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Effect of *CYP1A1* Gene Polymorphisms on Estrogen Metabolism and Bone Density*

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Abstract

In this study, we evaluated the effect of polymorphisms of the *CYP1A1* gene, linked to hormone-related cancers, on estrogen metabolism and BMD. We found that variants carrying the *A* allele (CA and AA) for the C4887A polymorphism have a significantly higher degree of estrogen catabolism and lower femoral BMD.

Introduction: Polymorphisms of the *CYP1A1* gene, one of the key enzymes that metabolize estrogen, have been linked with hormone-related cancers. We investigated the impact of these polymorphisms on estrogen metabolism and BMD, which is another hormone-dependent health issue.

Materials and Methods: One hundred seventy postmenopausal women (mean age, 63.5 ± 0.6 years) participated in the study, but analysis was limited to 156 white women. Genotyping was performed by restriction fragment length polymorphism analysis, urinary estrogen metabolites by enzyme immunoassay, serum estradiol by ultrasensitive radioimmunoassay, serum sex hormone-binding globulin by immunoradiometric assay, and BMD by DXA. Differences in the levels of urinary metabolites and BMD among the different variants were analyzed by analysis of covariance, whereas differences in free estradiol index, urinary N-telopeptide of type 1 collagen (NTx), and bone size were compared by one-way ANOVA.

Results: We found that subjects carrying the *A* allele (CA or AA) for the C4887A polymorphism of the *CYP1A1* gene have significantly lower free estradiol index (0.323 ± 0.08 versus 0.506 ± 0.04; $p = 0.04$; pmol/nmol) and higher levels of total urinary estrogen metabolites (ng/mg Cr) than CC subjects (27.92 ± 2.03 versus 21.15 ± 1.04; $p = 0.03$), suggestive of an accelerated estrogen catabolism in these (CA + AA) individuals. They also had significantly lower BMD (g/cm^2) in all regions of the femur than subjects with the CC genotype, (total hip: 0.809 ± 0.02 versus 0.865 ± 0.01; neck: 0.671 ± 0.02 versus 0.722 ± 0.01; trochanter: 0.614 ± 0.02 versus 0.656 ± 0.01; and intertrochanter: 0.969 ± 0.03 versus 1.039 ± 0.01; all $p < 0.05$). No significant effect of this gene

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polymorphism was detected on lumbar spine BMD. Urinary NTx, a marker for bone resorption, was also significantly higher in the CA + AA compared with the CC variants (186.09 ± 16.15 versus 124.00 ± 11.87 nmol of bone collagen equivalent/mmol of creatinine; $p = 0.003$). Genotype frequencies for this polymorphism showed CC as the most common genotype (127/156), followed by CA (28/156), whereas AA was rare (1/156).

Conclusion: Women with the *A* allele seem to have increased estrogen catabolism, as indicated by higher urinary estrogen metabolites and lower free estradiol index. This is associated with increased bone resorption and lower femoral BMD in those with the *A* allele. Our data, therefore, suggest that, through its effect on the rate of estrogen catabolism, the C4887A polymorphism of the *CYP1A1* gene may represent a possible genetic risk factor for osteoporosis.

Keywords

genetic research; epidemiology; osteoporosis; hormones and receptors; menopause

INTRODUCTION

Rates of bone loss in the postmenopausal period vary widely among individuals, even when controlled for age, years since menopause (YSM), and body mass index (BMI). Although estrogen exposure during the fertile period is important for the achievement and maintenance of peak bone mass, the role of postmenopausal estrogen level remains controversial. Results from epidemiological studies are conflicting about the correlation between bone loss and circulating estrogen levels.[1,2] One reason for such discrepancies stems from the existence of many metabolic pathways that convert estradiol (E_2) and estrone (E_1) to metabolites, with widely diverse estrogenic activity.[3,4] We previously reported that the ratio of 2-hydroxyestrone ($2OHE_1$) to 16α-hydroxyestrone ($16\alpha OHE_1$), originating from the two major mutually exclusive estrogen oxidative pathways, is an important determinant of bone loss in the postmenopausal period.[5] Because 2-hydroxylated compounds, $2OHE_1$ and 2-methoxyestrone ($2MeOE_1$), are inactive or estrogen antagonists,[4,6] whereas 16-hydroxylated estrogens, $16\alpha OHE_1$ and estriol (E_3), are agonists,[4,7] the rate of either metabolic pathway may dictate the overall estrogen “tone” after menopause.

