Association of an aromatase TTTA repeat polymorphism with circulating estrogen, bone structure, and biochemistry in older women

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Dick, I. M., A. Devine, and R. L. Prince. Association of an aromatase TTTA repeat polymorphism with circulating estrogen, bone structure, and biochemistry in older women. Am J Physiol Endocrinol Metab 288: E989–E995, 2005. First published December 21, 2004; doi:10.1152/ajpendo.00550.2004.—Osteoporosis is a disease that is strongly genetically determined. Aromatase converts androgens to estradiol in postmenopausal women, therefore polymorphisms of the gene for this enzyme may be associated with bone mass and fracture. We investigated the association of the TTTA microsatellite polymorphism in intron 4 of the aromatase (CYP19) gene with bone mineral density (BMD) and fracture in 1,257 women aged 70 yr and greater. The data obtained were stratified based on the presence or absence of a [TTTA]n of 7 (A2), determined from a preliminary analysis of hip dual-energy X-ray absorptiometry BMD, which was present in 27% of the population. The presence of an A2 allele was associated with a higher free estradiol index (0.52 ± 0.49, P = 0.049) compared with the absence of an A2 allele (0.47 ± 0.45); higher BMD at all sites of the hip (3.4% total hip, 2.3% femoral neck, 3.6% intertrochanter, 4.1% trochanter) and the lumbar spine (12.7%); higher values for the calcaneal quantitative ultrasound parameters broadband ultrasound (1.3%), speed of sound (0.4%), and stiffness (3.7%); and higher peripheral quantitative computed tomography measures for total (3.4%), trabecular (3.3%), and cortical BMD (3.3%) and the derived stress strain index (SSI) parameters SSI polar (6.4%) and SSI × (6.8%) values. A lower deoxyxypyrindoline creatinine ratio was observed in subjects with an A2 allele (30.3 ± 10.4 vs. 27.1 ± 9.1, P = 0.03). The A2 allele was associated with a lower prevalence of vertebral fracture in subjects who were osteoporotic (odds ratio 0.27, confidence interval 0.09–0.79). Therefore, a common polymorphism of the aromatase gene, perhaps in linkage disequilibrium with a functionally significant CYP19 polymorphism, is associated with bone structure and bone turnover, either by local effects or by effects on circulating bioactive estrogen.

estradiol; fracture; bone density

OSTEOPOROSIS IS A COMMON DISEASE of bone that is strongly genetically determined as shown by various family studies (4, 13, 21). We and others showed a strong relationship between circulating estrogen concentration, bone density, and fractures (5). The human aromatase (CYP19) gene is localized on 15q21.2 and catalyzes the conversion of testosterone to estradiol and androstenedione to estrone. A polymorphism in the 5'-untranslated region of the CYP19 gene has been reported to be associated with bone mass and vertebral fracture risk in a small case control study, but no association with circulating estradiol was demonstrated (30). A TTTA microsatellite polymorphism in intron 4 of the gene for the CYP19 gene has been associated with bone mineral density (BMD) and vertebral fracture in a group of relatively young (mean age 57) postmenopausal women (16). The CYP19 TTTA microsatellite polymorphism has also been shown to modify the BMD response to hormone replacement therapy in early postmenopausal women (29) and to be associated with circulating estrogen concentration in postmenopausal women (25). An association of the TTTA microsatellite with estrogen concentration and BMD has also been demonstrated in men (6).

It is not known if there is an association between CYP19 polymorphisms, estradiol concentration, and measures of bone mass and vertebral fracture in older women. On the basis of previous studies in younger postmenopausal women and the demonstrated importance of endogenous estradiol concentration in determining BMD and fracture risk in older women (5), we hypothesize that the CYP19 TTTA microsatellite is associated with estrogen concentration and consequently bone density, strength, and vertebral fracture in women many years past the menopause. Therefore, we examined the association of this TTTA microsatellite of the CYP19 gene in an unselected, elderly female population with circulating estradiol concentration, measures of bone mass and strength, and vertebral deformities.

