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Associations between genetic variants and the effect of letrozole and exemestane on bone mass and bone turnover

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Abstract

Adjuvant therapy for hormone receptor (HR) positive postmenopausal breast cancer patients includes aromatase inhibitors (AI). While both the non-steroidal AI letrozole and the steroidal AI exemestane decrease serum estrogen concentrations, there is evidence that exemestane may be less detrimental to bone. We hypothesized that single nucleotide polymorphisms (SNP) predict effects of AIs on bone turnover. Early stage HR-positive breast cancer patients were enrolled in a randomized trial of exemestane versus letrozole. Effects of AI on bone mineral density (BMD) and bone turnover markers (BTM), and associations between SNPs in 24 candidate genes and changes in BMD or BTM were determined. Of the 503 enrolled patients, paired BMD data were available for 123 and 101 patients treated with letrozole and exemestane, respectively, and paired BTM data were available for 175 and 173 patients, respectively. The mean change in lumbar spine BMD was significantly greater for letrozole-treated (−3.2 %) compared to exemestane-treated patients (−1.0 %) ($p = 0.0016$). Urine N-telopeptide was significantly increased in patients treated

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Compliance with ethical standards

Conflict of Interest NLH received research funding from Astra-Zeneca. DFH received research funding during the conduct of this trial from Novartis and Pfizer, and currently receives research funding from Veridex and Janssen Diagnostics. DAF receives research funding from Novartis and Pfizer and is a member of the Scientific Advisory Board for Quest Diagnostics, Inc. VS receives research funding from Abbott, Abraxis, Merck, Novartis, and Pfizer. JMR received a research grant from Pfizer. TNH receives research funding from Shire. TCS, JD, LL, KMK, CVP, CG, ATN, ZD, SO, SP, JSC, and AMS reported no conflicts of interest.

with exemestane ($p = 0.001$) but not letrozole. Two SNPs (rs4870061 and rs9322335) in ESR1 and one SNP (rs10140457) in ESR2 were associated with decreased BMD in letrozole-treated patients. In the exemestane-treated patients, SNPs in ESR1 (Rs2813543) and CYP19A1 (Rs6493497) were associated with decreased bone density. Exemestane had a less negative impact on bone density compared to letrozole, and the effects of AI therapy on bone may be impacted by genetic variants in the ER pathway.

Keywords

Aromatase inhibitors; Breast cancer; Bone health; Polymorphism; Pharmacogenomics

Introduction

Aromatase inhibitors (AI) are superior to tamoxifen for the treatment of hormone receptor (HR) positive post-menopausal breast cancer in the neoadjuvant, adjuvant, and metastatic settings [1]. In the adjuvant setting, AIs are currently prescribed for 5 years, and studies are ongoing to determine whether patients will benefit from more prolonged therapy [2]. However, there is concern that along with the potential for additional benefit there could also be detrimental effects of long-term therapy, including negative effects on bone health.

AIs suppress production of estrogens, which is believed to result in toxicity in a subset of treated patients [3]. These include, but are not limited to, osteoporosis and the attendant fracture risks [4]. Large trials of AI versus tamoxifen therapy have demonstrated a statistically significantly increased fracture incidence in AI-treated patients [5]. Similarly, in a study of letrozole versus placebo following 5 years of tamoxifen, bone mineral density (BMD) was significantly lower in both the hip (-3.6 vs. 0.7 %) and spine (-5.35 vs. -0.7 %) after 24 months of letrozole compared to placebo. In addition, after both 12 and 24 months, the bone turnover marker (BTM) N-telopeptide (NTX) was increased in the letrozole-treated patients [6].

AIs can be divided into steroidal (exemestane) and non-steroidal (anastrozole and letrozole) inhibitors. There is evidence that exemestane may have less detrimental effects on bone compared to non-steroidal AIs. In rat models, exemestane treatment prevented bone loss induced by ovariectomy [7]. In healthy postmenopausal women, exemestane (but not letrozole or anastrozole) increased serum procollagen type I N-terminal propeptide (PINP) suggesting exemestane-specific effects on bone formation [8]. Nevertheless, exemestane has been shown to cause more bone loss than anticipated from aging alone [9, 10]. The open label Phase III randomized clinical trial MA.27 bone subprotocol (MA.27B), in which exemestane and anastrozole were directly compared, failed to demonstrate a statistically significant difference in spine or hip BMD at 2 years, although there were suggestions that exemestane may have mild bone sparing effects based on differences in rates of osteoporosis diagnosis and new prescription of bisphosphonate medication between the two groups [11, 12]. It is of note that MA.27B was originally planned for longer follow-up but was truncated when the parent study MA.27 closed [11]. Hence, further exploration of the differential bone effects of steroidal and non-steroidal AI remains clinically relevant [11, 12].

