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PRELIMINARY REPORT

Vitamin D Receptor and Aromatase Gene Interaction and Bone Mass in Older African-American Women

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Aromatization of androgens by the *CYP19* gene product, aromatase, is the major source of endogenous estrogen in postmenopausal women. We determined whether an Arg^{264}Cys polymorphism in the *CYP19* gene is associated with bone mineral density (BMD) and bone loss in older women. Because vitamin D regulates *CYP19* gene expression, we also tested for an interaction with a translation start site polymorphism in the vitamin D receptor (*VDR*) gene. Hip BMD was measured twice, an average of 1.9 years apart, in 100 African-American women aged ≥65 years. Neither polymorphism alone was significantly associated with BMD or bone loss. BMD measurements in women with the less frequent allele at both loci were 0.5 to 1.3 SD lower than in women with neither or only a single rare allele ($P < .001$ for interaction). These women also experienced more rapid hip bone loss than other women ($P < .05$ for interaction). We conclude that *VDR* and *CYP19* gene polymorphisms may jointly influence bone mass and the rate of bone loss in older African-American women.

AROMATIZATION of androgens by the *CYP19* gene product, aromatase, is the major source of endogenous estrogen in postmenopausal women and estrogen deficiency plays a central role in postmenopausal osteoporosis.[1] Aromatase deficiency due to rare nonsense mutations in *CYP19* causes severe osteoporosis[2] and a tetranucleotide repeat polymorphism in *CYP19* has been associated with bone mass.[3] In the present study, we determined whether a common Arg^{264}Cys polymorphism in aromatase is associated with bone mineral density (BMD) and bone loss among older women. Because the vitamin D receptor (VDR) regulates *CYP19* gene expression,[4] we also evaluated the interaction between a *VDR* translation start site polymorphism[5] and the aromatase polymorphism.

MATERIALS AND METHODS

African-American women were recruited by mailings to population-based lists.[5] Women who were aged ≥65 years, able to walk unassisted, community living, and who had not undergone a bilateral hip replacement were eligible. Of the 156 participants, 100 provided blood for DNA extraction. The characteristics of women with and without DNA were similar.[5]

DNA was extracted from leukocytes and the *CYP19* Arg^{264}Cys[6] and *VDR* translation start site (FokI)[5] polymorphisms genotyped as described. Hip BMD was measured twice, 1.9 years apart (range, 0.8 to 2.5 years), on a Hologic QDR-2000 densitometer (Hologic, Inc, Bedford, MA). Women completed standardized anthropometric assessments and a questionnaire about lifestyle habits, selected medications, and dietary calcium.[5]

Hardy-Weinberg equilibrium was tested by a χ^2 statistic. Few women were homozygous for the minor *VDR* and *CYP19* alleles. Thus, statistical comparisons were made between the *VDR* FF and Ff/ff genotypes and between the CYP19 Arg^{264}Arg and Arg^{264}Cys/Cys^{264}Cys genotypes using independent *t* tests or analysis of covariance (ANCOVA). We also tested for an interaction between the *VDR* and *CYP19* polymorphisms using 2-way ANCOVA to adjust for covariates. We included terms for *VDR* genotype (FF, Ff/ff), *CYP19* genotype (Arg^{264}Arg, Arg^{264}Cys/Cys^{264}Cys), and their interaction in the models.

RESULTS

Genotype distributions did not deviate from Hardy-Weinberg expectations (*CYP19*: Arg^{264}Arg, 72.0%; Arg^{264}Cys, 26.0%; Cys^{264}Cys, 2.0%; VDR: FF, 61.2%; Ff, 32.7%, ff,

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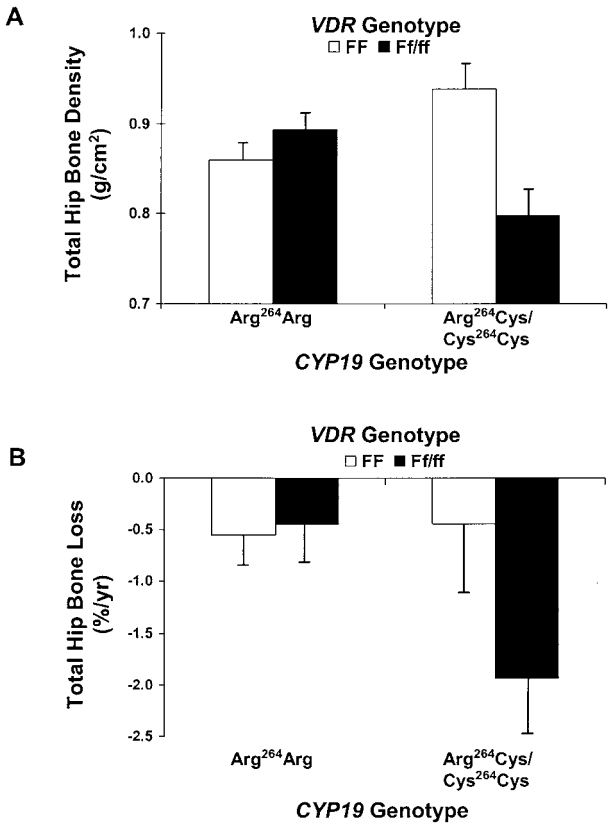

