

Jirong Long · Pengyuan Liu · Yuanyuan Zhang
Hui Shen · Yongjun Liu · Volodymyr Dvornyk
Hong-Wen Deng

Interaction effects between estrogen receptor α gene, vitamin D receptor gene, age, and sex on bone mineral density in Chinese

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Abstract We evaluated the interaction effects between the estrogen receptor α gene (ER- α), vitamin D receptor gene (VDR), age and sex on bone mineral density (BMD) in a sample of 340 unrelated males and 297 unrelated females from 401 Chinese nuclear families. Polymorphisms of *PvuII* and *XbaI* in the ER- α gene and *ApaI* in the VDR gene were detected by RFLP, and ER- α genotype was defined by the haplotype reconstructed according to the two loci. In the females, significant ER-VDR gene interaction ($P < 0.05$) was found on the lumbar spine BMD. Such interaction might account for approximately 1.0% of the BMD variation. At the femoral neck and trochanter, significant ER-age interaction effects were observed, which might explain 0.3% BMD variation for both skeletal sites. In the males, significant VDR-age interaction was found on femoral neck BMD ($P < 0.05$), and it accounted for 0.6% BMD variation. These interaction effects were largely dependent on gender groups, suggesting there may exist ER-VDR-sex, ER-age-sex, and VDR-age-sex complex interactions in our Chinese sample.

Keywords Interaction · ER- α gene · VDR gene · Bone mineral density (BMD) · Age · Sex

Introduction

Osteoporosis is among the major health problems throughout the world. It is characterized by low bone mineral density (BMD) and microarchitectural deterioration of bone structure (Kanis et al. 1994). BMD variation is under strong genetic determination with heritability of over 50% (Recker and Deng 2002). A number of candidate genes associated with BMD have been identified, such as estrogen receptor α (ER- α), vitamin D receptor (VDR), calcitonin receptor, collagen type 1 $\alpha 1$ (COL1A1), interleukin-6, transforming growth factor $\beta 1$, and others (Ralston 2002; Liu et al. 2003). Among them, polymorphisms of *PvuII* and *XbaI* sites in the ER- α gene (ER-*PvuII* and ER-*XbaI*), and *ApaI* site in the VDR gene (VDR-*ApaI*) have been the most extensively studied genetic markers in relation to BMD variation (Ralston 2002; Liu et al. 2003). However, the results so far have been largely inconsistent and controversial.

To some extent, the association between a candidate gene and BMD may depend on other factors, such as environment, gene-gene and gene-nongenetic factor interactions (Ralston 2002; Liu et al. 2003). Gene-gene interaction implies that genotypic effects of one locus may depend on the genotype of the other locus (or loci), while gene-nongenetic factor interaction means that only under particular conditions, such as specific age or sex, will the genotypes affect phenotypes. If such interaction effects exist and are not taken into account, they could confound, and even bias, the associations between the candidate genes and the phenotypes (Deng et al. 1998). Gene-gene interactions between the VDR, ER- α , and COL1A1 genes, as well as the interactions between genes and nongenetic factors, e.g., sex, age, race, calcium intake, vitamin D supplements, hormone replacement therapy (HRT), and exercise, were recently documented (Graafmans et al. 1997; Giguere et al. 2000; Efstathiadou et al. 2001; Dennison et al. 2001; Rapuri et al. 2001; Salmen et al. 2001; Tofteng et al. 2002). However, most

J. Long · H.-W. Deng
Laboratory of Molecular and Statistical Genetics,
College of Life Sciences, Hunan Normal University,
410081 Changsha, Hunan, P. R. China

J. Long · P. Liu · Y. Zhang · H. Shen · Y. Liu · V. Dvornyk ·
H.-W. Deng (✉)
Osteoporosis Research Center,
Creighton University Medical Center,
601 N. 30th St., Suite 6787, Omaha, NE 68131, USA
E-mail: deng@creighton.edu
Tel.: +1-402-280-5911
Fax: +1-402-280-5034

of these studies were performed in Caucasians, and scarce data are available about the interaction effects on BMD in Chinese (but see Zhao et al. 1997; Lau et al. 2001, 2002).

