Breast Cancer Res Treat (2011) 129:583–591
DOI 10.1007/s10549-011-1523-1

EPIDEMIOLOGY

The relationship between bone mineral density and mammographic density in Korean women: The Healthy Twin study

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Received: 15 September 2010 / Accepted: 12 April 2011 / Published online: 22 April 2011

Abstract Mammographic density is one of the strong risk factors for breast cancer. A potential mechanism for this association is that cumulative exposure to mammographic density may reflect cumulative exposure to hormones that stimulate cell division in breast stroma and epithelium, which may have corresponding effects on breast cancer development. Bone mineral density (BMD), a marker of lifetime estrogen exposure, has been found to be associated with breast cancer. We examined the association between BMD and mammographic density in a Korean population. Study subjects were 730 Korean women selected from the Healthy Twin study. BMD (g/cm^2) was measured with dual-energy X-ray absorptiometry. Mammographic density was measured from digital mammograms using a computer-assisted thresholding method. Linear mixed model considering familial correlations and a wide range of covariates was used for analyses. Quantitative genetic analysis was completed using SOLAR. In premenopausal women, positive associations existed between absolute dense area and BMD at ribs, pelvis, and legs, and between percent dense area and BMD at pelvis and legs. However, in postmenopausal women, there was no association between BMD at any site and mammographic density measures. An evaluation of additive genetic cross-trait correlation showed that absolute dense area had a weak-positive additive genetic cross-trait correlation with BMD at ribs and spines after full adjustment of covariates. This finding suggests that the association between mammographic density and breast cancer could, at least in part, be attributable to an estrogen-related hormonal mechanism.

Keywords Bone density · Breast neoplasms · Genetic variation · Mammography · Menopause

Abbreviation
BMD Bone mineral density

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Introduction

Higher mammographic density is a strong risk factor for breast cancer in both Caucasian and non-white women [1–7]. Mammographic density varies greatly among women, reflecting the relative amount of epithelial and connective tissue and fat tissue in a breast [8]. Mammographic density is associated with some of the established risk factors for breast cancer being related to endogenous and exogenous estrogen levels, such as parity and estrogen replacement therapy [8–11]. The associations of mammographic density with both breast cancer and hormone-related risk factors of breast cancer suggest that circulating estrogen exerts its effect on breast cancer development by

influencing mammary parenchyma [12, 13]. However, whilst estrogen is a potential unifying explanation for the association between mammographic density and breast cancer risk, there is limited evidence that circulating estrogen is directly associated with mammographic density [14–19].

Bone mineral density (BMD) is determined by the relative rate of bone resorption and formation, and is closely associated with the endogenous estradiol level [20–24] as well as exogenous estrogen therapy [25–27], which supports the use of BMD as a marker of lifetime estrogen exposure. BMD has also been shown to be positively associated with the risk of breast cancer [28–31] and this association is likely mediated by the effect of estrogen on both breast cancer development and bone remodeling.

Given the relation of BMD with estrogen and breast cancer, an evaluation of the association between BMD and mammographic density could give insight into whether or not a mechanism involving estrogen contributes to the association between mammographic density and breast cancer.

Several studies have evaluated the relationship between BMD and mammographic density with conflicting results [32–38].

In this context, we examined the association between BMD and mammographic density in a study of twins and their family members from a general Korean population. Indeed, this is the first study evaluating the association in an Asian population. Furthermore, the study design involving various family relationships enabled us not only to examine the association but also to explore the possible shared genetic factors influencing both BMD and the mammographic density.

Materials and methods

Study participants

Female participants of the Healthy Twin study with both mammogram and BMD measurements obtained during a routine health examination were included in this study. Details of the Healthy Twin study, a nationwide cross-sectional survey as a part of the Korean Genome Epidemiology Study, have been previously published [39] and only briefly summarized here. Participants were not ascertained by their health status or breast diseases. Between April 2005 and December 2007, a total of 2,278 Korean male and female adult twins ($\geq$30 years of age) and their first-degree adult family members were recruited. Among them, a total of 734 women had both mammogram and BMD measurements available. Four women were excluded because they were shown to be genetically unrelated with their family members. Finally, 730 women from 341 families were included in the analysis with 122 pairs of monozygotic twins, 28 pairs of dizygotic twins, and 430 female family members.

