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

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Running Title: CYP19 polymorphisms and bone

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Abstract

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 1257 women aged 70 years and greater. The data obtained was 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 (DXA) BMD, which was present in 27% of the population. The presence of an A2 allele was associated with a higher free estradiol index (FEI) (0.52 ± 0.49, p=0.049) compared to 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 (QUS) parameters BUA (1.3%), SOS (0.4%) and stiffness (3.7%) and higher peripheral quantitative computed tomography (pQCT) 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 x (6.8%) values. A lower deoxypryridinoline creatinine ratio (DpdCr) 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, CI 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.
Key Words: estradiol; aromatase; CYP19; polymorphisms; fracture; bone density
Introduction

Osteoporosis is a common disease of bone that is strongly genetically determined as shown by various family studies (4, 13, 21). We and others have shown 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. Based on 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 have 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 1499 Caucasian women aged between 70 and 85. They were recruited by letter from the population aged 70 years 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 years 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-year 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 1332 consented to having a blood sample taken for the isolation of DNA and the CYP19 TTTA microsatellite polymorphism was examined in 1170 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 base pair 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 1129 of these subjects using a Lunar Achilles Ultrasound machine (Lunar Corp, Madison, WI USA). The manufacturer’s quality assurance methods were employed. The averaged measurement of the Speed of Sound (SOS), Broadband Ultrasound (BUA) and Stiffness were reported. The C.V. for SOS and BUA using the manufacturer’s standards were 0.43% and 1.59% respectively. BMD measurements of the hip on 1065 subjects were carried out using an Hologic Acclaim 4500A detector fan beam densitometer (Hologic, Waltham, MA, USA), 12 months after the commencement of the study. The C.V. at the total hip site was 1.0% (9). Lumbar spine BMD measurements were available for 211 subjects. The C.V. at the spine was 1.1% (9). Peripheral quantitative computed tomography (pQCT) bone structure and density was measured on 1048 subjects at five years at the radius at a site 4% of the length of the tibial shaft to the ankle, using a Stratec XCT 2000 pQCT (Stratec Medizintechnik GmbH, Pforzheim Germany). The voxel size was set at 150µ in the x and y direction and 1000µ in the z direction, which increased the scan time to five minutes. 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/cm³ and an endosteal threshold of 169 mg/cm³ 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 (1200 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).
MXA analysis

Dual-energy high-definition lateral MXA scans of vertebra T4-L4 (Hologic QDR 4500 A) where available for 976 of the subjects who had a CYP19 genotype. The positions of six reference markers for each vertebra were placed at the corners and in the middle of the upper and lower surface of each vertebra as described by the operator’s manual. The mean coefficient of variation in MXA measurements of the anterior and posterior heights of vertebrae L4 to T12 was 4.8% determined by the one operator who undertook the scans and point placement on duplicate measurements from 30 subjects. Vertebral heights reference data were calculated according to a modification of the procedure described by McCloskey (17).

Demographic, Anthropometric and Lifestyle Factors

All subjects completed a lifestyle questionnaire that included age at menopause and smoking history. A quantitative food frequency questionnaire (11) was used to ascertain the average daily alcohol and calcium intake. Weight and height were measured. Body mass index (BMI) was calculated as height/(weight)².

