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GENOTYPES OF VITAMIN D AND ESTROGEN RECEPTORS IN PRE AND PERIMENOPAUSAL WOMEN FROM CORDOBA, ARGENTINA

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Abstract The aim of this study was to determine the frequency of vitamin D receptor and estrogen receptor genotypes and their relationship with the lumbar spine or femoral neck bone mineral density in healthy pre and perimenopausal women from Córdoba (Argentina) and adjacent areas. Genotypes were assessed by restriction fragment length polymorphism-polymerase chain reaction technique. Bsm I and Fok I for vitamin D receptor gene and XbaI and PvuII for estrogen receptor gene were used as restrictases. Two hundred and ten healthy pre and perimenopausal women were recruited and analyzed by age. Calcemia and serum parathyroid hormone did not change, but serum P and β-CrossLaps decreased with age. Femoral neck bone mineral density decreased significantly after 30 years old. Vitamin D receptor and estrogen receptor genotype frequencies were similar to those from other Caucasian women. No association between vitamin D receptor and estrogen receptor genotypes with the lumbar spine or femoral neck bone mineral density has been detected. Analysis of interaction between vitamin D receptor and estrogen receptor genes using covariates such as age, height and body mass index did not show any influence of the combination of those genotypes on bone mineral density. Lifestyle, smoking and alcohol intake had no effect on lumbar spine and femoral neck bone mineral density. To conclude, these data do not support the hypothesis that vitamin D receptor and estrogen receptor genotypes influence on lumbar spine and femoral neck bone mineral density in healthy pre and perimenopausal women from this area of Argentina.

Key words: VDR, ER, bone mineral density, pre and perimenopausal women

Resumen ***Genotipos de los receptores de vitamina D y de estrógeno en mujeres pre y perimenopáusicas de Córdoba, Argentina***. El propósito del estudio fue determinar la frecuencia de los genotipos de los receptores de vitamina D y de estrógeno y su relación con la densidad mineral ósea en mujeres sanas pre y perimenopáusicas de la ciudad de Córdoba y alrededores. Los genotipos se determinaron con la técnica de reacción en cadena de la polimerasa y análisis de los polimorfismos de longitud de fragmentos de restricción. Se usaron como restrictasas Bsm I y Fok I para el gen del receptor de vitamina D y Pvu II y Xba I para el gen del receptor de estrógeno. Se reclutaron y agruparon por edad doscientos diez mujeres pre y perimenopáusicas. Sus niveles séricos de Ca y de hormona paratiroidea fueron similares, pero los de fósforo y β-Cross Laps disminuyeron con la edad. La densidad mineral ósea de cuello femoral disminuyó después de los 30 años. Las frecuencias genotípicas de ambos receptores fueron similares a aquéllas de otras mujeres caucásicas. No hubo asociación entre los genotipos de los receptores y la densidad mineral ósea. Los análisis de interacción entre ambos genes no evidenciaron influencia sobre la densidad mineral ósea, utilizándose edad, talla e índice de masa corporal como covariables. Los estilos de vida y hábitos de fumar y beber alcohol tampoco afectaron la densidad mineral ósea. En conclusión, estos datos no sostienen la hipótesis de que los genotipos de los receptores de vitamina D y de estrógeno influencian la densidad mineral ósea de columna lumbar y cuello femoral en mujeres sanas pre y perimenopáusicas de esta región de Argentina.