MATERIALS AND METHODS

Patients. The subjects for this study were obtained from a population-based cohort consisting of 1,499 Caucasian women aged between 70 and 85 yr. They were recruited by letter from the population aged 70 yr and over using the Western Australian electoral roll, which includes over 98% of the population in this age group, as has previously been described (3). The subjects were included in the study if they were not currently taking medication that would affect bone turnover, including calcium supplements, estrogen, bisphosphonates, and vitamin D, and did not have any serious medical condition that meant they were unlikely to survive the 5 yr of the study. As this was a population-based study, subjects who had ever used estrogen (n = 30), vitamin D (n = 14), or calcium (n = 25), or other medications such as corticosteroids (n = 85), statins (n = 282), tamoxifen (n = 10), or diuretics (n = 171) that may affect bone were not excluded from the study. Clinical diagnosis of osteoporosis and other metabolic bone diseases, such as Paget’s disease and primary hyperparathyroidism, resulted in exclusion from the study. All subjects were enrolled in a 5-yr trial of the effects of calcium on fracture outcome and were randomized to receive either 1.2 g of calcium carbonate daily or a matched placebo. Of these subjects, 1,332 consented to having a blood
sample taken for the isolation of DNA, and the CYP19 TTTA microsatellite polymorphism was examined in 1,170 of these subjects who agreed to have a bone mass measurement performed using at least one of the modalities used in the study. Informed consent was obtained and the Human Rights Committee of the University of Western Australia approved the study.

Study design. The study design consisted of a preliminary evaluation of the association of microsatellite repeats on hip BMD measured by dual-energy X-ray absorptiometry (DXA). Once the association of a 171-bp repeat had been identified at the total hip site, an a priori hypothesis testing approach was applied to the biochemical data, spine BMD, quantitative ultrasound (QUS), and peripheral quantitative computed tomography (pQCT) measurements and vertebral fracture. Quantitative QUS, BMD, and pQCT. QUS of the calcaneum of the left foot was measured twice in 1,129 of these subjects using a Lunar Achilles Ultrasound machine (Lunar, Madison, WI). The manufacturer’s quality assurance methods were employed. The average measurement of the speed of sound (SOS), broadband ultrasound (BUA), and stiffness was reported. The coefficient of variation (CV) for SOS and BUA using the manufacturer’s standards were 0.43 and 1.59%, respectively. BMD measurements of the hip on 1,065 subjects were carried out using an Hologic Acclaim 4500A detector fan beam densitometer (Hologic, Waltham, MA), 12 mo after the commencement of the study. The CV at the total hip site was 1.0% (9). Lumbar spine BMD measurements were available for 211 subjects. The CV at the spine was 1.1% (9). pQCT bone structure and density were measured on 1,048 subjects at 5 yr at the radius at 4% of the length of the tibial shaft to the ankle, using a Stratec XCT 2000 pQCT (Stratec Medizintechnik, Pforzheim, Germany). The voxel size was set at 150 μm in the x- and y-direction and 1,000 μm in the z-direction, which increased the scan time to 5 min. Trabecular and cortical BMD was ascertained using the peel mode 1 algorithm of the manufacturer’s analysis software package, version 5.50. For the tibia, the CV error for total BMD was 1.5%, for trabecular BMD was 2.9%, and for cortical BMD was 2.5%. The cross-sectional area of cortical bone was measured using a periosteal bone density threshold of 710 mg/cm² and an endosteal threshold of 169 mg/cm² determined as the lowest density that consistently allowed delineation of an endosteal surface in these patients. A previously validated biomechanical parameter, the stress strain index (SSI), was calculated as the product of the section modulus and cortical density normalized to the maximal physiological cortical density of human bones (1,200 mg/cm³) for the polar moment and the bending moments in the x- and y-direction, where the y-direction is the widest part of the radius and the x-direction is perpendicular to this (1).

Morphometric X-ray absorptiometry analysis. Dual-energy high-definition lateral morphometric X-ray absorptiometry (MXA) scans of vertebra T4-L4 (Hologic QDR 4500 A) were available for 976 of the subjects who had a CYP19 genotype. The positions of six reference vertebra T4-L4 (Hologic QDR 4500 A) were available for 976 of the subjects who had a CYP19 genotype. The positions of six reference

