000	0.0000 0.0000	.	.
BMI	1	-0.0171	0.0078	-0.0325 -0.0018	4.77	0.0290*
Scale factor	1	0.1409	0.0104	0.1220 0.1628		

*A p-value with * denotes a significant p-value (α=0.05); a p-value with ** denotes a highly significant p-value (α=0.01).*

Table 4 - Orthogonal contrast between aromatase genotypes.

Contrast	Df	Chi-square	Pr>Chi-square
genotype1+genotype 2 vs. genotype 3	1	4.62	0.0316*
genotype 1 vs. genotype 2	1	0.04	0.8466

Table 5
A) ANOVA analysis: LS-BMD after one year HRT as dependent variable

Source	Df	SS	MS	F-Value	Pr>F
Model	9	0.78226895	0.08691877	4.11	0.0002
Error	81	1.71448234	0.02116645		
Total	90	2.49675130			

B)

Source	Df	Type III SS	MS	F-Value	Pr>F
D	2	0.41470153	0.20735076	9.80	0.002
Height	1	0.14132376	0.14132376	6.68	0.016
Genotype	3	0.15950749	0.05316916	2.51	0.0644
Height*genotype	3	0.16408324	0.05469441	2.58	0.0589

D: diagnosis; genotype: ERα genotype and height*genotype: independent variables

C) ANOVA analysis considering *difference* as dependent variable

Source	df	Type III SS	MS	F-value	Pr > F
BMI	1	0.06931337	0.0693133	5.25	0.0246
D	2	0.18596114	0.0929805	7.04	0.0015
Weight	3	0.03128502	0.0104283	0.79	0.5031
genotype	3	0.11067964	0.0368932	2.79	0.0457

BMI: body mass index; genotype: ERα genotypes as independent variables

The results showed a highly significant relevance (p-value =0.0002; α=0.01) of the D variable, and a significant effect of the height (p-value= 0.0116; α =0.05) in the influence the LS-BMD after 1 year of HRT. All these variables were statistically significant using the Fisher's test providing to refuse the null hypothesis. On the other hand, *ER*α genotypes and *ER*α genotype*height interaction resulted to be the less informative variables and were not statistically significant (p=0.064 and 0.058 respectively) (Table 5A, B).

A second step of this analysis evaluating the modification of LS-BMD after 1 year of HRT as a dependent variable was performed through GLMs and the results confirmed those obtained through ANOVA, in which the *ER*α genotype variable had a trend only close to the significant level of α=0.05 (data not shown).

Interesting results were obtained considering the *difference* as a dependent variable. In this analysis the BMI, D, the weight and *ER*α genotypes were involved as independent variables. Table 5C showed the results applying ANOVA analysis. We observed that *ER*α genotype variables were able to influence the *difference* of the LS-BMD evaluated before and after HRT (p=0.0015 and 0.0457 respectively). On the other hand no significant effect was observed for the weight variable (p= 0.5).

Using GLMs method with *difference* as dependent variable we found the results reported in Table 6. The link function is assumed equal to the Identity. The final statistical model was the following:

$Y_{difference} = \beta_0 + \beta_{1\ D\_1} D + \beta_{2\ D\_2} D + \beta_{3\ D\_3} D + \beta_{1\ bmi}\ BMI + \beta_{1\ weight\_<=50} WEIGHT + \beta_{2\ weight\_50\text{-}l65}\ WEIGHT + \beta_{3\ weight\_65\text{-}l75}\ WEIGHT + \beta_{4\ weight\_75\text{-}l90}\ WEIGHT + \beta_1\ ER\text{-}\alpha_{\_E}\ ER\text{-}\alpha + \beta_2\ ER\text{-}\alpha_{\_O}\ ER\alpha + \beta_3\ ER\text{-}\alpha_{\_d}\ ER\text{-}\alpha + \beta_4\ ER\text{-}\alpha_{\_0}\ ER\text{-}\alpha + \varepsilon$

ERα "d" indicates the genotypes: PP-Xx; PP-xx, Pp-XX; Pp-xx; pp-Xx; pp-XX; ERα "o" indicates the genotypes: pp; xx.