The present study was aimed to determine if there are interaction effects between the ER- α and VDR genes, as well as between these genes and age, on BMD variation in healthy Chinese males and females.

Materials and methods

Subjects

The subjects were selected from 401 nuclear families (each with both parents and at least one female child) used in our early study (Qin et al. 2003), which was approved by the Research Administration Department of Shanghai's Sixth People's Hospital and Hunan Normal University. All subjects signed informed-consent documents. The following data from the subjects were obtained: age, sex, medical history, and family history. Exclusion criteria were used to minimize any known potential confounding factors on the study phenotypes as detailed by Deng et al. (2002a). In brief, subjects with the following diseases were excluded: diabetes mellitus, cancer, exostosis, hepatopathy, high blood pressure, hyperthyroidism, kidney disease, parathyroid disease, rheumatoid arthritis, ovary resection, and tuberculosis. To get unrelated individual samples, only phenotypes from parents of each nuclear family were used in this study. Excluding those under 50 years old (because of the small sample size), and pre/perimenopausal women (to keep the subjects homogeneous), the phenotypes of 340 males and 297 postmenopausal females were analyzed.

Genotyping

Genotypes of all subjects in the 401 nuclear families were determined using PCR-RFLP reaction protocols as described by Lei et al. (2003). The genotypes were designated as *PP*, *Pp*, *pp* for ER-*PvuII*, *XX*, *Xx*, *xx* for ER-*XbaI*, and *AA*, *Aa*, *aa* for VDR-*ApaI*. Uppercase and lowercase letters denote the absence and presence of the restriction sites, respectively. PedCheck software (O'Connell and Week 1998) was used to verify Mendelian inheritance of the alleles within each family.

Table 1 Descriptive characteristics of the study subjects

Trait	Male (<i>n</i> = 340)	Female (<i>n</i> = 297)
Age (year)	62.91 ± 6.11	60.27 ± 5.78
Height (cm)	165.57 ± 10.80	154.36 ± 5.49
Weight (kg)	68.18 ± 10.33	59.21 ± 8.44
Lumbar spine (L1-L4) BMD (g/cm ²)	0.931 ± 0.153	0.799 ± 0.145
Total hip BMD (g/cm ²)	0.876 ± 0.124	0.737 ± 0.129
Femoral neck BMD (g/cm ²)	0.751 ± 0.116	0.664 ± 0.116
Trochanter BMD (g/cm ²)	0.649 ± 0.101	0.535 ± 0.102
Intertrochanteric region BMD (g/cm ²)	1.025 ± 0.149	0.869 ± 0.156
ER- α gene allele/haplotype frequencies (%)		
<i>P</i>	34.26	40.07
<i>p</i>	65.74	59.93
<i>X</i>	22.21*	26.26*
<i>x</i>	77.79	73.74
<i>PX</i>	15.15	18.01
<i>pX</i>	58.68	51.68
<i>Px</i>	18.97	21.89
<i>pX</i>	7.20	8.42
VDR gene allele frequencies (%)		
<i>A</i>	30.15	27.61
<i>a</i>	69.85	72.39

Values shown are mean ± standard deviation; values are raw data
 * χ^2 -test for Hardy-Weinberg equilibrium, $P < 0.05$ at ER-*XbaI* both in the males and the females, $P > 0.1$ at ER-*PvuII* and VDR-*ApaI* both in the males and the females

Measurement

A Hologic QDR 2000+ dual-energy X-ray absorptiometry (DXA) scanner (Hologic Corporation, Waltham, MA, USA) was used to measure BMD of lumbar spine (L1-L4) and total hip (including femoral neck, trochanter and intertrochanteric regions), and reports BMD in grams per centimeter squared. The machine was calibrated daily. The coefficient of variability (CV) of the accuracy of DXA measurement at the spine, total hip, femoral neck, trochanter, and intertrochanteric region was 0.9%, 0.8%, 1.93%, 1.48%, and 1.31%, respectively. Weight and height were measured at the same time of BMD measurement.