A number of studies have suggested that single nucleotide polymorphisms (SNPs) may impact efficacy and/or side effects of AI treatment [13–18]. We hypothesized that SNPs associated with loss or preservation of BMD can be identified in AI-treated patients, and that the SNPs may differ depending on the class of AI medication. To test this hypothesis, a pre-planned secondary analysis was performed within the prospective multicenter exemestane and letrozole pharmacogenetics (ELPh) trial conducted by the consortium on breast cancer pharmacogenomics (COBRA), in which patients were randomized to treatment with exemestane or letrozole. Within the ELPh trial, associations between patient factors, including germline SNPs, and both BMD and BTMs were assessed.

Materials and methods

Study design and patient selection

The details of this study's design and conduct have previously been described [19, 20]. Briefly, postmenopausal women with HR-positive breast cancer who were starting AI therapy were recruited between 2005 and 2009 and enrolled in the ELPh trial (clinicaltrials.gov #NCT00228956). All indicated surgery, chemotherapy, and radiation therapies for breast cancer were completed before enrollment. Following enrollment, women were randomly assigned to treatment with exemestane (25 mg) or letrozole (2.5 mg) daily for 2 years. Randomization was stratified based on prior adjuvant tamoxifen (yes/no), prior chemotherapy (yes/no), and current bisphosphonate therapy (yes/no). Vitamin D and calcium intake either in the diet or as a supplement was advised per current clinical practice, but use was not recorded. Although patients participating in ELPh were stratified by bisphosphonate use, this analysis of bone-focused endpoints excluded patients who were taking bisphosphonate therapy at baseline or who started treatment during study participation (for detail see consort diagram in Fig. 1). The protocol was approved by the IRBs of all participating study sites, and all enrolled patients provided written informed consent. The clinical trial was reviewed by an independent data and safety monitoring committee on a bi-annual basis.

Bone analysis

BMD was measured in the left hip and lumbar spine at baseline (BL) and after 24 months of therapy, using dual-energy x-ray absorptiometry (DXA). Standardized protocols were implemented, and the BMIL QA/QC Phantom [21] was used at each site for longitudinal calibration. A separate identical phantom was circulated among the study sites for scanner cross-calibration. The blinded bone densitometry data were sent to a centralized laboratory, the BioMedical Imaging Laboratory, Dayton, OH, for analysis (by TH). Based on the cross-calibration phantom data, all patient data were adjusted to one of the Hologic scanners, and *T* scores were extracted from the individual scan reports.

Sample collection and handling

Whole blood was collected at BL for DNA which was extracted from whole blood using QIAamp DNA Blood Maxi kit (Qiagen, Inc., Valencia, CA), as previously described [20]. In addition to whole blood for germline DNA, phlebotomy was performed for biochemical markers related to bone metabolism including bone alkaline phosphatase (BAP), total

alkaline phosphatase, phosphate, calcium, creatinine, 25-hydroxy vitamin D, and 1,25-dihydroxyvitamin D. Urinary type I cross-linked N telopeptides (NTx) and serum bone-specific BAP were analyzed using enzyme-linked immunoassay (ELISA) kits (Quidel® Corporation, San Diego, CA). Serum 25-hydroxy vitamin D and 1,25 dihydroxy vitamin D were analyzed using radioimmuno assays (RIA) (DiaSorin, Stillwater, MN). Serum phosphorus, alkaline phosphatase (Alk phos), calcium and creatinine, and urinary phosphorus, calcium, and creatinine were measured using a COBAS MIRA chemistry spectrophotometric analyzer (Roche Diagnostics, Indianapolis, IN). NTX concentrations were corrected for urine dilution by adjusting for the corresponding urine creatinine concentrations. The blood and corresponding 2-h urine specimens were collected in a non-fasting state at the time convenient for the patient at baseline and at 3 months.