Study variables

Mammograms were obtained using the same full-field digital mammography system (Senographe 2000D/DMR/DS, General Electric Company, Milwaukee, WI, USA) in female participants if they were aged $\geq$40 years of age at the time of participation in the study or were willing to undergo a mammogram for screening purposes. A single observer blinded to all identifying information measured the mammographic density in one cranio-caudal view of the right breast for each woman using a computer-assisted thresholding technique (Cumulus). Using the technique, the total area and area of absolute dense tissue of the breast were directly measured, then, the non-dense area and percent dense area were derived. This measure has been shown to be highly reproducible and reliable [40]. Mammograms were first randomized by family into reading sets of approximately 100 insuring that all twins and/or relatives of the same family were measured in the same set. A 10% random sample of repeats was included in each set and between every third set to test the reliability of the measurement; the estimated intra-class correlation coefficient for the total area, dense area, non-dense area, and percent dense area was 0.99, 0.98, 0.97, and 0.98, respectively.

BMDs (g/cm^2) of the whole body, rib, spine, pelvis, leg, and arm were measured using dual-energy X-ray absorptiometry (Lunar Radiation, Madison, WI, USA; and Delphi W; Hologic, Boston, MA, USA). These devices were maintained using the standard quality control procedures as recommended by the manufacturer to assure that the BMD calibration remained constant and the reported coefficient of variation was 1.0%.

Body weight (kg) was measured to the nearest 0.1 kg using a digital scale with the participant in light clothing and wearing no shoes. Height (cm) was measured to the nearest 0.1 cm using a stadiometer, while the participant stood with heels together, arms to the side, legs straight, shoulders relaxed, and the head in 'look straight ahead' position. Body mass index was calculated as the weight divided by the height squared (kg/m^2).

A self-administered questionnaire collected information regarding health behaviors (smoking, alcohol consumption, and physical activity) and reproductive history (age at menarche, age at the first childbirth, number of live children, duration of breast feeding, menopause, use of oral contraceptives, and use of hormone replacement therapy). Among women who reported no menstruation for the last

12 months, only those women who reported natural menopause, had received hormonal replacement therapy, or who were aged 55 or older were considered postmenopausal. The other women were considered pre-menopausal regardless of whether or not they had undergone a hysterectomy.

Zygosity of twin pairs was identified by 16 short tandem repeat (STR) markers, including 15 autosomal STR markers and one sex-determining marker (Perkin Elmer, Waltham, MA, USA) in 67% of the twin pairs. For the remaining 33% of the twin pairs, the zygosity was determined by a self-administered zygosity questionnaire that was validated to be 94.3% accurate through a STR marker study [41].

All participants provided written informed consent when they visited the study center. The study protocol was approved by the Korea Center for Disease Control and the Institutional Review Board of the three participating centers (Samsung Medical Center, Pusan Paik Hospital, and Dankook University Hospital).

Statistical analysis

Selected characteristics were compared between premenopausal and postmenopausal women using student *t*-test and χ^2 test. In order to see the overall relationship, the age-adjusted mammographic density measures by quartile level of BMD at each site of measurement were calculated using analysis of covariance, and linear trend was examined using age-adjusted linear regression analysis. The association between BMD and mammographic density was evaluated using mixed linear model [42]. Each of the mammographic density measures was examined for normality and subsequently log transformed. Correlation structures from family relationships were adjusted by considering family (as family number) and twins (as twin number) as random effects. Covariates (age, smoking, alcohol consumption, physical exercise, age at menarche, age at the first full-term childbirth, number of live children, duration of breast feeding, and use of oral contraceptives) selected on the basis of previously reported probable associations with BMD and mammographic density [8, 20, 43] were put in the model as fixed effects. For postmenopausal women, the use of hormone replacement therapy was additionally included in the model as a fixed effect. To minimize the reduction of study power that may occur by missing information for variables included in the multivariable model, we imputed missing values for the age at menarche and age at the first full-term childbirth with median values of women of the same age. We also imputed missing values for the duration of breast feeding with median values of women who had the same number of live children. We further examined the association between BMD and mammographic density measures in two subgroups stratified by menopausal status (premenopausal and postmenopausal) and tested the statistical significance of interaction terms (BMD * menopausal status) in the multivariable model.