Statistical analyses

The χ^2 test was performed to examine Hardy-Weinberg equilibrium (HWE) at the three marker loci. ER- α haplotypes were reconstructed according to all subjects from the nuclear families using the program SimWalk2 (Sobel and Lange 1996). Multiple linear regression analyses were performed to examine the interaction effects on BMD variation in males and females. The regression variables were age, height, weight, ER- α (defined by the haplotype), VDR, ER-age interaction, VDR-age interaction, and ER-VDR interaction. The frequency of genotype *AA* for VDR gene was low: 7.35% in males and 7.74% in females. If the subjects were divided to three groups, *aa*, *Aa*, and *AA*, the sample sizes for some groups were small, and it was not appropriate to analyze the interaction effects. Then genotypes *Aa* and *AA* were combined into one group and coded as "1", another group, *aa*, was coded as "2". To analyze the effect of a special ER- α haplotype, the subjects with this haplotype were denoted as "1"; those without it were denoted as "2". In males, there were 97, 276, 118, and 49 subjects denoted as "1" for the haplotypes *PX*, *pX*, *Px*, and *pX*, respectively, while in females, there were 95, 220, 116, and 50 individuals denoted as "1", respectively. The interaction terms of ER-age, VDR-age, and ER-VDR were the products of ER- α and age, VDR and age, VDR and ER- α . These statistical analyses were performed using SAS version 8.0 (SAS Institute, Cary, NC, USA).

Results

Descriptive characteristics of the subjects are summarized in Table 1. The male subjects in the study were

aged 62.91 ± 6.11 (mean \pm SD) years and ranged from 50.25 to 78.83 years. The female subjects were averaged 60.27 ± 5.78 years, with the range from 50.00 to 76.67 years. The frequencies of the alleles and/or haplotypes are also summarized in Table 1. The χ^2 tests showed that the ER-*PvuII* and VDR-*ApaI* polymorphisms were in HWE ($P > 0.1$) both in males and females, whereas ER-*XbaI* was not ($P < 0.05$).

The results of multiple linear regression analyses in the females are shown in Table 2. We observed a significant ER-VDR interaction effect on lumbar spine BMD ($P < 0.05$). Only when the VDR genotype was *aa* was the difference statistically significant for the lumbar spine BMD between the subjects with haplotype *pX* and without it, (0.840 ± 0.143 versus 0.779 ± 0.139 g/cm², $P < 0.05$). Significant gene-age interaction effects were found for ER- α *pX* haplotype on both femoral neck BMD ($P < 0.05$) and trochanter BMD ($P < 0.05$). No such effect was observed either for any other ER- α haplotypes (data not shown) or the VDR gene at any skeletal site studied (Table 2).

The results of regression analyses in the males were quite different from those in the females (Table 3). The only significant interaction effect in the males was observed between VDR and age on femoral neck BMD ($P < 0.05$). No evidence was obtained for the ER-age and ER-VDR effects on the BMD variations at any skeletal site (data not shown).

As expected, when the interaction terms were removed from the multiple regression model, the percentage of variation explained by the model, as reflected by the adjusted R², was decreased (Table 2). The differences in the R² values of the multiple regression analyses with and without the interaction terms approximately equaled to the percentage of BMD variation, which may be attributed to the significant interaction effects. In the females, about 1.0% spine BMD variation may be due to the ER-VDR interaction, while the ER-age interaction can account for about 0.3%

femoral neck BMD and 0.3% trochanter BMD variation (Table 2). In the males, 0.6% BMD variation at the femoral neck was due to the VDR-age interaction. Another finding of this study is, in the females, when the interaction factors were incorporated in the regression model, BMD variations were significantly affected by ER- α genotypes at the femoral neck and trochanter (Table 2). When the interaction factors were removed from the regression model, the ER- α effect appeared to be nonsignificant.