Biochemical assessment. A randomly selected subgroup of 242 subjects had a blood and urine sample collected after an overnight fast at baseline. The urine samples were analyzed for creatinine, calcium, and phosphorus using routine methods (BM/Hitachi 747 Analyser, Boehringer Mannheim, Mannheim, Germany). Urine deoxypyridinoline was measured by HPLC (20) and corrected for creatinine excretion. The blood samples were analyzed for alkaline phosphatase, creatinine, calcium, and phosphorus using routine methods (BM/Hitachi 747 Analyser). Serum osteocalcin was determined using RIA techniques as previously described (14, 19). Serum estradiol was measured by RIA (Orion Diagnostica, Espoo, Finland) on 1,146 subjects. This assay had a sensitivity of 5 pmol/l and an interassay CV of 20% at 18 pmol/l and 6.6% at 100 pmol/l and an intra-assay CV of 5% at 100 pmol/l. Sex hormone-binding globulin (SHBG) was measured on these subjects using an immunochromelumimetric assay (Imulite, Los Angeles, CA). The intra- and interassay CV were 7.1 and 6.8%, respectively, at 24 nmol/l. Serum intact parathyroid hormone was measured by immunochromelumimetric assay (27) with intra- and interassay CV of 3.6 and 6.2%, respectively.

Genotype analysis. Genomic DNA was extracted and purified from EDTA whole blood samples. The tetranucleotide [TTTA]n repeat begins at the 682 bp of the human CYP19 gene and has been reported to be repeated up to 24 times (18). This region was amplified by PCR using previously described TET-labeled primers (18). Amplification conditions were as follows: 94°C for 4 min and then 94°C (30 s); 55°C (30 s); 72°C (30 s) for 5 cycles; 94°C (5 s); 55°C (30 s); 72°C (45 s) for 20 cycles; 94°C (5 s); 55°C (45 s); and 72°C (1 min 20 s) for 15 cycles followed by a 7-min extension. The PCR product was electrophoresed in preheated (55°C) 6% polyacrylamide gel for 50 min at 70 W. Samples were visualized on the Hitachi FMBIO (Tokyo, Japan) using a 585-nm filter. The smallest allele has previously been reported to not be part of the [TTTA]n polymorphism and is due to a 3 base insertion/deletion ∼50 bp upstream of the [TTTA]n repeat (8). This insertion/deletion polymorphism has been reported to be in complete linkage disequilibrium with a [TTTA]n of 7 in a caucasian population (2).

Statistical analysis. Hardy Weinberg equilibrium for the CYP19 TTA allele was tested using the Genepop program (http://wbiomed. curtin.edu.au/genepop/genepop_op1.html). A preliminary analysis of the association of CYP19 TTA alleles with low total hip BMD (T ≤ −1) was performed using the CLUMP program, which uses a Monte Carlo simulation technique to compare the departure of observed values from expected values conditional on the marginal totals. This approach overcomes the problems of multiple comparisons (22). All subsequent statistical analyses were performed using SPSS Windows version 11 (SPSS, Chicago, IL). The subjects were divided into two groups depending on whether they had at least one allele with a TTTA allele of 7 repeats that included the 3-bp insertion 50 bp from the TTTA microsatellite (A2), which was a cutoff chosen based on the preliminary analysis of the hip BMD data and applied to all other analyses. The data were also analyzed on the basis of a dichotomized allele length of [TTTA]n of 10 or longer, or less than 10, as previously described (6). Significant differences in QUS, height, weight, and biochemical parameters between the groups were determined using unpaired Student’s t-test. Significant differences in age, number of years since menopause, alcohol consumption, cigarette smoking, and calcium intake between the groups were examined using the Mann-Whitney U-test, as these variables were not normally distributed. The free estradiol index (FEI) was calculated as the molar ratio of serum estradiol concentration divided by serum sex binding globulin concentration. The log10 values for the deoxypyridinoline creatinine ratio were adjusted for calcium supplementation or placebo and the groups were compared by Wald t-test using the general linear modeling (GLM) procedure. In some analysis, BMD, QUS, and pQCT values were adjusted for the FEI and means were compared using GLM. Those subjects with a T score for hip BMD of ≤1 SD below the
premenopausal mean (±0.855) were classified as being osteopenic and those subjects with a T score for hip BMD of ±2.5 SD below the premenopausal mean (±0.675) were classified as being osteoporotic according to World Health Organization (WHO) criteria (12) applied to a reference population of young normal women collected at our laboratory. The effect of a CYP19 TTTA A2 on the distribution into the normal compared with the osteopenic and osteoporotic groups was calculated using logistic regression analysis, adjusting for the FEI and the results were reported as the odds ratio (OR) and 95% confidence intervals (CI).