The importance of estrogen metabolism in the pathogenesis of hormone-related diseases has emerged primarily from studies on breast cancer.[8,9] Only recently have a few studies suggested that the oxidative metabolism of estrogens may also be an important determinant of postmenopausal BMD. Women with preferential metabolism through the 2-hydroxyl pathway were found to have lower bone mass compared with those with increased hydroxylation through the 16α-hydroxyl pathway.[5,10] Furthermore, a lower $2OHE_1/16\alpha OHE_1$ ratio has been shown to protect from bone loss in women studied over a 1-year period.[5]

There are four major enzymes involved in estrogen metabolism, each encoded by a different gene: (1) cytochrome P450, family1, subfamily A, polypeptide 1 (*CYP1A1*) at chromosome 15q22–24; cytochrome P450, family 1, subfamily B, polypeptide 1 (*CYP1B1*) at chromosome 2p22-p21; cytochrome P450, family 1, subfamily A, polypeptide 2 (*CYP1A2*) at chromosome 15q22-ter; and cytochrome P450, family 3, subfamily A,

polypeptide 4 (*CYP3A4*) at chromosome 7q22.1.[11,12] Although all of these enzymes can be found in the liver, CYP1A1 and CYP1B1 are predominantly extrahepatic in location.[11,12] Each enzyme has a different hydroxylation activity, thus implying that the overall estrogen metabolic profile in each individual is imparted by the relative activity of each of these enzymes. Several hormone-related neoplasias have been linked to genetic polymorphisms of these enzymes,[13–19] and a vast majority of them, in relation to polymorphisms of the *CYP1A1* gene. For example, incidence of cancers of the breast,[13,17] prostate,[18] ovary,[19] and endometrium[14] are found to be elevated with certain polymorphisms of the CYP1A1 enzyme, suggesting a hormonal pathogenesis for these associations. However, to our knowledge, none have been reported for osteoporosis, which is another hormone-related condition.

The objective of this study, therefore, is to evaluate the biological significance of the three polymorphisms of the *CYP1A1* gene that are associated with hormone-related diseases (i.e., T6235C in the 3' UTR and the A4889G and C4887A in the protein coding region) on estrogen metabolism and BMD. We hypothesize that, by altering enzymatic activity, certain polymorphisms of the *CYP1A1* gene will result in specific estrogen metabolic profiles, which may in turn result in differences in BMD.

MATERIALS AND METHODS

Study population

This is a cross-sectional study conducted on community-dwelling, otherwise healthy, postmenopausal women living in the St Louis metropolitan area. Participants were volunteers who responded to advertisements and direct mailing. The protocol was approved by the Washington University School of Medicine Institutional Review Board, and informed consent was obtained from each participant. This study recruited healthy women who were at least 1 year from the last menstrual period. Twenty-six women were participants in a previous related study and were asked to return for blood draw for genetic studies.[5] The previous clinical and laboratory data for these women were also used in this study. Subjects who were taking any medication that affect bone metabolism such as estrogen, selective estrogen receptor modulators (SERMS; including raloxifene and tamoxifen), bisphosphonates (alendronate, risedronate, pamidronate, or zoledronate), aromatase inhibitors, GnRH analogs, glucocorticoids (>5 mg daily of prednisone or equivalent for >1 month), or phenytoin were excluded from the study. Intake of medications known to affect estrogen hydroxylation (phytoestrogens, cimetidine, thyroid hormones, monooxygenase inhibitors) and drugs known to affect cytochrome P450 enzyme activity were also exclusionary criteria, as were diseases or conditions known to interfere with bone metabolism, including hyperthyroidism, osteomalacia, chronic liver disease, renal failure, hypercortisolism, malabsorption, immobilization, and alcoholism. Current tobacco users were excluded, whereas past smokers who stopped smoking for at least 6 months were allowed into the study. Subjects consuming more than one serving per day of vegetables containing high levels of phytochemicals, including indole-3-carbinol, which are known to preferentially enhance 2-hydroxylation of estrogen[20] such as cabbage, cauliflower, Brussels sprouts, broccoli, and kale were excluded from participation.

Clinical, dietary, and anthropometric data

Dietary calcium and vitamin D intake were estimated from a 7-day dietary record, which was mailed to the participants at least 1 week before the study visit. The record contains a list and serving sizes of common dietary sources of calcium. The participants were asked to record daily intake of these foodstuffs, and the average daily intake was determined for 7 days. Diet history also included intake of vegetables such as cabbage, cauliflower, Brussels sprouts, broccoli, and kale. Anybody consuming more than one serving per day of these vegetables was not allowed into the study for reasons cited under the Study population section. Alcohol intake was expressed as the average number of alcoholic-drink equivalents consumed over a 1-week period. A can of beer (336 ml), a glass of wine (112 ml), and 28 ml of a heavy alcoholic beverage were considered one-drink-equivalent. Previous smoking was expressed in pack-years and estimated as the number of 20-cigarette packs smoked per day multiplied by the number of years of smoking. Physical activity was expressed as a numerical score and defined as sedentary (sitting or lying most of the day, score 1); moderately active (being on feet more than one-half a day, score 2); and very active (engaging in regular physical exercise, score 3).[21]