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 analysed for creatinine, calcium, and phosphorus, using routine methods (BM/Hitachi 747 Analyser, Boehringer Mannheim GmbH, Mannheim Germany). Urine deoxypridinoline (DPD) was measured by HPLC (20) and corrected for creatinine excretion. The blood samples were analysed for alkaline phosphatase (ALP), creatinine, calcium, and phosphorus, using routine methods (BM/Hitachi 747 Analyser, Boehringer Mannheim GmbH, Mannheim Germany). Serum osteocalcin (OC) was determined using radioimmunoassay (RIA) techniques as previously described (14, 19). Serum estradiol was measured by radioimmunoassay (Orion Diagnostica, Espoo, Finland) on
1146 subjects. This assay had a sensitivity of 5 pmol/l and an inter assay C.V. of 20% at 18 pmol/l and 6.6% at 100 pmol/l and an intra-assay C.V. of 5% at 100 pmol/l. Sex hormone binding globulin (SHBG) was measured on these subjects using an immunochemiluminometric assay (Imulite, Los Angeles, CA). The intra- and inter-assay C.V. were 7.1% and 6.8% respectively at 24 nmol/l. Serum intact parathyroid hormone (PTH) was measured by immunochemiluminometric assay, (27) with an intra- and interassay coefficients of variation 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 base pair 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 labelled primers as described previously (18). Amplification conditions were as follows: 94°C for 4 mins 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); 72°C (1 min 20 s) for 15 cycles followed by a 7 min extension. The PCR product was electrophoresed in pre-heated (55°C) 6% polyacrylamide gel for 50 mins at 70W. Samples were visualised on the Hitachi FMBIO (Tokyo, Japan) using a 585nm 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 approximately 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 TTTA allele was tested using the Genepop program (http://wbiomed.curtin.edu.au/genepop/genepop_op1.html). A preliminary analysis
of the association of CYP19 TTTA 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 Inc, Chicago USA). The subjects were divided into two groups depending on whether or not 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 was 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 deoxypyridonoline creatinine ratio and the FEI index were calculated to normalize the distribution of these variables. QUS paramteres were compared between groups using Student’s T test. BMD and pQCT were measured at one year and five years respectively after commencement of calcium treatment, therefore the estimated means of BMD and pQCT parameters were calculated after adjustment 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 less than or equal to 1 S.D. below the premenopausal mean (≤ 0.855) were
classified as being osteopenic and those subjects with a T score for hip BMD of less than or equal to 2.5 S.D. below the premenopausal mean ($\leq 0.675$) were classified as being osteoporotic according to 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 to the osteopenic and osteoporotic groups was calculated using logistic regression analysis, adjusting for the free estradiol index and the results reported as the Odds Ratio (OR) and 95% Confidence Intervals (CI).
Results

CYP19 [TTTA]n allele distribution

TTTA genotyping resulted in 8 different PCR fragments ranging in size from 168 to 191 base pairs, designated A1 to A8. The frequency of each TTTA allele is illustrated in figure 1. This resulted in the presence of 20 different genotypes being present in the population, which was in 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 was 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 to 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 to the A2 allele absent groups.

CYP 19 [TTTA]n alleles and Demographic, Anthropometric and Biochemical data

Demographic, anthropometric and biochemical data is presented in Table 1. There was no difference in age, years since menopause, BMI, height or weight between the A2 allele present group compared to the A2 allele absent group. The FEI was higher and the deoxypyridinoline creatinine ratio was lower in the A2 allele present group compared to 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.

**The effect of CYP 19 [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 to the A2 absent group (figure 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 to A2 absent groups was 3.4% for the total hip, 2.3% for the 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 to 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 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 to A2 absent group with differences of 1.3% for BUA, 0.4% for SOS and 3.7% for stiffness (figure 3). These all remained significantly different after adjustment for weight. After adjustment for FEI, the
The relation between CYP 19 [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 (ie 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 free estradiol index, 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 (O.R. 0.60, 95% C.I. 0.38.-0.95). The association of low repeat numbers with osteopenia did not reach statistical significance (O.R. 0.78, 95% C.I. 0.58-1.04) compared to 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).

The relation between CYP 19 [TTTA]n alleles and vertebral fracture
Vertebral fracture, determined by morphometric x-ray absorptiometry (MXA), was assessed on 976 subjects. Of these, 16.1% had one or more anterior wedge fractures (AW), 17.8% had one or more crush fractures and 5.6% had one or more central collapse fractures. A combination of AW and crush fractures occurred in 3.7% of subjects. The various types of fracture were subsequently analyzed together, with 33.5% of subjects having one or more vertebral fractures. A strong interactive effect between WHO defined bone density groups and vertebral fracture with an A2 allele was observed (figure 6). In the osteoporotic group, the presence of an A2 allele was associated with a lower risk of vertebral fracture (OR 0.27, CI 0.09-0.79). This was not observed in osteopenic or normal subjects (figure 6).
There was no difference in the prevalence of vertebral fractures in the osteoporotic subjects who had an A2 allele compared to those subjects who had an A2 allele and had a hip BMD T score greater than -2.5 ($\chi^2 = 1.65, p=0.2$) (figure 6). The high prevalence of vertebral fractures in the osteoporotic subjects who did not have an A2 allele was highly significantly different from those subjects who did not have an A2 allele and who had a hip BMD T score greater than -2.5 ($\chi^2 = 11.26, p=0.001$) (Figure 6). After adjustment for the FEI, the association in the osteoporotic group between the A2 allele and vertebral fracture remained significant (OR 0.21, CI 0.07-0.68). When a high repeat containing allele group was compared to the low repeat allele group, no significant difference in vertebral fracture was observed, either considered as a whole group (OR 0.90, CI 0.69-1.18), or in the osteoporotic group alone (OR 0.53, CI 0.23-1.24), for the short compared to the long repeat allele groups.
Discussion