Palabras clave: VDR, ER, densidad mineral ósea, mujeres pre y perimenopáusicas

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Low peak bone mass achieved in early adulthood and bone loss after menopause, are major determinants in women for development of osteoporosis later in life[1]. Both determinants have been suggested to be under strong genetic influence. Only on rare occasions osteoporosis occurs as the result of mutations in a single gene such as in *osteogenesis imperfecta* and in other osteoporosis associated with inactivating mutations in the aromatase and estrogen receptor α genes. Even in such extreme cases, polygenic effects have been identified on disease severity. Furthermore, many approaches have looked for evidences of an association between phenotypic characteristic of osteoporosis and a series of polymorphic genetic markers. The phenotypes that have been mostly studied are a continuous variable such as bone mineral density (BMD) or a categorical variable such as fracture[2]. Morrison et al.[3] were the first to reveal that vitamin D receptor (VDR) gene could be one of the genetic determinants of BMD. Several studies have demonstrated that pre and postmenopausal women with genotype BB have lower BMD than women with genotype bb and are prone to accelerated bone loss after the menopause[4, 5, 6]. However, in some cases the correlation between VDR polymorphisms and BMD has been in the opposite direction to that originally demonstrated[7]. Other authors have shown that the effect is strongest in premenopausal women, declines with age and has no effect by the age of 70[8, 9]. VDR gene polymorphism has also been related to a higher prevalence in vertebral fractures, but probably acting independently of BMD[10]. Recently, it has been shown VDR gene polymorphism effects on pharmacological response to anti-osteoporotic treatment[11, 12].

The estrogen receptor alpha (ERα) gene is also a strong candidate because of the well-established relationship between estrogen status and growth, development, maturation and maintenance of the skeleton, not only in women but also in men[13]. ERα is present in osteoblasts, osteoclasts, chondrocytes and it is well known the beneficial effects of hormone replacement therapy on bone mass in postmenopausal women[14]. Associations between VDR and ERα genotypes and BMD have been extensively studied in different populations of USA, Europe, Asia and in a few Latin American countries. Since there is no information on VDR and ERα genotypes in relation to BMD in the Argentine population, this study was performed in order to elucidate the influence of VDR and ERα polymorphisms on bone mass of healthy pre and perimenopausal women of Córdoba and adjacent areas, located in the central part of Argentina. Because of gene-by-gene interaction has potential effects on the BMD[14], the possible interaction between the ERα and VDR genes on BMD was also studied. In addition, the influence of lifestyle, smoking and alcohol intake, were investigated in relation to lumbar spine and femoral neck BMD.

Materials and Methods

Recruitment was achieved through voluntary response to advertisement for a study on genetic markers affecting bone health in pre and perimenopausal women. The consideration of pre or perimenopausal women was according to the criteria of Willing et al.[15]. Women were excluded when had hepatic or renal diseases, malabsorption, hyperparathyroidism, malignity in the last 5 years and use in the last 3 months of estrogen, glucocorticoids or other drugs known to influence on calcium metabolism. The Committee of Bioethics from the School of Medicine of the Universidad Nacional de Córdoba approved the protocol and the informed consent. Each subject signed informed consent before entering the project. Two hundred and ten healthy pre and perimenopausal women aged 20-55 years old from the city of Córdoba and adjacent areas were recruited. They were divided into three groups: A) 20-30 years old (young population that was finishing the acquisition of bone mass), B) 31-40 years old (population with equilibrium between mineralization and bone resorption), and C) 41-55 years old (late premenopausal and perimenopausal women).

Questionnaires: Calcium intake was determined by a food-frequency questionnaire[16]. Calcium content of foods was calculated as described by Zeni et al.[17]. Tobacco addiction was evaluated by using a short test[18]. Alcohol consumption was evaluated by the AUDIT Test[19]. Physical activity was determined by Baecke questionnaire[20].

Bone mineral density: The BMD of the lumbar spine (L2-L4) and the proximal femur (femoral neck) were measured by trained personnel using dual-energy X-ray absorptiometry (DXA) (Norland XR36 Quick Scan; Fort Madison, Wisconsin, USA). The coefficients of intra-assay variation were 1.43% for the lumbar spine and 1.39% for the femoral neck. The long-term stability of the equipment was checked by daily scans of an anthropomorphic spine phantom and the coefficient of variance, CV%, was less than 0.48% during the study period.