A family history of osteoporosis was coded as positive in the presence of a blood relative diagnosed with osteoporosis, kyphosis, and fragility fractures in the absence of secondary causes. Data on estrogen exposure were assessed through a number of variables as age at menarche, average number of periods per year during the reproductive years, number of years of birth control pill use, total number of pregnancies, number of pregnancies to term, months of lactation, age at menopause, and YSM. BMI was calculated as the weight in kilograms divided by the square of the height in meters (kg/m^2). The waist-to-hip ratio was calculated as the ratio between the waist circumference, taken at the umbilical level, and the hip circumference, measured 6 in below the anterior superior iliac spine.

Biochemical data

Urinary estrogen metabolites were measured on a 24-h urine specimen using the ESTRAMET immunoassay kits (Immuna Care, Bethlehem, PA, USA). The ESTRAMET series of test kits are monoclonal antibody-based competitive enzyme immunoassays for estrogen metabolites in microtiter plate format. The antibodies and assays for urinary 2- and 16α-hydroxyestrogen have been described.[22] The monoclonal antibody to 2-hydroxyestrogens recognizes the 2-hydroxy forms of E_1, E_2, and E_3 equivalently. Similarly, the monoclonal antibody to 2-methoxyestrogens recognizes the 2-methoxy forms of estrogen metabolites equivalently and exhibits <0.1% cross-reactivity with any other estrogen, including 2-hydroxyestrogens. The monoclonal antibody to E_3 exhibited <2% cross-reactivity with any other estrogen. All urinary estrogen assays were performed according to methods described previously.[5] Briefly, urine samples were incubated with enzymes that deconjugated estrogen metabolite sulfates and glucuronides to their respective free forms. The amount of estrogen metabolite in the enzymic hydrolysate was determined by competition between deconjugated estrogen in the hydrolysate and estrogen-labeled alkaline phosphatase for binding to specific monoclonal antibodies attached to the microtiter plate. Greater than 90% of the metabolites in the urine exist as glucuronides and were recovered totally by this method. The inter- and intra-assay CVs for these enzyme-linked

immunoassays were <9% and 13%, respectively. Each urinary metabolite value was corrected for 24-h urinary creatinine (mg/24 h; ng/mg Cr).

Serum samples were collected in a nonfasting state. Serum estradiol was measured by ultrasensitive radioimmunoassay technique (Diagnostic Systems Laboratory, Webster, TX, USA), and sex-hormone-binding globulin (SHBG) was measured by immunoradiometric (IRMA) assay (Diagnostic Systems Laboratory). The inter- and intra-assay CVs for serum estradiol and SHBG were <10%. The free estradiol index (FEI) was calculated as the molar ratio of total estradiol to SHBG.[23] Urinary N-telopeptide of type 1 collagen (NTx) was measured on a 24-h urine sample by an enzyme-linked immunosorbent assay (Osteomark NTx; Ostex International, Seattle, WA, USA) and expressed as nanomoles of bone collagen equivalent (BCE) per millimolar of creatinine. The CV in our laboratory for this assay is <10%.

BMD

BMD of the lumbar spine and the proximal femur were measured by DXA using the Hologic QDR 4500 (Hologic, Waltham, MA, USA). BMD of the lumbar spine was performed using the anteroposterior projection and was calculated as the average of L_1–L_4 vertebrae. The nondominant hip was used for proximal femur scans, and values were calculated on the total femur, femoral neck, trochanter, and intertrochanteric areas. BMD values were expressed in grams per square centimeter. The CV of this technique using the QDR 4500 densitometer is 1.09% for the lumbar spine and 1.2% for the total femur at our center.

Genotyping for CYP gene polymorphisms

Genomic DNA was extracted from peripheral leukocytes using the Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, MN, USA) and used as a template for genotyping procedures. Genotype characterizations to identify polymorphisms were performed using PCR and restriction fragment length polymorphism (RFLP). DNA fragments were identified by gel electrophoresis.