RESULTS

CYP19 [TTTA]n allele distribution. TTNA genotyping resulted in eight different PCR fragments ranging in size from 168 to 191 bp, designated A1 to A8. The frequency of each TTNA allele is illustrated in Fig. 1. This resulted in the presence of 20 different genotypes being present in the population, which was in the Hardy-Weinberg equilibrium. An initial statistical analysis of total hip BMD identified that the A2 allele (171 bp, number of TA repeats = 7) was significantly different from the other alleles (P = 0.03). Consequently, the data were stratified on the presence or absence of the A2 allele for all further analyses. The A2 allele was observed in 27% of the subjects and 2.2% of subjects were homozygous for A2. There was no difference in the A2 present compared with A2 allele absent groups in their history of medication use that might influence bone metabolism. The A2 allele was initially examined using both a codominant and a dominant model. A dosing association of the number of A2 alleles with the phenotypic variables was not evident, with only the dominant model being associated with significant differences. As both the A1 allele and A2 allele have seven repeats, we specifically examined the combination of the presence of either an A1 and/or A2 allele with other allele combinations, or the presence of an A1 allele with other allele combinations, and in neither case did we find significant associations with any bone or biochemical measures. Therefore, all analyses reported are comparing the A2 present compared with the A2 allele absent groups.

CYP19 [TTTA]n alleles and demographic, anthropometric, and biochemical data. Demographic, anthropometric, and biochemical data are presented in Table 1. There was no difference in age, years since menopause, BMI, height, or weight between the A2 allele present group compared with the A2 allele absent group. The FEI was higher and the deoxypyridinoline creatinine ratio was lower in the A2 allele present group compared with the A2 allele absent group. No other biochemical variables were different between the groups. In addition, the FEI was negatively correlated with the deoxypyridinoline creatinine ratio (r = −0.25, P < 0.001). The analysis based on low and high repeats was not able to demonstrate a significant difference between the two groups.

Effect of CYP19 [TTTA]n alleles on BMD, QUS, and pQCT parameters. BMD was higher at all of the hip sites, except for the femoral neck where the significance was marginal, and at the lumbar spine, in the A2 present compared with the A2 absent group (Fig. 2). This remained significant after adjustment for weight at all sites except for the femoral neck. The higher mean hip BMD between the A2 present compared with A2 absent groups was 3.4% for the total hip, 2.3% for the

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Table 1. Characteristics of the subjects with and without a TTNA A2 allele

<table>
<thead>
<tr>
<th></th>
<th>TTNA A2 Absent</th>
<th>TTNA A2 Present</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>75.1±2.7</td>
<td>75.2±2.6</td>
<td>NS</td>
</tr>
<tr>
<td>Years since menopause</td>
<td>27.3±6.4</td>
<td>26.7±6.6</td>
<td>NS</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>68.3±12.2</td>
<td>69.2±12.4</td>
<td>NS</td>
</tr>
<tr>
<td>Height, cm</td>
<td>158.8±6.0</td>
<td>158.5±5.8</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>27.1±4.6</td>
<td>27.5±4.8</td>
<td>NS</td>
</tr>
<tr>
<td>Dietary calcium intake, mg/day</td>
<td>863±335</td>
<td>853±311</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking, pack-yr</td>
<td>6.3±15.8</td>
<td>6.0±15.6</td>
<td>NS</td>
</tr>
<tr>
<td>Alcohol consumption, g/day</td>
<td>6.0±8.4</td>
<td>6.7±9.6</td>
<td>NS</td>
</tr>
<tr>
<td>Estradiol, pmol/l</td>
<td>275±18.6</td>
<td>28.6±15.9</td>
<td>NS</td>
</tr>
<tr>
<td>Sex hormone binding globulin, nmol/l</td>
<td>55.3±25.1</td>
<td>52.8±23.9</td>
<td>NS</td>
</tr>
<tr>
<td>Free estradiol index, mmol/mol</td>
<td>0.47±0.45</td>
<td>0.52±0.49</td>
<td>0.049</td>
</tr>
<tr>
<td>Parathyroid hormone, pmol/l</td>
<td>4.1±1.9</td>
<td>3.7±1.1</td>
<td>NS</td>
</tr>
<tr>
<td>Osteocalcin, μg/l</td>
<td>5.5±3.5</td>
<td>5.0±3.3</td>
<td>NS</td>
</tr>
<tr>
<td>Alkaline phosphatase, U/l</td>
<td>79.2±22.8</td>
<td>80.4±19.1</td>
<td>NS</td>
</tr>
<tr>
<td>Calcium creatinine ratio</td>
<td>268±216</td>
<td>282±189</td>
<td>NS</td>
</tr>
<tr>
<td>Deoxypyridinoline creatinine ratio</td>
<td>30.34±10.4</td>
<td>27.1±9.1</td>
<td>0.027</td>
</tr>
<tr>
<td>Plasma calcium, mmol/l</td>
<td>2.29±0.17</td>
<td>2.32±0.15</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma phosphorus, mmol/l</td>
<td>1.15±0.15</td>
<td>1.14±0.13</td>
<td>NS</td>
</tr>
</tbody>
</table>