Discussion

In the recent past, extensive molecular genetic studies have been launched to identify genes underlying BMD variations (Liu et al. 2003). However, only a few studies analyzed the interaction effects of gene-gene and gene-nongenetic factors. Such interactions may vary in different populations (Dennison et al. 2001) and may influence the association between the candidate genes and BMD variation. For example, Willing et al. (1998) found that ER-*PvuII* and ER-*XbaI* interacted with VDR-*BsmI* to have an effect on BMD variations at the femoral neck, lumbar spine, and total body in Caucasian females. In Italian postmenopausal women, subjects with genotype *AABBtt-PPXX* had significant lower spine BMD than those with *aabbTT-ppxx* genotype (Gennari et al. 1998). In Korean women, effects of ER- α genotype on BMD at the Ward's triangle were observed only when combined with the VDR *FokI* genotype FF (Kim et al. 2001). Such VDR-ER interaction effects, between VDR-*ApaI* with ER-*PvuII* and ER-*XbaI* were also observed on BMD changes in both the placebo and HRT groups in postmenopausal Caucasian women (Deng et al. 1998). The results of all these studies are in general agreement with the findings of the present study. The mechanism of ER-VDR interaction on BMD variation is still unclear; however, it is consistent with the

Table 2 Results of multiple regression analyses in the females

Trait (BMD)	Intercept	ER- α [†]	VDR	Age	Height	Weight	ER-age	VDR-age	ER-VDR	Adj. R ^{2§}
With interaction factors										
Lumbar spine	0.414	0.192	-0.171	-0.005**	-0.001	0.008**	-0.004	0.003	0.085*	0.288
Total hip	0.652**	-0.201	-0.080	-0.007**	-0.001	0.008**	0.003	-0.002	0.022	0.395
Femoral neck	0.508**	-0.299*	0.046	-0.006**	-0.001	0.006**	0.005*	-0.001	0.001	0.365
Trochanter	0.469*	-0.268*	0.062	-0.005**	-0.001	0.006**	0.004*	-0.001	0.017	0.352
Intertrochanteric region	0.742**	-0.163	0.093	-0.008**	0.001	0.009**	0.003	-0.002	0.023	0.366
Without interaction factors										
Lumbar spine	0.322	0.004	0.002	-0.004**	0.001	0.008**				0.278
Total hip	0.704**	0.018	-0.012	-0.007**	-0.001	0.008**				0.394
Femoral neck	0.532	0.012	-0.013	-0.006**	-0.001	0.006**				0.362
Trochanter	0.505**	-0.008	-0.012	-0.005**	-0.001	0.006**				0.349
Intertrochanteric region	0.802**	0.024	-0.009	-0.008**	0.001	0.009**				0.365

[†] The results are given only for haplotype *pX*. The other haplotypes yielded no significant results (data not shown)

[§] Adj.R² is the proportion of variation of the dependent variable explained by the regression model. Other numbers in the table are the partial regression coefficients for the parameters of each factor

* $P < 0.05$

** $P < 0.01$

Table 3 Results of multiple regression analyses in the males

Trait (BMD) With interaction factors	Intercept	ER- α [†]	VDR	Age	Height	Weight	ER-age	VDR-age	ER-VDR	Adj.R ^{2§}
Lumbar spine	0.667**	0.282	-0.012	0.001	-0.001	0.006**	-0.005	0.001	-0.004	0.139
Total hip	1.132**	0.047	-0.140	-0.005**	-0.002**	0.007**	-0.001	0.002	0.011	0.295
Femoral neck	0.951**	0.094	-0.216	0.005**	-0.002**	0.006**	-0.002	0.003*	0.011	0.253
Trochanter	0.806**	0.043	-0.127	-0.003**	-0.002**	0.005**	-0.001	0.002	0.011	0.217
Intertrochanteric region	1.349**	-0.045	-0.147	-0.006**	-0.003**	0.008	0.001	0.002	0.012	0.282

[†] The results are given only for haplotype *pX*. The other haplotypes yielded no significant results (data not shown)

[§] Adj.R² is the proportion of variation of the dependent variable explained by the regression model. Other numbers in the table are the partial regression coefficients for the parameters of each factor

**P* < 0.05

***P* < 0.01

evidence that estrogens modulate the activity of 1,25-dihydroxyvitamin D receptors in osteoblast-like cells (Liel et al. 1992). In addition, vitamin D and its reception may influence the balance between androgens and estrogens in peripheral tissues, which in turn modulates the availability of steroid hormones for their receptors (Willing et al. 1998).