To ascertain evidence of a common genetic regulation between BMD and mammographic density, we conducted bivariate variance-component-based genetic analysis using Sequential Oligogenic Linkage Analysis Routines (SOLAR; version 2.0) [44]. The bivariate variance-component analysis allows the phenotypic correlations to be partitioned into genetic (ρ_G) and environmental correlations (ρ_E). It can also examine whether or not the correlation between two or more phenotypes of an individual is concurrently determined by shared genes and the environment. If a significant genetic correlation existed, it was considered evidence of pleiotropy, genetic effect of a single gene on multiple phenotypic traits, or common genetic factors influencing both phenotypes through shared pathways. To estimate independent genetic correlations, age was adjusted first, and then other covariates were adjusted.

Results

Table 1 shows selected characteristics of the participants included in this analysis. Of the 730 participants, 462 (63.3%) were premenopausal and 268 (36.7%) were postmenopausal. The mean age was 40.0 and 59.8 years for premenopausal and postmenopausal women, respectively. Compared to postmenopausal women, premenopausal women had a higher absolute mammographic dense area, percent dense area, BMD at all measured sites, and body mass index, while the premenopausal women had a smaller non-dense mammographic area. Ever-smoking and current alcohol consumption were more prevalent among premenopausal women. Postmenopausal women had menarche at an older age, the first childbirth at a younger age, a greater number of live children, a longer duration of breast feeding, and were more likely to be an ever-user of oral contraceptives.

Figure 1 shows the age-adjusted levels of the mammographic density measures by quartiles of BMD at each site of measurement. With increasing levels of the BMD at ribs, pelvis, arms, and legs, both non-dense and dense areas of mammographic measures increased (P for trend <0.05). Percent dense area decreased with increasing levels of pelvis BMD (P for trend <0.05), but no association was found between percent dense area and the BMD at other sites.

Table 2 shows the relationship between the BMD at each site and each mammographic density measure according to menopausal status. In premenopausal women,

Table 1 Characteristics of study participants

Characteristics	Overall	Premenopausal women	Postmenopausal women	*P* value[b]
Number of participants	730	462	268	
Age, mean (SD), years	47.2 (11.9)	40.0 (6.9)	59.8 (7.3)	<0.01
Mammographic density measures				
Total area, mean (SD), cm^2	110.6 (40.2)	100.9 (35.8)	127.3 (42.0)	<0.01
Absolute dense area, mean (SD), cm^2	33.9 (23.4)	43.0 (21.6)	18.2 (17.2)	<0.01
Non-dense are, mean (SD), cm^2	76.7 (46.4)	58.0 (34.7)	109.1 (46.2)	<0.01
Percent dense area, mean (SD), %	34.4 (23.2)	44.9 (20.1)	16.2 (15.6)	<0.01
Bone mineral density, mean (SD), g/cm^2				
Whole body	1.06 (0.14)	1.11 (0.10)	0.98 (0.16)	<0.01
Ribs	0.62 (0.08)	0.64 (0.08)	0.58 (0.06)	<0.01
Spine	0.94 (0.21)	1.00 (0.20)	0.84 (0.19)	<0.01
Pelvis	1.07 (0.15)	1.11 (0.13)	0.99 (0.16)	<0.01
Arms	0.71 (0.10)	0.74 (0.11)	0.65 (0.07)	<0.01
Legs	1.08 (1.13)	1.12 (0.10)	1.02 (0.15)	<0.01
Body mass index, mean (SD), kg/m^2	23.6 (3.3)	23.0 (3.1)	24.7 (4.4)	<0.01
Ever-smoker[a], *N* (%)	71 (9.8)	53 (11.6)	18 (6.8)	<0.01
Current alcohol consumption[a], <2/week, *N* (%)	267 (36.9)	198 (43.1)	69 (26.0)	0.04
Current alcohol consumption[a], ≥2/week, *N* (%)	63 (8.7)	48 (10.5)	15 (5.7)	
Regular physical exercise[a], 1–2/week, *N* (%)	49 (6.8)	38 (8.4)	11 (4.2)	<0.01
Regular physical exercise[a], ≥3/week, *N* (%)	198 (27.7)	104 (23.0)	94 (35.7)	
Age at menarche[a], mean (SD), years	14.7 (2.0)	14.0 (1.6)	16.0 (2.1)	<0.01
Age at first childbirth[a], mean (SD), years	26.3 (3.3)	27.1 (3.2)	25.2 (3.2)	<0.01
Number of live children, mean (SD), persons	2.6 (1.8)	2.0 (1.7)	3.6 (1.6)	<0.01
Duration of breast feeding[a], mean (SD), months	25.6 (31.1)	13.7 (13.3)	42.3 (39.9)	<0.01
Ever-use of oral contraceptives[a], *N* (%)	115 (16.3)	60 (13.3)	55 (21.4)	<0.01
Ever-use of estrogen replacement[a], *N* (%)	69 (9.6)	0 (0.0)	69 (26.5)	–
Family history of breast cancer, *N* (%)	23 (3.2)	17 (4.7)	6 (2.2)	0.28