Biochemical determinations: Plasma calcium and phosphorus were measured using standard methods. The assay for intact PTH was obtained from Nichols Institute Diagnostics (San Juan de Capistrano, CA, USA). Serum β-CrossLaps was measured by an electrochemiluminiscence immunoassay (Roche Diagnostics Corporation, Indianapolis, IN, USA).

Genotyping: Genomic DNA was extracted from whole blood using a standard red cell lysis and proteinase K digestion technique. The primers used for the polymerase chain reaction (PCR) to amplify VDR gene fragments (Bsm I site) were: forward 5´-CAACCA AGACTACAAGTACCG-CGTCAGTGA-3´ and reverse 5´AACCAGCGGGAAGAGGT CAAGGG-3´, and for the VDR (Fok I site) were: forward 5´AGCTGGCCCTGG CACTGACTCTGCTCT 3´ and reverse 5´ATGGAAACACCTTG-C TTCTTCTCCCTC 3´. To amplify Erα gene fragments (Pvu II and Xba I sites) were: forward 5´-CTGCCAC CCTATCTGTA-TCTTTTCCTATTCTCC-3´ and re-verse 5´- TCTTTCTCT-GCCACCCTGGCGTCGATTATCTGA-3´ (CyberSyn Inc., Lenni, Pensilvania, USA). PCR amplifications were carried out in a Hybaid thermal cycler (Omnigene, Hampton Hill, Middlesex, U.K.) in 50 µL of buffer solution composed of 25 mmol/l Tris-HCl pH 8.3, 25 mmol/l KCl, 2.5 mmol/l $MgCl_2$ and 100 µg/l gelatin (Perkin Elmer, Inc., Roche, Massachusetts, USA), 0.8 mmol/l of deoxyribonucleotides (Promega, Madison, WI, USA), 0.4 µmol/l each of oligonucleotide primer, 2.5 U of Amplitaq (Perkin Elmer, Inc., Roche, Massachusetts, USA) and water. Thermal profiles for amplification of VDR gene fragments consisted of an initial denaturation step at 94 °C for 60 sec followed by 40 cycles of 60 sec at 93.5 °C (denaturation), 60 sec at 56 °C (annealing) and 60 sec at 72 °C (extension), with a final extension step of 2 min at 72 °C. The cycling conditions

for amplification of ERα gene fragments consisted of similar initial denaturation and final extension steps, but with 30 cycles of 30 sec at 94 °C (denaturation), 40 sec at 61 °C (annealing), and 90 sec at 72 °C (extension). Genotypes for BsmI polymorphisms were termed BB, Bb and bb and for Fok I were FF, Ff, ff, while PvuII and XbaI were termed PP, Pp and pp and XX, Xx and xx, respectively. Uppercase letters represent absence, and lowercase letters represent presence of restriction sites.

Statistical analysis: All the analysis were carried out by using SPSS software version 9.0 (SPSS, Inc., Chicago, IL, USA). Genotype frequencies for the four loci were tested against Hardy-Weinberg ratios by the χ^2 test . Concordance between Bsm I and Fok I sites for VDR and XbaI and Pvu II sites for ER was tested by Kappa statistics. Tests for association between genotypes at each genetic locus and BMD values were performed by using one way ANOVA followed by the Tukey *post hoc* Test. The general linear model (GLM)-ANOVA procedure was used to study gene-by-gene interactions by including genotypes from each studied polymorphic site in the analysis along with relevant covariates such as age, height and body mass index (BMI). Differences were considered statistically significant at $p < 0.05$.

Results

Frequencies of VDR and ERα genotypes in the total population are shown in Table 1. The distribution of genotypes agreed with that expected according to the Hardy Weinberg equilibrium. The allele b frequency of the VDR gene was 0.58 and that of allele f was 0.31, while the frequencies of allele x and p from the ERα gene were 0.65 and 0.63, respectively. There was a strong concordance between the polymorphic sites Xba I and PvuII of ERα genotypes ($p < 0.001$), showing concordance xx/pp, XX/PP and Xx/Pp in 157 from 202 women (Kappa = 0.59). On the contrary, Bsm I and Fok I genotypes of VDR did not show concordance (Kappa = 0.006, p= 0.902).