On the other hand, no VDR-ER interaction effects were found in Greek postmenopausal women (Efsthadiadou et al. 2001) and an elderly white women population (Brown et al. 2001). The inconsistent results of the different studies may be due to interstudy differences in ethnicity, sample size, age, population structure and employed statistical methods. For example, if the sample size is insufficiently large, the power to detect the interaction effects will be very low, especially for genetic markers with low allele frequencies. On the other hand, the effects of the main genotypes and/or gene-gene interaction may be masked and not detected in a case, when actually existing significant nongenetic effects or gene-nongenetic interaction effects are not incorporated into the analyses. Population admixture/stratification may lead to deviation of genotype frequencies from HWE (Schaid and Jacobsen 1999). Such deviations from HWE have been observed in some studies of the ER- α polymorphisms (e.g., Ho et al. 2000; Kim et al. 2001) and in the present study as well. Our samples were recruited from a local population of Shanghai City, a metropolitan area and home to tens of millions of people from different geographical areas. It is possible that there exists heterogeneity at ER-*XbaI* locus but not at the other two loci examined in the present study.

In addition to gene-gene interactions, gene-nongenetic factor interactions may also affect BMD variation. In the present study, we found significant ER-age effects on trochanter BMD and femoral neck BMD in the females, and VDR-age interaction on femoral neck BMD in the males. These gene-age interactions suggest that the dynamics of BMD change with aging may not be the same in groups with different ER- α genotype (with or without haplotype *pX*), or VDR genotype (*aa* or other two genotypes). Similar ER-age interaction effects on BMD variation have also been reported in other studies. Chinese women of 70–79 years old with the XX genotype had 80% of a standard deviation (SD) higher spine

BMD than those of other genotypes, while such difference was only 40% of an SD in younger women aged 55–59 years (Lau et al. 2001). In Japanese women, the effects of ER- α genotypes on BMD variation were observed in those aged 60 or over but not in those under 60 years (Yamada et al. 2002). VDR-age interactions were also observed in other studies. The VDR-*FokI* polymorphism was associated with BMD only in elderly Chinese women (70–79 years), but not in elderly men or younger women aged 50–59 years (Lau et al. 2002). VDR-*ApaI* and VDR-*BsmI* were associated with BMD only in the young Chinese women but not in the postmenopausal women (Zhao et al. 1997). In Caucasians, two studies indicated that the correlation of the VDR gene polymorphism with BMD tended to disappear with age and might exist only in the premenopausal (Riggs et al. 1995) or even the prepubertal period (Ferrari et al. 1998).

It is worth mentioning that in this study, the gene-age, gene-gene interaction effects were different in the males and the females as outlined before, which implied that such interactions were influenced by sex, i.e., there may exist complex interactions such as VDR-age-sex, ER-age-sex, and ER-VDR-sex. The gene-age-sex interaction has been observed in our previous study (Deng et al. 2002b) by performing a complex segregation analysis in proband ascertained pedigrees from Caucasians. Although the exact mechanism underlying the sex- and age-genotype interactions remains to be elucidated, one possible explanation may be lower expression of genes at certain ages and/or differences in gene action in the different genders (Karasik et al. 2000; Deng et al. 2002b; Livshits et al. 2002). Interstudy differences in gender, age, ethnicity, sample size, population structure, and employed statistical methods may also be the reasons for the inconsistent results on the gene-nongenetic factor interaction effects on BMD variation.

When the interactions are significant but not incorporated in the analyses, the main effects of the genotypes may be clouded and thus not detected. This is demonstrated for the association between ER- α and BMD variations at the femoral neck and the trochanter (see Results). Therefore, several significant interaction factors were incorporated into the analyses and thus are expected to yield more convincing results. Further

studies in other homogeneous populations with larger sample sizes are needed to confirm the results found here.

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