SD standard deviation

[a] For some participants, information about this variable was missing

[b] The difference between pre- and post-menopausal women for each variable was examined by *t*-test or χ^2 test

there were significant associations between the BMD at several sites and mammographic density measures. Absolute dense area was positively associated with the BMD at the ribs, pelvis, and legs. Percent dense area was positively associated with pelvis and leg BMD. Non-dense area was inversely associated with arm BMD. However, in postmenopausal women, there was no significant association between BMD and mammographic density measures. When pre- and post-menopausal women were combined, absolute dense area and percent dense area were positively associated with the BMD at ribs, pelvis, arms, and legs, whereas the non-dense area was inversely associated with arm BMD. When we examined the presence of any interaction between the BMD and menopausal status, the interaction term (BMD * menopausal status) was not statistically significant (data not shown).

Table 3 shows the total and additive genetic cross-trait correlation between the BMD at each site and mammographic density measures. Among the BMD at sites that showed significant association with mammographic density measures, only the rib BMD had a positive genetic cross-trait correlation with absolute dense area when covariates were fully adjusted. There was no significant genetic correlation between percent dense area and the BMD at any site.

Discussion

In this Korean twin and family study, we found that a positive association existed between BMD and mammographic density, even after a wide range of covariates were considered. Although the significant association tended to be confined to premenopausal women, these findings are consistent with the association of BMD and mammographic density with estrogen and breast cancer.