Genotyping

At the time of study design, candidate single nucleotide polymorphisms (SNP) in 24 genes were identified from publications in the literature based on their critical role in ER signaling and their potential functional significance in a variety of AI-associated effects, including bone density, breast density, and hot flashes. In total, 174 candidate variants in 24 individual genes were identified and genotyped, as previously described [20], and are presented in Supplementary Table S1. Before the data were analyzed, 15 SNPs were excluded from the analysis as described in Supplementary Table S2, and thus a total of 159 variants in 24 genes were included in the final analysis.

The majority of SNPs were genotyped using the OpenArray™ platform (Applied Biosystems, Inc, Foster City, CA). Assays for which OpenArray™ assays could not be reliably developed were run using Taqman/PCR assays, as previously described [20]. To assure quality control, approximately ~10 % of the samples were re-genotyped, which showed 97 % concordance.

Statistical analysis

BTM and BMD measures are described with means and standard deviations for women who had data at BL and 3 months, and BL and 24 months, respectively. Two women were excluded from the BTM analyses for BAP and two excluded from the NTX analyses due to extreme outlying measures (defined as greater than two-fold larger than the next nearest values), which greatly skewed the distributions of percent changes in outcomes (BAP 279 and 855 %; NTX 1463 and 2017 %). Percent changes in BMD and BTM from BL to 24 or 3 months, respectively, were tested to be significantly different from zero using the sign test. To investigate the difference between treatments in the percent changes in BMD and BTM, *t* tests or Wilcoxon rank-sum tests were used. Three genetic models, additive, dominant, and recessive were applied to test for associations between SNPs and percent changes in BMD and BTM and absolute changes in hip and spine *T* scores. Osteopenia was defined as a *T* score between -1 and -2.5 and osteoporosis was defined as a *T* score less than or equal to -2.5. Proportions of women who fell into these categories were compared at BL and 24 months for women who had measures at both time points. Comparisons between the proportion of women who were osteopenic or osteoporotic at BL and at 24 months were made using Chi-Square or Fisher's exact test and between treatments using a logistic model.

In order to account for multiple genetic comparisons with 159 SNPs, a p value <0.00031 was used based on a Bonferroni correction. Data analyses were performed in SAS v 9.3 and R.0.2 using the SNPassoc and ggplot2 packages. R was also used to create the boxplots, where Q1 is the 1st quartile, Q3 is the 3rd quartile, IQR is the middle 50 % (interquartile range), and the end of the whiskers are at $(Q1-1.5*IQR)$ and $(Q3+1.5*IQR)$.

Results

Patient characteristics

Five hundred and three subjects were enrolled on the ELPh trial, and 500 were randomized to either exemestane or letrozole. Baseline characteristics of patients included in the analyses are given in Table 1. BMD was measured at BL and after 24 months of AI therapy (see consort diagram in Fig. 1; Table 2). For the letrozole group, paired BL and 24 month BMD assessments in the spine and hip were available for 123 and 122 eligible patients, respectively. For the exemestane group, paired BL- and 24-month BMD assessments in the spine and hip were available for 101 and 100 eligible patients, respectively. As previously reported, 32 % of patients discontinued treatment before the 24-month assessment, primarily because of intolerable side effects [19]. In addition, patients on bisphosphonate treatment at the time of study enrollment or who initiated treatment during study participation were ineligible for the bone analysis and were thus excluded. Of the 37 women who started on bisphosphonate after study enrollment, 24 (64.9 %) were on letrozole and 13 (35.1 %) were on exemestane ($p = 0.07$).

BTMs were measured at BL and after 3 months of therapy (Tables 3, S6). Serum BAP and urinary NTX were analyzed as the key markers of interest. For serum BAP, data from both time points were available for 175 letrozole-treated and 173 exemestane-treated patients (Fig. 1). For urinary NTX, data were available for 163 letrozole- and 160 exemestane-treated patients. The genotype–phenotype analyses were performed using data from all patients with paired measurements, with the exception of 26 patients for whom germline DNA was not available.

BMD in AI-treated patients

Letrozole-treated patients lost an average of 4.1 % (SD 4.4; $p < 0.001$) and 3.3 % BMD (SD 4.4; $p < 0.001$) in the hip and lumbar spine, respectively, during 24 months of therapy (Table 2; Suppl Fig. S1). In contrast, 24 months of exemestane therapy resulted in an average decrease in BMD of 3.2 % (SD 3.2; $p < 0.001$) and 1.2 % BMD (SD 6.1; $p < 0.001$) in the hip and the lumbar spine, respectively. Letrozole-treated patients lost significantly more BMD in the spine compared to exemestane-treated patients ($p = 0.001$), and there was a trend toward a greater loss at the hip with letrozole ($p = 0.075$).