Three polymorphisms in the *CYP1A1* gene known to be associated with hormone-related disorders were evaluated as described.[24] They are T6235C in the 3’ UTR, A4889G (Ile 462 Val) in exon 7, and C4887A (Thr 461 Asn), also in exon 7. The T6235C polymorphism in the 3’ UTR was detected using the forward primer 5’-GGCTGAGCAATCTGACCCTA-3’ and the reverse primer 5’-TAGGAGTCTTGTCTCATGCCT-3’. The PCR reaction was performed using standard protocols with 1.5 mmol of $MgCl_2$ and 1 μmol of each primer. The PCR reactions started with an initial denaturation at 94°C for 5 minutes, followed by 35 cycles for 0.5 minutes at 94°C, 1 minute at 63°C, and 1 minute at 72°C, with a final extension of 7 minutes at 72°C. PCR products were digested by *Msp*I (New England Bio-labs), following the manufacturer’s protocol. Fragments were separated by electrophoresis on a 2.5% agarose gel and visualized by ethidium bromide staining. There was an undigested 899-bp product for the TT, 693- and 206-bp fragments for the CC, and three bands (899, 693, and 206 bp) for the TC genotypes (Fig. 1A).

The polymorphisms, A4889G and C4887A, located in exon 7 were identified by amplifying a 204-bp fragment using the forward primer 5'-CTGTCTCCCTCTGGTTACAGGAAGC-3' and the reverse primer 5'-TTCCACCCGTTGCAGCAGGATAGCC-3'. PCR conditions were 35 cycles for 0.5 minutes at 94°C, 0.5 minutes at 63°C, and 0.5 minutes at 72°C. The PCR products were digested with *Bsr*DI (New England Biolabs) for the A4889G and with *Bsa*I (New England Biolabs) for the C4887A polymorphisms. The digested products were separated on a 3.0% Nusieve GTG agarose gel (BMA, Rockland, ME, USA). Those with the AA genotype for the A4889G polymorphism generated two fragments of 149 and 55 bp when cut with *Bsr*DI, whereas those with GG genotype produced an undigested 204-bp product (Fig. 1B), and those with the AG genotype had three bands (204, 149, and 55 bp). When digested with *Bsa*I, those with CC genotype for the C4887A polymorphism yielded two fragments of sizes 139 and 65 bp, those with AA genotype remained uncut at 204 bp (Fig. 1C), whereas those with the CA genotype generated three bands (204, 139, and 65 bp).

Statistical analysis

Results are expressed as means ± SE. BMD (Z score) at each anatomical site was regressed against each urinary metabolite, the $2OHE_1/16\alpha OHE_1$ ratio, and the sum total of all metabolites ($2OHE_1 + 2MeOE_1 + 16\alpha OHE_1 + E_3$). Values for urinary metabolites were not normally distributed, and therefore, were log-transformed. The log-transformed values were then used in analysis of covariance comparing metabolite values among the different gene variants adjusted for covariates as age, YSM, BMI, and pack-years of past smoking. Differences in BMD (g/cm^2) between the gene variants were also compared by analysis of covariance adjusted for the above covariates, whereas differences in bone size, serum hormone levels, SHBG, and urinary NTx were compared by one-way ANOVA. The clinical features of the different gene variants were compared using Student's *t*-test for continuous variables (e.g., age, BMI) and using χ^2 test for categorical variables (e.g., family history of osteoporosis and the percentage of past smokers) as appropriate. In the C4887A polymorphism, CA and AA genotypes were combined in every analysis, because there was only one subject with the AA genotype. The data were managed and analyzed using Excel 2000 (Microsoft, Redmond, WA, USA) and SPSS 11.5 (SPSS, Chicago, IL, USA). The significance level was set at $p < 0.05$. Analysis of Hardy-Weinberg equilibrium was carried out using the Polymorphism and Haplotype Analysis Suite (http://krunch.med.yale.edu/hswiml).

RESULTS

A total of 170 postmenopausal women, who were at least 1 year from the last menstrual period, participated in the study. There were 156 whites, 7 blacks, and 7 Asians. The clinical features of the entire study population are shown in Table 1. Our participants were for the most part elderly (i.e., mean age, 63.5 ± 0.6 years), several years away from the last menstrual period (13.1 ± 0.8 years), and overweight (mean BMI, 27.6 ± 0.4 kg/m^2). Because we have only a small number of minority participants, analysis was limited to white participants, who comprised the majority of our subjects.

Simple correlation analysis revealed weak inverse correlations between levels of urinary E_3 and BMD of the total hip and intertrochanter ($r = -0.16$ and $r = -0.20$, respectively; both $p < 0.05$). Total urinary metabolites ($2OHE_1 + 2MeOE_1 + 16\alpha OHE_1 + E_3$), likewise, showed weak inverse correlations with BMD of the total hip, femoral neck, and intertrochanter ($r = -0.17$, $r = -0.17$, and $r = -0.18$, respectively; $p < 0.05$). There were no significant correlations observed between the spine BMD with any of the metabolites, and for $2MeOE_1$, $16\alpha OHE_1$, and $2OHE_1/16\alpha OHE_1$ ratio, with any skeletal site analyzed.