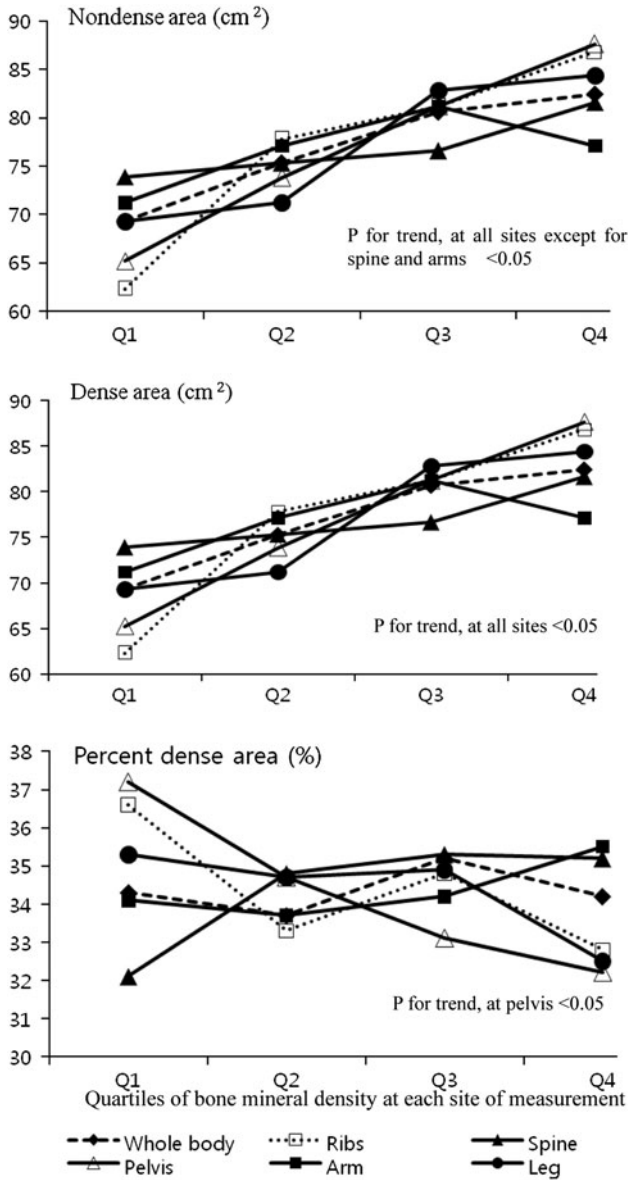

Fig. 1 Age-adjusted levels of the mammographic density measures by quartiles of bone mineral density

As mentioned previously, several studies have examined the association between BMD and mammographic density with conflicting results [32–38]. Crandall and colleagues showed a positive association at the lumbar spine and femur in a study of 594 postmenopausal women [36]. However, this association was limited to postmenopausal women not using hormone replacement therapy. Larger study by Kerlikowske and colleagues ($N \sim 15{,}000$, mostly postmenopausal women) [38] revealed no evidence of an association. Dite and colleagues studied twins and showed that there was no overlap of the genetic determinants between BMD and mammographic density [34]. They also applied similar statistical analyses used by Crandall and colleagues to a larger set of twin data and found no evidence of an association between BMD and mammographic density [33]. Larger study by Buist and colleagues ($N = 2{,}000$ postmenopausal women) showed an inverse association confined to women with normal body mass index (<25 kg/m^2) [32]. Two more recent studies found evidence of an inverse association between BMD and mammographic density [35, 37]. Crandall and colleagues found evidence of an inverse association in approximately 401 perimenopausal women, but this association depended on whether hormone users were included in the model [37]. They found no evidence of an association in 100 premenopausal women after adjusting for body mass index and other covariates. Yong and colleagues reported weak inverse correlations between percent dense area and BMD at lumbar spine, pelvis, and head in 192 premenopausal women [35].

Separate evaluation of the association between BMD and mammographic density according to the menopausal status is essential for the following reasons: both BMD and mammographic density are markedly influenced by the menopausal transition [8]; body mass index is closely associated with BMD and mammographic density and shows a different association with breast cancer as a function of menopausal status [45]; and it is uncertain whether the response of a breast to endogenous steroid hormones differs between pre- and post-menopausal women.

The current study is the first study to report a positive association between BMD and mammographic density in premenopausal women. Our study has an adequate size of 462 premenopausal participants providing 80% power to detect medium effect size ($f^2 = 0.15$) at the 5% level and a wide range of potential confounders were considered.

On the contrary, there was no association between the BMD and mammographic density in postmenopausal women, for which several explanations could be considered. Firstly, it has been suggested that the positive association between BMD and mammographic density might be obscured by recent postmenopausal hormone use that exerts a persistent effect on breast tissue [36]. However, Dite and colleagues found no positive association between mammographic density and BMD both for women who were recent or current users and for women who were never or past users of hormone replacement therapy [33]. To clarify this controversy, we re-analyzed our data after excluding women who ever-used hormone replacement therapy and we found no evidence supporting that the association between BMD and mammographic density was obscured by hormone use (data not shown). Therefore, it is less likely that the residual effect of recent or current hormone use has obscured the association between BMD and mammographic density in postmenopausal women. Secondly, considering that the direction and size of the

Table 2 Association[a] between bone mineral density and mammographic density measures according to the menopausal status of the participants