Using T score data (Tables S3, S4), the number of patients with osteopenia increased from 34 to 71 at the hip ($p < 0.001$), and from 51 to 71 in the spine ($p = 0.043$) after 24 months of therapy, and there was no significant difference between letrozole- and exemestane-treated patients.

Analysis of associations between loss of BMD and covariates, including age, body mass index (BMI), and prior treatment with chemotherapy or tamoxifen, revealed a trend toward an association between prior tamoxifen use and a decrease in BMD in the hip ($p = 0.077$) (Table S5). In the spine, there were significant associations between change in BMD and age (for each additional year older, there was a greater decrease in BMD, $p < 0.001$), prior chemotherapy (less loss of BMD if prior chemotherapy, $p = 0.022$), and prior tamoxifen (less loss of BMD if prior tamoxifen therapy, $p < 0.001$).

Bone turnover marker (BTM) measurements

There was no significant change in BAP with either AI treatment group (Fig. 2a; Table 2). There was a significant increase in NTX in exemestane-treated patients ($p = 0.001$) but not in letrozole-treated patients (Fig. 2b; and Table 2).

Serum (total AP, phosphate, calcium, 25OH vitD, and 1,25(OH)₂ vitD) and urine (phosphate, calcium) markers of bone metabolism were assayed at BL and at 3 months (Table S6). We observed a statistically significant increase in serum AP and phosphate concentrations in patients treated with letrozole for 3 months ($p < 0.001$) but not in patients treated with exemestane. We also detected statistically significant increases in calcium and 25OH vitamin D concentrations with 3 months of AI therapy in both letrozole- and exemestane-treated patients, without statistically significant differences between the two groups.

Associations between SNPs and change in BMD in AI-treated patients

A total of 159 SNPs were analyzed for association with changes in BMD and BTM (Table S1). Using dominant, recessive, and additive statistical genetic models, we identified three SNPs in ESR1 (rs4870061, rs9322335, rs2813543), one SNP in ESR2 (rs10140457), and one in CYP19A1 (rs6493497) that were associated with change in BMD in AI-treated patients (Table 3). The association between the ESR1 intronic SNP rs4870061 and change of hip BMD in letrozole-treated patients was also identified when using *T* score measurements and is shown in Fig. 3.

Associations between SNPs and change in BTM in AI-treated patients

For the genetic association studies with BTM, we focused the analysis on associations between SNPs and changes in urine NTX and serum BAP. Using the recessive model, there was a significant increase in percent change in BAP in letrozole-treated patients with HTR2A SNP rs3742278 in VT/VT ($n = 6$) ($29.7 \pm 38.7\%$) compared to WT/WT ($n = 120$) and WT/VT ($n = 29$) ($2.9 \pm 15.6\%$) ($p = 0.0002$). This association was not seen in exemestane-treated patients or in the entire cohort. No statistically significant associations were identified between urinary NTX and SNPs for all patients or by AI medication.

Discussion

Here, we present data supporting the hypotheses (i) that the steroidal AI exemestane is associated with less bone loss compared to the non-steroidal AI letrozole, and (ii) that SNPs in the ER pathway are associated with changes in BMD and BTM in AI-treated patients.

The strengths of our study include the prospective nature of the analyses, the availability of accurate BMD measurements, and the relatively large study size for comparison of bone phenotypes between steroidal and non-steroidal AI-treated groups.

Our observation that AI therapy had detrimental effects on BMD over 2 years but that exemestane had a less negative impact compared to letrozole is consistent with prior reports. In MA.27 trial, patients treated with exemestane were less likely to report a diagnosis of osteoporosis compared to those treated with anastrozole [12]. However, in the MA.27B bone substudy results, in which a subset of patients were closely evaluated for change in bone parameters during AI therapy, there was no difference in change in BMD identified between anastrozole- and exemestane-treated patients [11, 22]. MA.27B did provide suggestions that exemestane may have mild bone sparing effects as demonstrated by the fact that fewer women with BMD *T* scores of -2.0 or higher who took exemestane started bisphosphonate therapy than women on anastrozole (14.5 and 20.6 %, respectively). One possible explanation for the differences between MA.27B and our study could be differences in sample size, since the ELPh trial had more evaluable patients. In addition, unlike MA.27B, the ELPh analysis excluded women treated with bisphosphonates and did not stratify by baseline BMD. Finally, the discrepancy could be due to differences between the two non-steroidal AI medications, since studies have demonstrated greater estrogen suppression with letrozole compared to anastrozole [23].