Women D1 had a *difference* lower 0.11 gr/cm² with respect to the reference value (D3) (p=0.0001) (Table 6). Subjects with lower LS-BMD at the baseline had a major response to the HRT in comparison with subjects with dia1. Considering the weight variable, the heavier classes were significant; therefore, a woman with a weight of 65-75 or 75-90 Kg had a difference estimated value greater 0,11 gr/cm² and 0,16 g/cm² with respect to a woman with weight less or equal to 50 kg (p=0.04 and 0.03 respectively) (Table 6)

According to the BMI we observed that the higher BMI was follow by a decrease of 0.013 gr/cm² difference on the LS-BMD (p=0.035) (Table 6).

Statistically significant results in the *difference* variable were observed only in subjects with genotype d (p= 0.05). We showed that a woman with the *ER*α genotype d had a mean value of difference lower -0,0695 gr/cm² in comparison with a woman that has the *ER*α genotype o. This finding could be interpreted as a different response to the HRT according to the two *ER*α genotypes d and o. In particular, therapy had a lower effect when the *ER*α genotype was d.

The orthogonal contrast supported the previous data indicating that women with dia1 had a significant *difference* in the effect of HRT on LS-BMD in comparison to the dia2 and dia3 (p=0.0002). No significant differences were observed between dia2 and dia3 women (p=0.2428) (Table 7A)

Finally, by orthogonal analysis, even though we cannot observed high significant p-values, we estimated a p-value very close to the level of significance of α=0.05 for the category o of the *ER*α genotype. In particular, a trend characterized by variability in the *difference* was observed between genotype o and d (p=0.05), whereas no significant variability was observed for all the other genotypes (Table 7B).

Conclusions

In the present study we evaluated the influence of the *CYP19* and *ESR1* gene polymorphisms on the effects of HRT on LS-BMD in postmenopausal women using various statistical approaches. It has been well established that genetic factors account for as much as 50% of the observed variation in BMD (15) and genes involved in estrogen metabolism and response are potentially contributors to the abnormal pathophysiological processes associated with osteoporosis (6, 16).

CYP19 gene, encoding aromatase enzyme, is involved in the estrogen response pathway. In fertile women, ovary represent the major source of circulating estrogens, whereas in postmenopausal women extraglandular aromatization of circulating androgens becomes the most important metabolic mechanism for the estrogen production (17). Either bone tissue or bone derived cells express aromatase gene and enzyme activity (6). We previously demonstrated that the allelic variant of the aromatase gene containing longer TTTA repeats segregated with higher BMD and a lower fractures risk in both postmenopausal women and men (18).

In the present study, we evaluated the influence of TTTA repeats on the LS-BMD variation with regard to the response to HRT. Thus, we observed that genotypes with low number of TTTA repeats (genotype 1 and 2) had a lower LS-BMD modification in comparison with women with higher number of TTTA repeats genotype (genotype 3) after one year of HRT.

These data have been obtained using two different statistical models, ANOVA and GLMs. Only by GLMs we observed a statistical significant differences, suggesting that subjects with genotype 3 may be more hormone-sensitive, then benefit the protective effect of HRT on LS-BMD when compared to women with genotype 1 and 2.

Our finding was reinforced by orthogonal analysis that showed a significant statistical difference between genotype 3 and genotypes 1/2. Tofteng et al. found similar results in a group of Danish posmenopausal women emphasizing the role of genes in the regulation of either bone mass or bone response to the therapy (19).

However, no significant results were observed considering the change of LS-BMD during the year of therapy (variable

Table 6 - GLM analysis considering *difference* as dependent variable.

Parameter	Df	Difference in LS-BMD (gr/cm2)	Standard Error	Confidence limits Wald 95%	χ^2-value	Pr > χ^2-value
Intercept	1	0.2873	0.1271	0.0382; 0.5364	5.11	0.0238
D1	1	-0.1101	0.0284	-0.1657; 0.0544	15.04	0.0001
D2	1	-0.0360	0.0307	-0.0963; 0.0242	1.37	0.2410
D3	0	-	-	-; -	-	-
				-		
weight						
51-l65	1	0.0510	0.0354	0.0183; 0.1204	2.08	0.1492
65-l75	1	0.1169	0.0571	0.0050 ; 0.2288	4.20	0.0405
75-l90	1	0.1663	0.0787	0.0121; 0.3205	4.47	0.0346
<=50	0	-	-	- ; -	-	-
Genotype E	1	-0.0107	0.0297	-0.0689; 0.0475	0.13	0.7177
Genotype O	1	0.0563	0.0326	-0.0076 ; 0.1201	2.98	0.0841
Genotype d	1	-0.0695	0.0361	-0.1402; 0.0012	3.71	0.0541
Genotype o	0	-	-	- ; -	-	-
BMI	1	-0.0130	0.0062	-0.0252; 0.0009	4.42	0.0355
Scale factor	1	0.1077	0.0080	0.0932; 0.1246		

Table 7A - Orthogonal contrast between diagnosis.