Comparing phenotypes of the different polymorphisms for the *CYP1A1* gene revealed that the C4887A polymorphism was associated with significant differences in urinary estrogen metabolites, serum free estradiol index, and BMD in the variants. Levels of individual metabolites ($2OHE_1$, $16\alpha OHE_1$, and E_3) were higher in the CA + AA relative to the CC subjects, and as a result, the mean level of the total metabolites ($2OHE_1 + 2MeOE_1 + 16\alpha OHE_1 + E_3$) was significantly higher in the CA + AA subjects (Table 2). The urinary $2OHE_1/16\alpha OHE_1$ was unaffected because mean values for this ratio were comparable between the genotypes. The CA + AA women also had significantly lower serum free estradiol index and higher SHBG. Urinary NTx, a marker of bone resorption, measured on a subset of 80 women (28 in the CA + AA and 52 age-matched women in the CC group), was significantly higher in the CA + AA variants.

Analysis of BMD showed that adjusted values in all regions of the femur (i.e., total femur, neck, trochanter, and intertrochanter) were significantly lower in the CA + AA subjects relative to the CC subjects (Table 3). On the other hand, no difference in the spine BMD was observed between the two groups. There were also no differences in bone size at either the lumbar spine or any region of the proximal femur between the two groups (Table 3).

There were no differences in clinical features such as age, BMI, waist-to-hip ratio, estrogen exposure, family history of osteoporosis, degree of physical activity, alcohol intake, and dietary factors between the CC and the CA + AA subjects (Table 4). There was, however, a significantly higher number of past smokers in the CA + AA subjects, but there were no differences in the levels of urinary metabolites, free estradiol index, or BMD at any site between the nonsmokers and past smokers within this subgroup (CA + AA). For this polymorphism, CC seemed to be the most common genotype (127/156), followed by CA (28/156), whereas AA was rare (1/156). The genotype frequencies calculated for this polymorphism were in agreement with Hardy-Weinberg equilibrium.

Meanwhile, differences in phenotypes were not observed for the variants of the T6235C and the A4889G polymorphisms, because urinary estrogen metabolites, serum hormone levels, and BMD values were comparable among the different variants for these polymorphisms (data not shown).

DISCUSSION

Although there are substantial data on the association between polymorphisms of the CYP450 enzymes that metabolize estrogen and certain hormone-related cancers,[13–18] so far little or no information is available on the effect of these polymorphisms on BMD.

In this study, we have identified the C→A transversion at position 4887 of the *CYP1A1* gene, which results in an amino acid change from threonine to asparagine at codon 461, (Thr461Asn) as a possible genetic risk factor for low BMD. Our data show that women with the *A* allele have lower BMD in all sites of the femur. They also have significantly higher levels of urinary estrogen metabolites and significantly lower free estradiol index, suggestive of an increased estrogen catabolism, as well as increased bone resorption. Because estrogen is critical to bone health and estrogen metabolism is an important determinant of bone mass,[5] we hypothesize that differences in BMD, and consequently the risk of osteoporosis associated with this polymorphism, is a result of the difference in estrogen metabolism.

An altered enzyme activity causing interindividual difference in the rate of metabolism and bioactivation of the different compounds including estrogen and some carcinogens has been proposed as the putative pathogenesis for the varying risks of cancers associated with polymorphisms of the CYP450 enzymes.[14,18,25] This hypothesis, however, has not been thoroughly tested in vivo, although indirect evidence from in vitro studies point to this potential mechanism.[26,27] For example, individual variants as Gly48 for the Arg48Gly polymorphism, Ser119 for Ala119Ser, Leu432 for Val432 Leu, Ser453 for Asn453Ser, and one particular genotype Gly48-Ser119-Leu432-Ser453 of the *CYP1B1* gene, have catalytic efficiencies in vitro that far exceeded their genotypic counterparts, as suggested by higher absolute levels of 4-, 2-, and 16α-hydroxylated products of estrogen metabolism.[26] Higher ratios of both 4- to 2-hydroxyestradiol and 4- to 2-hydroxyestrone in the *CYP1B1* Val432 than for the Leu432 variants were also noted in another study.[27] In other words, some of the allelic variants of these key enzymes may change enzyme kinetics, resulting in variable accumulation of different estrogen metabolites. On the other hand, there is currently very little, if any, data on the estrogen metabolic profile in individuals with different CYP450 genotypes. Our data fills this gap by showing variable levels of the different estrogen metabolites among the *CYP1A1* C4887A gene variants in vivo. The finding that the increased urinary metabolites among CA + AA women are accompanied by lower free estradiol index is suggestive of an enhanced estrogen catabolism. On the other hand, the higher circulating SHBG may also contribute to lower free estradiol index in these subjects. Interestingly, a positive correlation was recently reported between SHBG and the activity of another estrogen-metabolizing enzyme, CYP1A2, measured by the caffeine metabolic ratio.[28] Similar to our study, women with the highest levels of SHBG also had the lowest levels of free estradiol index. However, the reason for the higher SHBG levels in these polymorphic CYP variants remains unclear.