The differences observed in the ELPh study between exemestane and letrozole at 2 years were more pronounced in the spine and less in the hip. This effect of AI therapy is anticipated since estrogen has a greater effect on cancellous bone, which is found in the spine, compared to cortical bone, which is found in the hip, and more rapid loss of spine BMD at 2 years has been documented within other adjuvant AI studies [6, 24–28]. However, the mechanism underlying the differences in effects on bone between the two AIs remains unknown.

The bone formation marker, serum BAP, in ELPh demonstrated a similar result to that of MA.27.B which examined non-fasting serum N-terminal of procollagen type I propeptide (PINP) and the bone resorption marker serum NTX at baseline, 6, and 12 months [11]. Neither study identified a statistically significant difference in the formation of BTMs between the steroidal and non-steroidal AIs, or significant changes over time. In ELPh, but not in MA.27B, the marker of bone resorption demonstrated a statistically significant increase over time in the exemestane arm. However, this biochemically noted increase in bone resorption over 3 months in ELPh did not correlate with a change in BMD. Instead, the exemestane group experienced less bone loss. The finding that the serotonin gene HTR2A SNP rs3742278 is associated with an increase in BAP in the letrozole group is limited by the small sample size but is considered hypothesis-generating given the importance of serotonin in bone metabolism [29]. Interpretation of the BTM results may have been influenced by the methods of collection, which did not mandate the time of day of specimen donation or a fasting state, although the majority of patients were fasting at the time of sample collection.

We identified SNPs in estrogen-related genes ESR1, ESR2, and CYP19A1 (aromatase) that were associated with altered effects of AI treatment on bone. A recent report also identified

a SNP in the aromatase gene (rs700518) that was associated with bone loss in AI-treated breast cancer patients [30]. However, in this analysis we were unable to validate this association. Further, this SNP is not in LD ($R^2 = 0.008$; HAPMAP CEU) with the CYP19A1 SNP we found to be associated with changes in BMD (rs6493497). Nevertheless, the shared findings of association between CYP19A1 SNPs and response to aromatase inhibition in bone in both studies support the idea that aromatase is involved in mediating risk for bone loss in AI-treated women.

A recent GWAS study identified a number of SNPs in estrogen-regulated genes (CTSZ-SLMO2-ATP5E, TRAM2-TMEM14A, and MAP4K4 genes) associated with fractures in AI-treated women enrolled in the MA.27 trial [15]. However, these were not included in the list of candidate genes at the time of ELPh study design, and therefore, we did not test these SNPs in our study.

The most robust association we identified was between the loss of BMD and change in T score in letrozole-treated patients and an intronic ESR1 SNP (rs4870061). To our knowledge, there are no published studies associating this ESR1 SNP with bone or any other phenotypes [31], and it is also different from the ESR1 intronic SNP which we previously reported to be associated with discontinuation of treatment because of toxicity in AI-treated patients (rs9322336) [20]. The SNP is in close proximity to a DNase-hypersensitive site, with binding sites for MYC, STAT3, p300, FOS, and glucocorticoid receptor (<http://genome.ucsc.edu/>), thus making it likely that rs4870061 is a functional polymorphism. There is increasing evidence, for example provided through the results from the ENCODE project (www.genome.gov/encode), that intragenic regions harbor many highly conserved regulatory regions. There is also increasing support for germline (and somatic) variants in non-coding regulatory regions, such as identification of a highly significant association between 4 intronic SNPs in FGFR2 and breast cancer risk [32]. The lack of an identifiable association between rs4870061 and loss of BMD in exemestane-treated patients suggests that this intronic ESR1 SNP may play a role in the negative effect of letrozole on BMD. Alternatively, inability to observe an effect in patients treated with exemestane could be the result of an overall decreased effect of exemestane on bone loss and thus decreased statistical power to observe it.