Clinical characteristics, biochemical parameters related to Ca and P metabolism and densitometric data of lumbar spine and femoral neck from the population are summarized in Table 2. As expected, all the studied variables were within the normal range, but there were some significant differences with age. Height was identical in the three groups of women, while weight and BMI increased with age. Serum Ca was similar in the three groups. Serum P was highest in the group of 20-30 years old. Serum PTH did not change with age, but showed a tendency to increase in the two older groups. Values of serum β-Cross Laps, a marker of bone resorption, were much lower in women older than 30 years old. Lumbar spine BMD was very similar in the three groups, while

TABLE 1.– *Frequency of VDR and ERα genotypes in pre and peri-menopausal women from Córdoba (Argentina) and adjacent areas*

Gene	Genotypes		
VDR (Bsm I)	BB	Bb	bb
	15.16% (32)	53.08 % (112)	31.75% (67)
VDR (Fok I)	FF	Ff	ff
	44.74% (84)	48.42% (92)	6.84% (13)
ER (Xba I)	XX	Xx	xx
	12.21% (26)	46.00% (98)	41.78% (89)
ER (Pvu II)	PP	Pp	pp
	11.32% (24)	51.89% (110)	36.79% (78)

The frequency of genotypes is expressed as percentage of the total population. Bsm I, Fok I, Xba I and Pvu II were employed as restriction enzymes. ( ) = number of cases.

TABLE 2.– *Clinical characteristics and parameters related to calcium and phosphorus metabolism in pre and peri-menopausal women from the city of Córdoba (Argentina) and adjacent areas*

Variables	20-30 years (n=70)	31-40 years (n=69)	41-55 years (n=72)
Height (m)	1.630 ± 0.008	1.623 ± 0.007	1.621 ± 0.009
Weight (kg)	57.61 ± 1.12	60.58 ± 1.32	65.08 ± 1.23**†
BMI (kg/m^2)	21.75 ± 0.47	23.05 ± 0.49	24.79 ± 0.49**†
Serum Ca (mg/dl)	9.25 ± 0.08	9.41 ± 0.12	9.44 ± 0.11
Serum P (mg/dl)	3.94 ± 0.08	3.47 ± 0.09**	3.61 ± 0.10 *
Serum PTH (pg/ml)	37.87 ± 2.22	43.42 ± 2.47	42.65 ± 2.34
β-CrossLaps (ng/ml)	0.42 ± 0.05	0.26 ± 0.02**	0.25 ± 0.02**
Lumbar spine BMD (g/cm^2)	1.079 ± 0.015	1.078 ± 0.018	1.102 ± 0.017
Femoral neck BMD (g/cm^2)	0.963 ± 0.014	0.909 ± 0.014*	0.897 ± 0.014*

*Values are the means ± S.E. ( ) = number of cases. * $p < 0.05$ vs women of 20-30 years old. ** $p < 0.001$ vs women of 20-30 years old. † $p < 0.05$ vs women of 31-40 years*

the femoral neck BMD was significantly lower in women older than 30 years old.

Table 3 shows the relationship between VDR and ERα genotypes with femoral neck and lumbar spine BMD in the three groups of women. Data indicate that femoral neck BMD did not vary with VDR or ERα genotypes in any age group. Similarly, no relationship between VDR and ERα genotypes and lumbar spine BMD was found in the three groups of women.