Contrast	Df	Chi-square	Pr> Chi-square
D1 vs. D2 + D3	1	13.93	0.0002**
D2 vs. D3	1	1.36	0.2428

Table 7B - Orthogonal contrast between ERα genotypes.

Contrast	Df	Chi-square	Pr> Chi-square
d vs.o	1	3.64	0.0565
O vs.d + o	1	0.69	0.4053
E vs. O + d + o	1	0.05	0.8189

difference). This result agrees with a previous study in early postmenopausal Finnish women, in which no association could be established between genotype and circulating estradiol level, bone mass, or changes of HRT (20).

Thus, we may speculate that environmental factors influence the effect of HRT on bone. In an *in vitro* study, we previously demonstrated that fibroblasts from patients with high number of TTTA repeats genotype produced a larger amount of estrogens in comparison with fibroblasts derived from patients with opposite genotype (18). It has been shown that osteoblasts, lining cells and adipocytes adjacent to bone trabecule express aromatase mRNA (19, 21) and an *in situ* production of estrogens has been suggested to be important for maintaining normal BMD (21).

It is likely that women with genotype 3 may be able to synthesize higher amount of local bone estradiol that has to be added to the estrogens amount present in the HRT producing a major effect on the bone turnover with a magnitude of bone gain in response to HRT.

The major amount of local bone estradiol in subjects with genotype 3 could explain the higher LS-BMD at the end of the first year of HRT without significant *difference* between the three genotypes.

In addition, due to the increasing evidence of a common lack of replication of results obtained in small studies, the delineation and establishment of common genetic risk factors for complex disorders, such as osteoporosis, require large-scale investigations to clarify clinically important genetic effects (22). Our population was relatively small and an evaluation of a larger number of women for a longer time may modify this results.

Looking at the effect of *ESR1* gene polymorphisms in the present study, we observed that diagnosis at the baseline was the most important factor influencing the modification of LS-BMD at the end of one year of HRT. These data were observed both applying ANOVA and GLMs analysis and were independent from *ERS1* gene polymorphism.

Our findings are in agreement with the results obtained in a GENOMOS study (12). In this large study on an European population (GENOMOS), XbaI *ESR1* gene polymorphism was correlated with an increase of vertebral fractures risk independently from BMD.

It is likely that ERα could play a role in the quality of bone more than in the bone density since *ESR1* alleles have been associated with ultrasound properties of bone and bone loss (23). However, *ER*α polymorphism may have a role influencing the *difference* variable and, in particular, patients with genotype d had a mean value of *difference* lower in comparison with genotype o and such a difference was bigger in osteopenic and osteoporotic women. Genotype o (xx) has been previously associated with higher risk of fractures (12). It is likely that these subjects would have an higher bone turnover with a baseline osteopenic and osteoporotic LS-BMD which is followed by a major response to HRT.

For both *CYP19* and *ESR1* gene polymorphisms we found a statistical significant association with weight. However, the result was not confirmed when we considered the BMI. The latter result is not coherent with the results obtained by the weight variable. Such an incoherence may be explained considering that, based on real data, we have low values of the BMI variable related to high values of height and weight. Another possibility to explain this inconsistent result may consists of the low number of samples analyzed.

In conclusion, we have demonstrated that *CYP19* gene polymorphisms are able to influence the effect of 1 year of HRT on the LS-BMD without influencing the differences between pre- and post- HRT LS-BMD.

Per converse, *ESR1* gene polymorphisms seem do not influence the LS-BMD after 1 year of HRT, while they may influence the modifications during the year of therapy.

These data underling the complexity either of the genetics of bone mass or the response to the HRT.

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