Our study has some limitations, including the relatively small sample size for the phenotype–genotype association study. This occurred, in part, because the analysis of bone phenotypes was a secondary outcome in this trial. Therefore, patients were permitted to take bisphosphonate medications as clinically indicated, although it was necessary to exclude those patients from this analysis. In addition, because of the high rates of development of treatment-emergent side effects, a substantial proportion of patients discontinued study participation before the second BMD assessment. Second, we focused the genetic component of our study on the analysis of preselected SNPs involved in estrogen metabolism and activity, which, while circumventing false discovery problems associated with GWAS studies, results in inability to detect additional potentially significant associations. One additional issue that could affect interpretation of our results is the fact that participants were encouraged to consume adequate calcium and vitamin D, although the amount consumed by participants was not carefully recorded.

In summary, data from our prospective randomized trial support the possibility that the steroidal AI exemestane has a less negative impact on BMD compared to letrozole. We identified a number of SNPs potentially associated with bone loss in AI-treated patients, but these findings require confirmation in studies. Future decisions on choice of adjuvant endocrine therapy could be influenced by identifying genetic predisposition to treatment toxicity, including risk of rapid loss of BMD. A greater understanding of the interaction between AIs and bone could lead to better bone-health management options for postmenopausal breast cancer survivors who receive prolonged therapy with AI medications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Consort diagram for subjects with bone turnover markers (BTM), bone mineral density (BMD), and genotype information. Percentages (in parentheses) in bone and phenotype-genotype analyses are in comparison to total number of patients in exemestane- and letrozole-treated patients

Fig. 2. Change in bone turnover markers in letrozole- and exemestane-treated patients. Box-and-Whisker blots representing % change in blood BAP (**a**) and urine NTX (**b**)

Fig. 3.

Association between rs4870061 (ESR1 intron) and % change in bone mineral density and *T* score. Whisker blots representing % change in BMD (*left panel*), and change in *T* score (*right panel*) at the hip in letrozole-treated patients

Table 1
Baseline characteristics of patients included (and excluded) in bone turnover marker (BTM) and bone mineral density (BMD) analyses

Characteristic	Included in BTM analysis (<i>n</i> = 351)	Not included in BTM analysis (<i>n</i> = 60)	<i>p</i> [#]	Included in BMD analysis (<i>n</i> = 225)	Not included in BMD analysis (<i>n</i> = 186)	<i>p</i> [^]
Median age, range	59 (35–89)	55 (37–83)	0.010	59 (35–77)	56 (37–89)	0.35
Race			0.54			0.91
White	308 (87.7 %)	51 (85.0 %)		195 (86.7 %)	164 (88.2 %)	
Black	34 (9.7 %)	6 (10.0 %)		23 (10.2 %)	17 (9.1 %)	
Other	9 (2.6 %)	3 (5.0 %)		7 (3.1 %)	5 (2.7 %)	
Prior chemo	162 (46.2 %)	28 (46.7 %)	0.94	102 (45.3 %)	88 (47.3 %)	0.69
Prior tamoxifen	127 (36.4 %)	30 (50.0 %)	0.045	79 (35.3 %)	78 (42.2 %)	0.15
Mean BMI (kg/m ²)	30.72	29.38	0.10	30.6	30.5	0.67
Drug			0.29			0.025
Exemestane	173 (49.3 %)	34 (56.7 %)		102 (45.3 %)	105 (56.5 %)	
Letrozole	178 (50.7 %)	26 (43.3 %)		123 (54.7 %)	81 (43.5 %)	

p[#] value refers to comparison of indicated characteristic between patients included into BTM analysis, and those that were not

p[^] value refers to comparison of indicated characteristic between patients included into BMD analysis, and those that were not

BMI body mass index

Effect of letrozole and exemestane treatment on bone mineral density (BMD) after 24 months (M) of AI treatment (top two rows) and on bone turnover markers (BTM) after 3 months (M) AI treatment (bottom two rows)