Results are means ± SD. P values calculated by unpaired t-test or Mann-Whitney U-test as described in MATERIALS AND METHODS. NS, not significant.
femoral neck, 3.6% for the intertrochanter, and 4.1% for the trochanter. The presence of an A2 allele was associated with a mean lumbar spine BMD 12.7% higher compared with the absence of an A2. After adjustment for FEI, the differences between the A2 allele present and absent groups were no longer statistically significant at the total hip, intertrochanteric, or femoral neck sites. The FEI accounted for between 42 and 67% of the difference between the A2 present and A2 absent groups at the hip sites and all of the difference between the A2 present and A2 absent groups at the lumbar spine. There was a significant interaction between the presence or absence of an A2 allele and age with femoral neck BMD ($P = 0.026$), which demonstrated an increasing difference in femoral neck BMD between the two allele groups with increasing age, suggesting a protective effect of the A2 allele against bone loss associated with ageing at the femoral neck. This interaction was still present ($P = 0.006$) after adjustment for the FEI.

The QUS parameters were higher in the A2 present compared with A2 absent group with differences of 1.3% for BUA, 0.4% for SOS, and 3.7% for stiffness (Fig. 3). These all remained significantly different after adjustment for weight. After adjustment for FEI, the difference between the A2 absent and present groups remained significant for SOS and stiffness, with the FEI accounting for between 35 and 39% of the difference between the A2 present and A2 absent groups for the QUS parameters.

The tibia pQCT data showed that total, trabecular, and cortical BMD were 3.4, 3.3, and 4.3%, respectively, higher in the A2 present compared with the A2 absent groups (Fig. 4). These differences were no longer significant after adjustment for the FEI, with the FEI accounting for 62, 61, and 53% of the difference for the femoral neck, 2.3% at the intertrochanter, and 2.9% at the trochanter. Spine BMD was 5.7% higher in the low compared with high allele group. The QUS parameters BUA, SOS, and stiffness were not significantly different between the high and low repeat number present compared with the low repeat number group nor were the pQCT, BMD, or SSI parameters.

The analysis based on low and high repeats demonstrated that all of the hip BMD parameters were higher in the low repeat number present compared with the high repeat number group. The difference in mean hip BMD in the low repeat number present compared with high repeat groups was 2.3% at the total hip, 1.9% at the femoral neck, 2.3% at the intertrochanter, and 2.9% at the trochanter. Spine BMD was 5.7% higher in the low compared with high allele group. The QUS parameters BUA, SOS, and stiffness were not significantly different between the high repeat number present compared with the low repeat group nor were the pQCT, BMD, or SSI parameters.

**Relationship between CYP19 [TTTA]n alleles and osteopenia and osteoporosis.** In this study population, 34.5% had normal BMD, 52.7% were osteopenic, and 12.2% were osteoporotic at the total hip site according to WHO criteria. Those subjects with an A2 allele were less likely to be osteopenic (OR 0.72, 95% CI 0.52–0.99). The decreased risk of being osteoporotic with an A2 allele did not achieve statistical significance (OR 0.64, 95% CI 0.38–1.05). When the osteopenic and osteoporotic (i.e., low BMD) subjects were considered together, an A2 allele was associated with a decreased risk of low BMD (OR 0.70, 95% CI 0.52–0.95). After adjustment for the FEI, those subjects with an A2 allele were no longer less likely to have low BMD (OR 0.92, 95% CI 0.65–1.31).