The biologic significance of the C4887A polymorphism in the *CYP1A1* gene has been inferred from lung,[24] breast,[17] and endometrial[14] cancer studies showing a higher prevalence of the variant *A* allele in subjects with these cancers. In particular, women with the *A* allele were reported as having a 6-fold higher likelihood of being diagnosed with endometrial cancer relative to subjects with the *C* allele.[14] This conclusion may seem at odds with our findings, showing lower free estradiol levels in women with the CA + AA genotypes relative to the CC group. It should be noted that the estrogen metabolic profiles were not investigated in that study and that the increased bone resorption marker in the CA + AA group is consistent with a lower estrogenic state. Accordingly, an increased estrogen breakdown in the CA + AA women may be the mechanism leading to the lower

free estradiol, which in turn may result in increased bone resorption and lower BMD in these subjects. Because we studied postmenopausal women, we cannot establish whether the lower BMD is the result of accelerated postmenopausal bone loss or reduced peak bone mass, although the latter hypothesis may be more likely considering the important role of estrogen in skeletal development and maturation.

These results also suggest that the rate of estradiol catabolism is more important than the type of metabolites produced by estradiol hydroxylation ($2OHE_1/16\alpha OHE_1$ ratio), at least for the genetic polymorphism we have analyzed here. Although this conclusion diverges from our original premise that was derived from a previous observational study,[5] an accelerated estradiol hydroxylation would generate larger amounts of products that in most part are inactive or have an inferior estrogenic activity relative to estradiol.[4,29,30] The accelerated metabolism would therefore lead to an overall lower estrogenic exposure in the CA + AA subjects, a risk factor for low BMD.

Our results suggest that this polymorphism may have a site-specific effect on the skeleton because differences in BMD between the CC and CA + AA subjects were only seen in the proximal femur but not in the spine. Importantly, this difference in femoral BMD was not accounted for by differences in bone sizes, because femoral neck width and total femoral area in the DXA scans were comparable among the genotypes. It should be noted that, because of the age of the subjects we studied, degenerative changes and sclerosis of the posterior vertebral elements may have masked a potential intergenotype difference on DXA measurements of the spine.[31] Similar results were obtained for a polymorphism of the *CYP17* gene, an enzyme involved in estrogen synthesis. Significant differences in BMD were seen in the femur but not in the spine,[32] but even in this case, age-dependent artifacts in vertebral DXA measurements cannot be excluded. Results from human linkage studies show that quantitative trait loci controlling for BMD of the hip are different from that of the spine,[33] supporting the idea that the biologic effects of estrogen are different for different skeletal sites.

Our study has some limitations. First, our participants were self-selected, and consequently, our findings may be biased to a subset of women who may be more motivated or who may have special interest in this particular study by virtue of family history of bone disease. Second, the relatively small sample size may have resulted in failure to see a difference in vertebral BMD between the CC and CA + AA variants. Because the *A* allele is not common, it is possible that a larger sample size with more subjects carrying the *A* allele might show a significant difference in BMD of the spine as well. On the other hand, we were able to show significant differences in urinary metabolites and femoral BMD in the current sample size, which would imply that a larger sample size might not be necessary. Indeed, posthoc power calculations indicated that our sample size had sufficient power (0.99) with a sufficiently small probability of type 1 error ($\alpha < 0.05$) to detect a 10% difference in femoral neck BMD between groups, assuming equal variances of 0.12 in both groups. This is the first report on the association between the *CYP1A1* C4887A polymorphisms, patterns of estrogen hydroxylation, and BMD. Considering that a host of factors modulates estrogen metabolism[34] and BMD is a product of the interaction between genetics[35] and

environmental factors,[36] the use of animal models should be useful to further detail the pathobiologic relevance of this polymorphism on bone.

In summary, our results show that a polymorphism of the *CYP1A1* gene, the transversion from C to A at position 4887, has biologic consequences on estrogen and bone metabolism. The presence of at least a single *A* allele at this position is associated with increased bone resorption and low femoral BMD, likely a result of accelerated estrogen catabolism, and consequently, low free estradiol among individuals carrying this allele. This genetic trait may be helpful in the future to identify individuals at risk for low bone mass who may represent the best candidates for preventative approaches to osteoporosis.