Table 2

	Time point	All					Letrozole					Exemestane					p [#]	
		n	Mean	SD	p [^]	n	Mean	SD	p [^]	n	Mean	SD	p [^]	n	Mean	SD	p [^]	p [^]
BMD	BL	222	0.925	0.116	-	122	0.932	0.121	-	100	0.916	0.111	-	0.31				
Hip	24 M	222	0.891	0.118	-	122	0.894	0.123	-	100	0.887	0.112	-	0.66				
(g/cm ²)	% Change	222	-3.7	4.0	<0.001	122	-4.1	4.4	<0.001	100	-3.2	3.2	<0.001	0.075				
BMD	BL	224	1.013	0.138	-	123	1.021	0.148	-	101	1.004	0.124	-	0.36				
Spine	24 M	224	0.988	0.137	-	123	0.987	0.148	-	101	0.990	0.124	-	0.65				
(g/cm ²)	% Change	224	-2.4	5.3	<0.001	123	-3.3	4.4	<0.001	101	-1.2	6.1	0.046	0.001				
Serum	BL	346	22.7	9.9	-	173	23.0	10.9	-	173	22.5	8.8	-	0.73				
BAP	3 M	346	22.9	9.7	-	173	23.4	10.6	-	173	22.4	8.6	-	0.78				
(U/L)	% Change	346	2.5	18.0	0.23	173	3.9	17.2	0.11	173	1.0	18.6	1.0	0.10				
Urine	BL	321	44.8	24.4	-	161	46.9	27.4	-	160	42.7	20.8	-	0.52				
NTX	3 M	321	50.0	33.2	-	161	50.6	34.0	-	160	49.3	32.6	-	0.56				
(nM/mM)	% Change	321	22.4	64.3	<0.001	161	21.8	68.8	0.058	160	22.9	59.7	0.001	0.50				

Bone alkaline phosphatase (BAP) was measured in serum and N-telopeptide (NTX) in urine. *BL* baseline

[^] p value refers to comparison of BMD (or BTM) between BL and 24 M (or 3 M)

[#] p value refers to comparison between % change BMD (or BTM) in letrozole- and exemestane-treated patients

* Two outliers on letrozole excluded from BAP, two outliers from exemestane excluded from NTX analysis

Table 3

Significant associations between change in bone mineral density (BMD) or *T* score in aromatase inhibitor (AI)-treated patients, and single nucleotide polymorphisms (SNP) in candidate genes

AI	SNP/Gene	Location	Model	Genotype	<i>n</i>	Mean % Change BMD	SD	$p^{\wedge}$	<i>n</i>	Mean Change <i>T</i> score	SD	$p^{\#}$
Both	rs6493497	Hip	Rec	WT/WT, WT/VT	–	–	–	–	187	–0.37	0.44	4.6E–8
	CYP19A1			VT/VT	–	–	–	–	1	–2.90	0	
	rs4870061	Hip	Rec	WT/WT, WT/VT	–	–	–	–	191	–0.34	0.40	3.2E–7
	ESR1 intron			VT/VT	–	–	–	–	11	–1.07	1.01	
Letrozole			Add	WT/WT	–	–	–	–	134	–0.31	0.40	5.2E–5
				WT/VT	–	–	–	–	57	–0.39	0.40	
				VT/VT	–	–	–	–	11	–1.07	1.01	
	rs9322335	Spine	Rec	WT/WT, WT/VT	–	–	–	–	202	–0.29	0.47	3.5E–5
	ESR1 intron			VT/VT	–	–	–	–	8	0.44	0.63	
	rs4870061	Hip	Rec	WT/WT, WT/VT	100	–3.76	3.49	3.0E–4	98	–0.36	0.39	3.1E–6
	ESR1 intron			VT/VT	5	–10.94	12.71		6	–1.25	0.90	
	rs10140457	Spine	Add/Dom	WT/WT	105	–3.43	3.97	3.0E–4	–	–	–	
	ESR2 intron			WT/VT	6	3.08	6.92		–	–	–	
	Rs9322335	Spine	Rec	WT/WT, WT/VT	–	–	–	–	105	–0.35	0.40	5.2E–6
Exemestane	ESR1 intron			VT/VT	–	–	–	–	5	0.54	0.63	
	rs6493497	Hip	Rec	WT/WT, WT/VT	–	–	–	–	90	–0.30	0.38	1.6E–9
	CYP19A1			VT/VT	–	–	–	–	1	–2.90	0	
	rs2813543	Hip	Rec	WT/WT, WT/VT	–	–	–	–	97	–0.31	0.41	2.1E–4
	ESR1 downstream			VT/VT	–	–	–	–	2	–1.55	1.91	

Add additive, *Dom* dominant, *Rec* recessive

$p^{\wedge}$ refers to association between change in BMD and SNPs in candidate genes (genetic model is indicated)

$p^{\#}$ refers to association between change in *T* score and SNPs in candidate genes (genetic model is indicated)