Regarding lifestyle, smoking and alcohol intake, 43 % of the women of 20-30 years old were light smokers (less than 5 cigarettes/day) and the rest were non smokers; 69% used to drink one or two glasses of wine or beer per week while the others did not drink alcohol, and all of them had low or moderate physical activity (2-5 hours of gymnasia, walking or swimming/week). In the two older groups, 25-30% were light smokers, 75% used to drink one or two glasses of wine or beer per week and they also had low or moderate physical activity. Calcium intake was 557.14 ± 34.88 mg/day (mean ± SE) in women of 20-30 years old, 637.72 ± 40.58 mg/day in women of 31-40 years old and 570.71 ± 34.15 mg/day in women of 41-55 years old. There were no differences between groups (one way ANOVA and Tukey Test). As can be noted, calcium intake was low in all the groups, except in two individuals that were not included in the statistical analysis. Tobacco smoking, alcohol intake and physical activities were very similar in the different groups. As shown in Table 4, femoral neck and lumbar spine BMD were not different because of light tobacco smoking, low alcohol intake or low physical activity.

Finally, the effect of individual variables (VDR genotype Bsm I site, VDR genotype Fok I site, ERα genotype Pvu II site, ERα genotype Xba I site) or interactions between them on femoral neck or lumbar spine BMD was analysed by the general linear model (GLM)-ANOVA procedure. Age, height or BMI were included as covariables to eliminate them as confounding factors. The variability of lumbar spine BMD that could be probably attributable to independent factors such as genotypes and age is around 8% (partial coefficient Eta^2 of the corrected model), which is not significant ($p = 0.815$). The variability of femoral neck BMD that could be attributed to those independent factors is around 16% (partial coefficient Eta^2 of the corrected model) with a $p = 0.058$. Although this is not significant, the value indicates a tendency of the femoral neck BMD to be affected by those factors, mainly by the effect of age (partial $Eta^2 = 6.7$, $p < 0.001$), followed by far by the independent effect of the ERα genotype PvuII site ($p = 0.184$).

TABLE 3.– *Relationship between VDR and ERα genotypes with femoral neck and lumbar spine BMD in pre and perimenopausal women from the city of Córdoba (Argentina) and adjacent areas*

Genotypes	21-30 years		31-40 years		41-55 years	
	Femoral neck (n=70)	Lumbar spine (n=72)	Femoral neck (n=61)	Lumbar spine (n=61)	Femoral neck (n=69)	Lumbar spine (n=70)
VDR						
BB	0.931 ± 0.024	1.119 ± 0.037	0.882 ± 0.057	1.098 ± 0.099	0.906 ± 0.025	1.103 ± 0.032
Bb	0.990 ± 0.020	1.069 ± 0.021	0.939 ± 0.029	1.102 ± 0.020	0.915 ± 0.020	1.095 ± 0.028
bb	0.950 ± 0.030	1.057 ± 0.028	0.898 ± 0.029	1.045 ± 0.037	0.861 ± 0.025	1.081 ± 0.031
FF	0.963 ± 0.025	1.059 ± 0.026	0.916 ± 0.020	1.105 ± 0.026	0.914 ± 0.021	1.120 ± 0.026
Ff	0.955 ± 0.017	1.090 ± 0.019	0.908 ± 0.022	1.073 ± 0.024	0.859 ± 0.022	1.058 ± 0.028
ff	0.960 ± 0.058	1.028 ± 0.108	0.992 ± 0.141	0.978 ± 0.066	0.989 ± 0.097	1.102 ± 0.140
ER						
XX	0.905 ± 0.026	1.073 ± 0.029	0.988 ± 0.141	1.077 ± 0.082	0.928 ± 0.045	1.180 ± 0.060
Xx	0.977 ± 0.022	1.054 ± 0.021	0.940 ± 0.021	1.105 ± 0.022	0.911 ± 0.019	1.088 ± 0.028
xx	0.980 ± 0.025	1.091 ± 0.027	0.890 ± 0.022	1.062 ± 0.025	0.870 ± 0.023	1.097 ± 0.023
PP	0.946 ± 0.035	1.074 ± 0.038	0.925 ± 0.042	1.111 ± 0.061	0.949 ± 0.065	1.219 ± 0.075
Pp	0.988 ± 0.021	1.075 ± 0.021	0.951 ± 0.035	1.098 ± 0.022	0.908 ± 0.019	1.101 ± 0.027
pp	0.946 ± 0.026	1.067 ± 0.029	0.887 ± 0.026	1.052 ± 0.028	0.872 ± 0.021	1.081 ± 0.022