The analysis based on low and high TTTAn repeats demonstrated that those subjects with a low repeat number allele were less likely to be in the osteoporotic group (OR 0.60, 95% CI 0.38–0.95). The association of low repeat numbers with osteopenia did not reach statistical significance (OR 0.78, 95% CI 0.58–1.04) compared with those subjects who had low repeat number alleles. When the osteopenic and osteoporotic groups were considered together, a low number of repeats was associated with a decreased risk of low BMD (OR 0.74, 95% CI 0.56–0.98).

**Relationship between CYP19 [TTTA]n alleles and vertebral fracture.** Vertebral fracture, determined by MXA, was assessed on 976 subjects. Of these, 16.1% had one or more anterior
[TTTA]n microsatellite was also associated with vertebral fracture in those subjects who had osteoporosis as defined by a total hip DXA BMD.

A previous study examining the association of the CYP19 TTTA microsatellite with bone mass and fracture risk reported that a short TTTA repeat was associated with a higher risk of osteoporosis at the spine and vertebral fracture (16). This is opposite to the pattern observed in our study, where the shorter TTTA repeat was associated with a higher BMD. The ethnically homogeneous Italian population studied by Masi et al. (16) is considerably different from the population reported in the present study of postmenopausal women of Northern European origin. Our population was much older (75 compared with 57 yr of age) and had a much lower rate of WHO-defined osteoporosis (12.6 compared with 52.8%). As the prior clinical diagnosis of osteoporosis was an exclusion from this study, this is likely to account for the relatively low prevalence of DXA-defined osteoporosis in this population. Another population-based study that used the Danish Osteoporosis Prevention Study (DOPS) failed to find an association between CYP19 TTTA microsatellite and BMD in young postmenopausal women, but it did find an interaction with this polymorphism to the BMD response to hormone replacement therapy (29). The reason why our population-based study differed from the DOPS population-based study in the association of BMD with the TTTA microsatellite polymorphism may be due to the difference in ages of the two study cohorts or may be due to the difference in the analysis methods. The variation in results between this and other studies is similar to the discrepant results observed with the association of breast cancer and the CYP19 TTTA microsatellite polymorphism, where some studies show an increased breast cancer risk associated with a [TTTA]12 allele (7, 15), association with other allele lengths (2), or decreased breast cancer risk associated with the [TTTA]12 allele (23). The present study was comprehensive enough to allow a priori testing for effects on bone density at the spine site and QUS at the heel, together with effects on biochemical parameters and vertebral fracture, after the initial determination of the significant association of a TTTA A2 allele on hip BMD. The DOPS study analysis relied on a strategy of comparing “short” and “long” allele lengths (29), which may have obscured differences associated with specific

**DISCUSSION**

In this study, we demonstrated an association of the CYP19 [TTTA]n microsatellite polymorphism with a higher FEI, a lower deoxypyridinoline creatinine ratio, higher DXA hip and spine BMD, higher calcaneal QUS parameters, and higher pQCT BMD and SSI parameters in subjects who had a TTTA A2 allele. The association of the [TTTA]n microsatellite polymorphism with DXA and pQCT BMD and QUS parameters was related, in part, to its association with the FEI. The CYP19 [TTTA]n microsatellite was also associated with vertebral fracture in those subjects who had osteoporosis as defined by a total hip DXA BMD.

A previous study examining the association of the CYP19 TTTA microsatellite with bone mass and fracture risk reported that a short TTTA repeat was associated with a higher risk of osteoporosis at the spine and vertebral fracture (16). This is opposite to the pattern observed in our study, where the shorter TTTA repeat was associated with a higher BMD. The ethnically homogeneous Italian population studied by Masi et al. (16) is considerably different from the population reported in the present study of postmenopausal women of Northern European origin. Our population was much older (75 compared with 57 yr of age) and had a much lower rate of WHO-defined osteoporosis (12.6 compared with 52.8%). As the prior clinical diagnosis of osteoporosis was an exclusion from this study, this is likely to account for the relatively low prevalence of DXA-defined osteoporosis in this population. Another population-based study that used the Danish Osteoporosis Prevention Study (DOPS) failed to find an association between CYP19 TTTA microsatellite and BMD in young postmenopausal women, but it did find an interaction with this polymorphism to the BMD response to hormone replacement therapy (29). The reason why our population-based study differed from the DOPS population-based study in the association of BMD with the TTTA microsatellite polymorphism may be due to the difference in ages of the two study cohorts or may be due to the difference in the analysis methods. The variation in results between this and other studies is similar to the discrepant results observed with the association of breast cancer and the CYP19 TTTA microsatellite polymorphism, where some studies show an increased breast cancer risk associated with a [TTTA]12 allele (7, 15), association with other allele lengths (2), or decreased breast cancer risk associated with the [TTTA]12 allele (23). The present study was comprehensive enough to allow a priori testing for effects on bone density at the spine site and QUS at the heel, together with effects on biochemical parameters and vertebral fracture, after the initial determination of the significant association of a TTTA A2 allele on hip BMD. The DOPS study analysis relied on a strategy of comparing “short” and “long” allele lengths (29), which may have obscured differences associated with specific
TTTA repeat lengths. In our study, we found the comparison of short and long alleles to be a less powerful analytic method than selecting the precise allele that was associated with a difference in bone phenotypes.