In this study we have demonstrated an association of the CYP19 [TTTA]n microsatellite polymorphism with a higher FEI, a lower deoxypryridinoline creatinine ratio and 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 is considerably different to the population reported in the present study of postmenopausal women of Northern European origin. Our population was much older (75 compared to 57 years of age) and had a much lower rate of WHO defined osteoporosis (12.6% compared to 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 utilized the Danish Osteoporosis Prevention Study (DOPS) failed to find an association between CYP19 TTTA microsatellite and BMD in young postmenopausal women, but did find an interaction with this polymorphism to the BMD response to HRT (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 analytical method than determining 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 post-translational expression. Therefore, it is likely that the association we have 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 3 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.
Acknowledgements

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References


9. **Henzell S, Dhaliwal S, Pontifex R, Gill F, Price R, Retallack R, and Prince R.** Precision error of fan-beam dual X-ray absorptiometry scans at the spine, hip, and


Figure Legends

Figure 1
The distribution of CYP19 TTTA alleles in the population

Figure 2
The association of the CYP19 TTTA A2 allele with hip and spine bone mineral density. Results are mean± S.E.M.
*p<0.05, **p<0.01, ***p<0.001 compared to A2 absent

Figure 3
The association of the CYP19 TTTA A2 allele with calcaneal QUS. Results are mean± S.E.M.
*p<0.05, **p<0.01, ***p<0.001 compared to A2 absent

Figure 4
The association of the CYP19 TTTA A2 allele with tibial pQCT BMD. Results are mean± S.E.M.
*p<0.05, **p<0.01 compared to A2 absent

Figure 5
The association of the CYP19 TTTA A2 allele with tibial Stress Strain Index (SSI). Results are mean± S.E.M.
*p<0.05 compared to A2 absent

Figure 6
The interaction between total hip BMD T score and the presence or absence of a TTTA A2 allele and the presence of a vertebral fracture.
Results are the percentage of subjects within the A2 allele group category.

* p=0.01 compared to A2 allele absent group, using Fisher’s exact test

Breslow-Day test for homogeneity $\chi^2 = 6.68$, p=0.035
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<th>TTTA A2 absent</th>
<th>TTTA A2 present</th>
<th>P value</th>
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<td>Age (years)</td>
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<td>Years since menopause</td>
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<td>Height (cm)</td>
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<td>863±335</td>
<td>853±311</td>
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<tr>
<td>Alkaline phosphatase (U/l)</td>
<td>79.2±22.8</td>
<td>80.4±19.1</td>
<td>N.S.</td>
</tr>
<tr>
<td>Calcium creatinine ratio</td>
<td>268±216</td>
<td>282±189</td>
<td>N.S.</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>N.S.</td>
</tr>
<tr>
<td>Plasma phosphorus (mmol/l)</td>
<td>1.15±0.15</td>
<td>1.14±0.13</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

Table 1 Characteristics of the subjects with and without a TTTA A2 allele. Results are mean±S.D. P values calculated by unpaired T Test or Mann Whitney U test as described in the methods section.
Figure 1

Allele frequency (%)

CYP19 TTTA repeats

A1 A2 A3 A4 A5 A6 A7 A8
Figure 2

DXA BMD

Bone mineral density (mg/cm²)

TTTA A2 absent
TTTA A2 present

Total hip  Femoral neck  Trochanter  Intertrochanter  Lumbar spine

**  ***  **  **
Figure 3

QUIS of the ankle

![Graphs showing BUA (Db/MHz), SOS (m/sec), and Stiffness (% Mean young)]
Figure 4

pQCT of the tibia

- TTTA A2 absent
- TTTA A2 present

BMD (mg/cm³)

**

Total

Trabecular

Cortical

*
Figure 5

pQCT bone strength of the tibia

SSI (mm$^3$)
Figure 6

A 3D bar chart showing the percentage of vertebral fractures (% of A2 allele group) for three conditions: Osteoporosis, Osteopenia, and Normal, with two allele status: A2 Allele Absent and A2 Allele Present. The chart indicates a significant difference (*) in vertebral fracture rates between the two allele groups for the Osteopenia condition.