Values are the media ± SE. BMD values are expressed in g/cm². ( ) = number of cases. No differences were found in BMD value of each skeletal site in relation with the genotypes of the corresponding age groups

TABLE 4.– *Relationship between BMD and tobacco smoking, alcohol intake and physical activity in pre and peri-menopausal women from the city of Córdoba (Argentina) and adjacent areas*

	Lumbar spine BMD		Femoral neck BMD	
	n	M ± SE	n	M ± SE
Tobacco smoking				
Yes	80	1.0805 ± 0.0214	80	0.9014 ± 0.0163
No	128	1.0793 ± 0.0161	128	0.9046 ± 0.0120
Alcoholic intake				
Yes	155	1.0865 ± 0.0152	155	0.9118 ± 0.0105
No	57	1.0512 ± 0.0209	57	0.8639 ± 0.0205
Physical activity				
Low	119	1.0812 ± 0.0138	119	0.9059 ± 0.0110
Moderate	89	1.0795 ± 0.0126	89	0.9027 ± 0.0205

The values of BMD are in g/cm^2. ( ) = number of cases. M ± SE (media ± standard error). There were no significant differences between groups

Discussion

The present study was designed in order to assess the influence of VDR and ERα gene polymorphisms and lifestyle on BMD in healthy pre and perimenopausal women of Cordoba (Argentina) and adjacent areas. The population of this area is characterized by an important racial heterogeneity and, therefore, is considered an ethnically heterogeneous population. However, a strong contribution to the present population has emerged from Spanish and Italian people, who immigrated to Argentina mainly at the end of the nineteen hundred century and in the first half of the last century. African or Asian ancestry was found in none of the analysed women.

Regarding polymorphisms of VDR gene at Bsm I site, the frequency of allele b in our population has been found to be similar to that of Spanish women[21], which could be due, at least in part, to the Spanish immigration. This distribution of VDR Bsm I genotypes is quite similar to that of premenopausal women from Spain[22], France[23] and Brazil[24], the last one being also a very heterogeneous population. Similarly, the frequency of the minor allele, (f) of VDR gene polymorphisms at Fok I site is identical to that described for Spanish[21] and Caucasian population[25].

The high concordance between alleles P and X as well as alleles p and x found in our population indicates that the Pvu II and Xba I sites are strongly associated with each other. A linkage disequilibrium is expected since they are located separated by 50 base pairs in intron 1, although it is not complete, as previously suggested[26]. The frequency of ERα genotypes for both polymorphic sites is similar to that found by Willing et al.[15] in white premenopausal women from USA (XX 10%, Xx 48% and xx 42%; PP 18%, Pp 53% and pp 29%). Han et al[27] also found a similar distribution for the polymorphic site PvuII in pre, peri and postmenopausal women from Korea; however the genotype frequencies for RFLP XbaI were very different showing a high predominance of genotype xx (64.2%), followed in decreasing order by Xx (30.1%) and XX (5.7%). In Japan, the frequencies of ERα genotypes in healthy postmenopausal women were XX 3%, Xx 33% and xx 64% for the site XbaI while the distribution of ERα PvuII genotypes was PP 20%, Pp 51% and pp 29%[28]. As noticed, changes in the frequency of genotypes occur according to the ethnic groups. Van Meurs et al.[29] have also suggested that the degree of disequilibrium may vary among different ethnic groups which may explain, at least in part, the observed population specificity in the genotypes predicting low and high BMD.