The differences in DXA and pQCT BMD, calcaneal QUS, and SSI parameters observed in this study are associated with a lower mean deoxypyridinoline creatinine ratio and a higher FEI. This indicates that a CYP19 A2 allele is associated with decreased bone resorption, perhaps as a result of the higher endogenous estrogen concentration associated with this allele. It has been previously reported that the low endogenous concentrations of estrogen present in late menopausal women are associated with decreased bone resorption and that suppression of this endogenous estrogen production results in increased bone resorption, but not bone formation (10), consistent with our observations.

In this study we demonstrated, in those subjects who had osteoporosis, that the presence of an A2 allele was associated with a greatly reduced risk of vertebral fracture. The CYP19 TTTA microsatellite has been previously reported to be associated with vertebral fracture (16). The increased prevalence of vertebral fracture with an A2 allele was independent of the FEI. Aromatase has direct target organ-specific effects that can result in increased levels of estrogen in target tissues, without substantially affecting circulating estrogen concentrations (24). Such effects would therefore not be assessed by measurement of the FEI so it is possible that the TTTA microsatellite polymorphism is associated with other factors affecting aromatase activity not accounted for by the FEI measurement. We observed that the FEI was associated with between 35 and 39% of the difference observed in the QUS parameters, between 42 and 67% of the difference for hip BMD and accounted for all of the difference in lumbar spine BMD, between the A2 allele present and absent groups. It is important to note that circulating estradiol concentration was measured in a sensitive and reproducible assay. Nevertheless, the circulating concentrations in the two CYP19 TTTA microsatellite groups were not different. An association with circulating estrogen was only evident after adjustment for SHBG, which was slightly but not significantly lower in the A2 group. We are aware that these data do not completely support the concept that an A2 allele has its effects on bone as a result of effects on circulating bioactive estrogen.

The CYP19 TTTA repeat is present in an intronic region that is not associated with gene regulation or with posttranslational expression. Therefore, it is likely that the association we observed is the result of the CYP19 TTTA microsatellite polymorphism being in linkage disequilibrium with a potentially causative polymorphism. This could also account for the reversed association between TTTA allele lengths and bone parameters observed between this and other studies. It is not clear why the association we observed with higher bone mass was with the TTTAn = 7 allele and not also with the TTTAn = 7(-3) allele. This suggests that the base deletion/insertion polymorphism that results in the TTTAn = 7(-3) allele is associated with a putative causative polymorphic site independently of the TTTAn = 7 allele. A previous association of the insertion/deletion polymorphism with premenopausal breast cancer risk has been published, indicating that this is a possibility (28). Further studies are therefore required to ascertain potentially functional polymorphisms in the CYP19 gene and to validate their biological function in regulating estrogen synthesis and bone structure. An example of such a polymorphism is the C-T substitution at base pair 1558 in exon 10 (26), which has been reported to be in linkage disequilibrium with the TTTA microsatellite polymorphism (15).

In conclusion, a significant association of the CYP19 TTTA microsatellite with BMD, bone strength, and vertebral fracture was observed in elderly women. This may be associated with endogenous estrogen concentration and subsequent effects on bone turnover. Given that associations between polymorphisms in the CYP19 gene and bone-related phenotypes have now been observed in a number of studies, further investigation to elucidate the functional polymorphisms in the CYP19 gene and their mechanisms of action should be pursued.

REFERENCES


GRANTS

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