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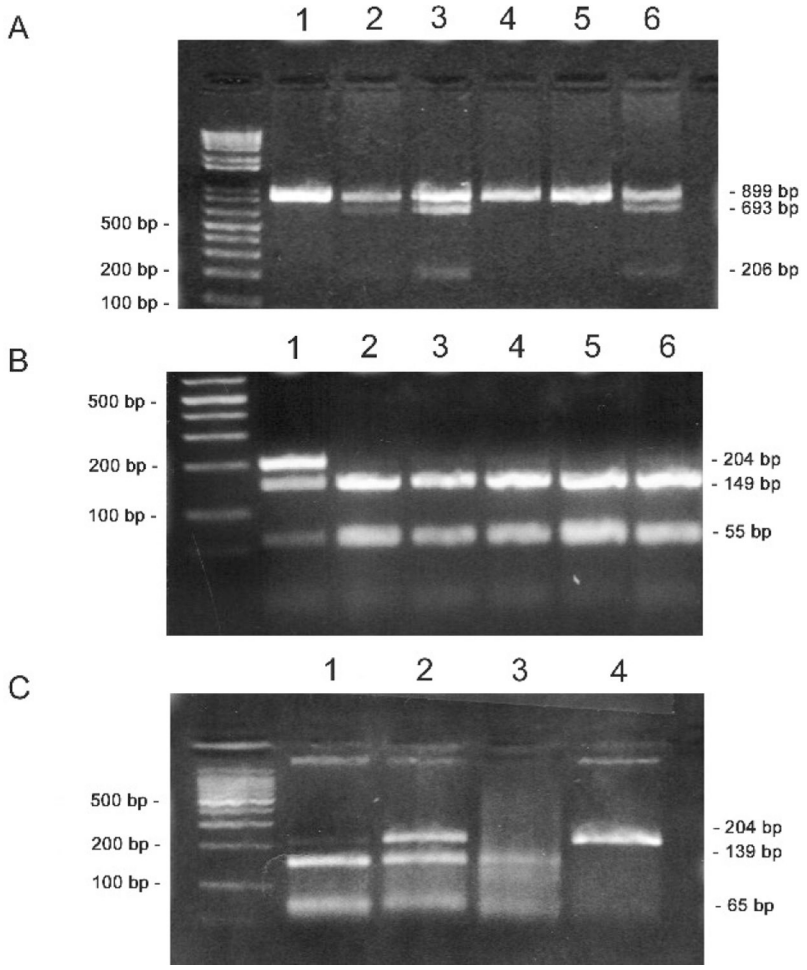

FIG. 1.

CYP1A1 gene polymorphisms analyzed by RFLP. For each polymorphism, DNA samples were amplified by PCR, digested with restriction enzymes (as indicated below), and analyzed by agarose gel electrophoresis followed by ethidium bromide staining. For each gel, the first lane is a DNA size marker; the remaining lanes are analyses for individual DNA samples. (A) T6235C polymorphism in the 3' UTR. PCR amplicons were digested with *Msp*I, generating the following: for TT, one band (899 bp), for TC, three bands (899, 693, and 206 bp), and for CC, two bands (693 and 206 bp). For lanes 2, 3, and 6, the TC genotype was detected (three bands), whereas the remaining lanes show the TT genotype (one band). (B) A4889G polymorphism in exon 7. PCR amplicons were digested with *Bsr*DI, generating the following: for AA, two bands (149 and 55 bp), for AG, three bands (204, 149, and 55 bp), and for GG, one band (204 bp). Lane 1 shows the AG genotype (three bands), whereas the remaining lanes show the AA genotype (two bands). (C) C4887A polymorphism in exon 7. PCR amplicons were digested with *Bsa*I, generating the following: for CC, two bands (139 and 65 bp), for CA, three bands (204, 139, and 65 bp), and for AA, one band (204 bp). Lane 2 shows the CA genotype (three bands), whereas in lane 4, the AA genotype was detected (1 band); the remaining lanes show the CC genotype (two bands).

Table 1.

Characteristics of All The Study Participants

Clinical features	***Means ± SE (N = 170)***
Age (years)	63.5 ± 0.6 (48–83)
YSM	13.1 ± 0.8
BMI (kg/m^2)	27.6 ± 0.4
Waist-to-hip ratio	0.82 ± 0.0
History of smoking	
Percent past smokers	44.7%
Total past smoking (pack-years)	7.8 ± 1.2
Average daily calcium intake (mg/day)	1089 ± 51
Alcohol intake (oz-Eq/week)	1.49 ± 0.2
Positive family history of osteoporosis	42.77%
Activity score	2.31 ± 0.1

Table 2.