In our population, the lumbar spine BMD is very similar in the three age groups, while femoral neck BMD is much lower in the thirties and later in life. This seems to be the natural history of bone loss at the femoral neck, whose onset has been observed to occur by the midtwenties[30]. However, the beginning age of bone loss in femoral neck in normal women is not always the same in different populations. Vega et al.[31] have found in normal women from Buenos Aires that the percentage fall of the BMD in femoral neck was 22% between the 3rd and 8th decades of age. Lofman et al.[32] did not observe significant changes in BMD in normal Swedish women from 20-49 years at any site of the skeleton, except a slight decline at Ward´s triangle.

In contrast to the association studies showing positive findings[1, 3, 6, 33], our study demonstrates lack of correlation between the BMD and VDR (Bsm I and Fok I) and ERα (Pvu II and Xba I) genotypes. This is an agreement with recent meta-analysis study which shows that there is no

association between Bsm I polymorphisms and lumbar spine and femoral neck BMD in premenopausal women[34]. The absence of significant association between the Xba I and/or Pvu II genotypes and BMD or bone accrual has been also observed in Italian[35], Belgian[36], and Australian population[37]. It is difficult to have a final picture looking at these contrasting findings. The reasons of these discrepancies could be differences in Ca intake, menopausal states (a rapid perimenopausal bone loss could hamper genetic effects not related to estrogen), race, age, ligament disequilibrium, allelic heterogeneity, etc. The existence of a significant gene-by-gene interaction effect between PvuII-Xba I RFLPs in ERa and VDR gene polymorphisms in the determination of BMD has not been observed in our population. Furthermore, we have not demonstrated gene-environment interactions. Gennari et al.[26] suggest that the influence of each single polymorphism on the total variation of bone-related traits is very limited so it is possible that very large samples are needed to demonstrate gene-environment interactions.

Dietary recommendation for calcium intake in pre and perimenopausal women is around 1 g/day[38]. However, calcium intake has shown to be very poor in pre and perimenopausal women of Córdoba (Argentina) and adjacent areas, independently of age. Similar findings have been previously observed in other regions of Argentina[17, 39]. The main cause of low Ca intake seems to be the dietary imbalance derived from alimentary habits common to all socioeconomic levels of the Argentine population[17]. This low Ca intake may partially contribute to the increasing number of osteoporotic fractures occurring in the later years of life in Argentine women. Nevertheless, association between low Ca diet and risk of osteoporotic fractures is controversial. Kalkwarf et al.[40] have shown that women with low milk intake during childhood and adolescence have less bone mass in adult life and greater risk of fracture. A recent meta-analysis of milk intake and fracture risk, on the contrary, indicates that low intake of Ca was not associated with a significant increased risk of fracture[41].

Although the variables related to calcium and phosphorus metabolism are within the normal range, it is noticeable the decrease in the β-CrossLaps values between the third and the fourth decade in the premenopausal women. The women in the twenties still are attaining peak bone mass and bone turnover must be high, consequently the bone marker of resorption is high, decreasing later when equilibrium between bone accretion and resorption is established. Serum P also declines with age, maybe as a result of a tendency to increase serum PTH.

Some habits of life such as tobacco smoking, alcohol intake and physical activities have not affected lumbar spine and femoral neck BMD in our population. However, it must be noticed that there were no heavy smokers or addicts to alcohol in the groups. Besides, all of them have only low or moderate physical activities. Our data are in agreement with those reported by Bernaards et al.[42] who did not find changes in BMD parameters in 36-year-old men and women because of current and lifetime smoking. However, they observed impaired bone quality by using measurements with ultrasound techniques.

In conclusion, VDR and ERα genotypes as the gene-by-gene interaction are not associated to the lumbar spine or femoral neck BMD in healthy pre and perimenopausal women from the city of Córdoba (Argentina) and adjacent areas.

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