Fig 1. (A) Total hip BMD and (B) rate of change in hip BMD according to vitamin D receptor (*VDR*) and aromatase (*CYP19*) gene polymorphisms in older African-American women. Values are adjusted for age, body weight, and height, and for weight change (bone loss analysis only). Uppercase letters indicate the absence and lowercase letters the presence of the *VDR* FokI restriction site. The number of women in each group is: FF/Arg^{264}Arg, 44; Ff or ff/Arg^{264}Arg, 25; FF/Arg^{264}Cys or Cys^{264}Cys, 15; Ff or ff/Arg^{264}Cys or Cys^{264}Cys, 12. BMD: *P* (*VDR*) = .05; *P* (*CYP19*) = .81; *P* (*VDR* × *CYP19* interaction) = .0008. Bone loss: *P* (*VDR*) = .07; *P* (*CYP19*) = .07; *P* (*VDR* × *CYP19* interaction) = .037.

6.1%). The joint distribution of the 2 polymorphisms was as follows: FF/Arg^{264}Arg, 45.9%; FF/Arg^{264}Cys, 13.3%; FF/Cys^{264}Cys, 2.0%; Ff/Arg^{264}Arg, 21.4%; Ff/Arg^{264}Cys, 11.2%; Ff/Cys^{264}Cys, 0.0%; ff/Arg^{264}Arg, 5.1%; ff/Arg^{264}Cys, 1.0%; ff/Cys^{264}Cys, 0.0%. Neither polymorphism was associated with baseline BMD or rate of change in BMD. There was a significant interaction between *VDR* and *CYP19* polymorphisms. BMD measurements in women with the less frequent allele at both loci were 0.5 to 1.3 SD lower compared to other women independent of age, body weight, and height (Fig 1A; $P < .001$ for interaction). Moreover, 25% of women with the rare allele at both loci had osteoporosis (t score ≤ -2.5) compared with only 0% to 8% of the other women. The rate of bone loss was significantly greater among women with the rare allele at both loci than other women independent of age, body weight, height, and weight change ($P < .05$ for interaction; Fig 1B). Additional adjustments for walking for exercise, smoking, alcohol consumption, dietary calcium, and use of thiazide diuretics and estrogen replacement yielded similar results.

DISCUSSION

We examined the independent and combined impact of *VDR* and *CYP19* gene polymorphisms on BMD in older African-American women. Neither polymorphism alone was significantly related to BMD or bone loss. When both polymorphisms were considered together, however, there was a highly significant gene-gene interaction. Women with the rare allele at both loci had lower BMD and a higher prevalence of osteoporosis than women with neither or only a single rare allele. The magnitude of effect is of probable clinical relevance because each standard deviation decrease in BMD is associated with a 2.5- to 3.0-fold increase in hip fracture risk among elderly women.[7] Women with both allelic variants also experienced more rapid hip bone loss. These findings suggest that *VDR* and *CYP19* gene polymorphisms have an interdependent effect on osteoporotic risk among older African-American women.

The *VDR* translation start site polymorphism has been associated with BMD in some but not all studies.[8] Complete consistency of findings across populations is not expected in studies of single susceptibility genes for complex polygenic diseases such as osteoporosis. A lack of association in some populations may have been due to the presence or absence of other allelic variants, which modified the effects of the *VDR* polymorphism. Indeed, the *VDR* translation start site polymorphism was only associated with BMD in women with the rare *CYP19* allele in our study. Others[9] have demonstrated interactive effects of *VDR* and estrogen receptor alleles on BMD. These findings suggest that the interaction of loci involved in estrogen metabolism may play a role in postmenopausal osteoporosis.

An interaction between *VDR* and *CYP19* polymorphisms is consistent with a *VDR* response element in the *CYP19* promoter,[10] the induction of aromatase by vitamin D,[4] and decreased *CYP19* expression in *VDR* knock-out mice.[11] Isoforms encoded by the rare *VDR* FokI f allele have decreased *trans*-activation capacity relative to more common F allele isoforms.[12] Thus, impaired transcriptional activation of aromatase may explain the decreased BMD in women with the rare allele of the *VDR* translation start site polymorphism.

The functional consequence of the aromatase Arg^{264}Cys polymorphism is less certain. This polymorphism lies near a substrate recognition site and may influence aromatase activity. The Cys^{264} allele has been associated with lower serum estradiol levels than the Arg^{264} allele.[13] However, in vitro experiments of this polymorphism have not revealed an effect on aromatization.[14] Further studies are needed to determine if the Arg^{264}Cys polymorphism has subtle functional effects on aromatase activity.

In summary, our results suggest that *VDR* and *CYP19* polymorphisms may jointly influence BMD and bone loss in older African-American women. Further studies are needed to confirm these observations in other population groups and to test if combined *VDR* and *CYP19* genotyping identifies women at increased risk of fracture.

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