Biochemical Data in the CC and CA + AA (*CYP1A1*, C4887A) Female White Subjects

	Untransformed values (means ± SE)		*Log transformed values (means ± SE)*		
Urine estrogen metabolites (ng/mg Cr)	***CC* (N = *127*)**	***CA + AA* (N = *29*)**	***CC* (N = *127*)**	***CA + AA* (N = *29*)**	**p**
$2OHE_1$	7.35 ± 0.43	9.24 ± 0.84	0.770 ± 0.340	0.903 ± 0.242	0.09
$2MeOE_1$	3.88 ± 0.22	4.86 ± 0.44	0.521 ± 0.227	0.622 ± 0.242	0.23
$16\alpha OHE_1$	3.97 ± 0.25	5.71 ± 0.49	0.518 ± 0.268	0.680 ± 0.267	0.01
E3	6.27 ± 0.365	7.20 ± 0.707	0.711 ± 0.251	0.838 ± 0.246	0.03
$2OHE_1/16\alpha OHE_1$	2.17 ± 0.11	1.73 ± 0.23	0.253 ± 0.214	0.206 ± 0.182	0.10
$2OHE_1$ + 2MeOE1 + $16\alpha OHE_1$ + E_3	21.15 ± 1.04	27.92 ± 2.03	1.272 ± 0.227	1.397 ± 0.212	0.03
Other biochemical data					
Serum estradiol (pg/ml)	14.63 ± 0.52	13.10 ± 0.98	—	—	0.25
Serum estradiol (pM)	53.69 ± 1.92	48.07 ± 3.61			
Free estradiol index (pmol/nmol)	0.506 ± 0.04	0.323 ± 0.08	—	—	0.04
SHBG (nM)	143.66 ± 7.44	181.81 ± 15.33	—	—	0.03
Urine NTx/creatinine (Nmol BCE/mmol creatinine)	124.00 ± 11.87	186.09 ± 16.15			0.003

Log-transformed values were used for urinary metabolite comparisons by analysis of covariance adjusted for age, BMI, YSM, and history of past smoking. Total serum estradiol, free estradiol index, SHBG, and urinary NTx were compared by one-way ANOVA.

Table 3.

BMD Values and Bone Size in the CC and CA + AA (*CYP1A1*, C4887A) Female White Subjects

	CC (N = *127*)	*CA + AA* (N = *29*)	p
BMD (g/cm^2)			
Spine	0.943 ± 0.01	0.923 ± 0.03	0.51
Total femur	0.865 ± 0.01	0.809 ± 0.02	0.01
Neck	0.722 ± 0.01	0.671 ± 0.02	0.01
Trochanter	0.656 ± 0.01	0.614 ± 0.02	0.03
Intertrochanteric	1.039 ± 0.01	0.969 ± 0.03	0.02
Bone area (cm^2)			
Spine	57.63 ± 0.51	57.20 ± 1.06	0.72
Femoral neck	5.08 ± 0.03	5.07 ± 0.06	0.89
Total femur	34.22 ± 0.26	33.80 ± 0.53	0.48
Trochanter	11.20 ± 0.11	10.89 ± 0.23	0.23
Intertrochanteric	17.94 ± 0.20	17.84 ± 0.41	0.83

BMD comparisons were adjusted for covariates as age, BMI, past smoking, and YSM using analyses of covariance. Skeletal sizes were compared by one-way ANOVA.

Table 4.

Clinical Features of the White Female Subjects Stratified According to Genotype

Clinical features	*CC (N = 127)*	*CA + AA (N = 29)*	p
Age (years)	63.2 ± 0.7 (48–83)	66.1 ±1.5 (49–82)	0.09
BMI (kg/m^2)	27.89 ± 0.45	26.91 ± 0.88	0.34
Waist-to-hip ratio	0.814 ± 0.01	0.817 ± 0.01	0.85
YSM	12.85 ± 0.92	14.39 ± 1.92	0.47
Age at menopause (years)	48.79 ± 0.53	50.06 ± 1.09	0.30
History of smoking			
Percent past smokers	38.6%	68.9%	0.01*
Total past smoking (pack-years)	7.59 ± 1.44	11.35 ± 2.99	0.26
Average daily calcium intake (mg/day)	1106.4 ± 59.9	1171.9 ± 121.3	0.63
Alcohol intake (oz-Eq/week)	1.47 ± 0.27	1.77 ± 0.55	0.63
Positive family history of osteoporosis (%)	43.5%	39.3%	0.84
Activity score	2.29 ± 0.05	2.41 ± 0.11	0.32

Differences were analyzed using independent *t*-test for continuous, and * χ^2 method for categorical variables.