Sites of BMD (g/cm^2) measurement	Analytic model	Non-dense area[d], cm^2			Absolute dense area[d], cm^2			Percent dense area[d], %		
		Pre-menopausal	Post-menopausal	Overall	Pre-menopausal	Post-menopausal	Overall	Pre-menopausal	Post-menopausal	Overall
Whole body	Age	0.84 (0.22,1.46)	0.52 (0.15,0.88)	0.62 (0.29,0.94)	0.32 (−0.29,0.93)	0.02 (−0.81,0.85)	0.53 (0.01,1.05)	−0.22 (−0.80,0.37)	−0.43 (−1.33,0.46)	0.02 (−0.51,0.55)
	Model 1[b]	0.76 (0.14,1.39)	0.55 (0.18,0.93)	0.63 (0.29,0.96)	0.35 (−0.27,0.97)	−0.18 (−1.05,0.68)	0.29 (0.24,0.81)	−0.21 (−0.80,0.39)	−0.65 (−1.57,0.28)	−0.24 (−0.78,0.30)
	Model 2[c]	−0.20 (−0.74,0.34)	0.09 (−0.23,0.40)	−0.03 (−0.32,0.26)	0.55 (−0.09,1.19)	0.13 (−0.76,1.01)	0.51 (−0.03,1.04)	0.46 (−0.10,1.03)	0.005 (−0.89,0.90)	0.38 (−0.14,0.90)
Ribs	Age	1.45 (0.79,2.12)	2.18 (1.25,3.11)	1.46 (0.96,1.96)	0.56 (−0.12,1.25)	0.22 (−1.98,2.41)	1.17 (0.38,1.97)	−0.35 (−1.02,0.32)	−1.62 (−3.98,0.73)	0.03 (−0.78,0.84)
	Model 1	1.45 (0.18,2.11)	2.16 (1.20,3.11)	1.48 (0.98,1.99)	0.51 (−0.18,1.20)	0.01 (−2.23,2.26)	0.91 (0.11,1.72)	−0.41 (−1.08,0.27)	−1.75 (−4.15,0.65)	−0.21 (−1.03,0.61)
	Model 2	0.15 (−0.45,0.74)	0.42 (−0.43,1.27)	0.21 (−0.24,0.67)	0.83 (0.11,1.56)	1.38 (−1.01,3.78)	1.46 (0.62,2.30)	0.59 (−0.07,1.24)	0.91 (−1.53,3.36)	1.11 (0.29,1.92)
Spines	Age	0.17 (−0.09,0.44)	0.44 (0.17,0.71)	0.20 (0.02,0.38)	0.10 (−0.17,0.37)	0.23 (−0.42,0.88)	0.26 (−0.03,0.54)	0.01 (−0.25,0.28)	−0.13 (−0.83,0.57)	0.07 (−0.22,0.36)
	Model 1	0.16 (−0.11,0.43)	0.41 (0.13,0.69)	0.20 (0.02,0.38)	0.10 (−0.18,0.37)	0.23 (−0.43,0.89)	0.19 (−0.10,0.48)	0.001 (−0.27,0.27)	−0.12 (−0.83,0.59)	0.003 (−0.29,0.30)
	Model 2	−0.10 (−0.33,0.12)	0.06 (−0.18,0.29)	−0.06 (−0.22,0.09)	0.15 (−0.13,0.43)	0.50 (−0.17,1.18)	0.28 (−0.01,0.58)	0.21 (−0.04,0.46)	0.42 (−0.27,1.11)	0.27 (−0.01,0.55)
Pelvis	Age	1.30 (0.86,1.73)	0.61 (0.24,0.97)	0.96 (0.68,1.24)	0.41 (−0.02,0.85)	0.30 (−0.55,1.14)	0.76 (0.32,1.21)	−0.24 (−0.66,0.18)	−0.25 (−1.15,0.66)	0.13 (−0.32,0.59)
	Model 1	1.25 (0.80,1.69)	0.61 (0.23,0.98)	0.94 (0.65,1.23)	0.45 (0.004,0.91)	0.19 (−0.67,1.05)	0.58 (0.13,1.02)	−0.20 (−0.64,0.23)	−0.34 (−1.27,0.59)	−0.05 (−0.51,0.41)
	Model 2	0.23 (−0.19,0.64)	0.10 (−0.22,0.41)	0.19 (−0.07,0.45)	0.75 (0.27,1.23)	0.59 (−0.30,1.48)	0.91 (0.44,1.39)	0.59 (0.16,1.02)	0.45 (−0.46,1.36)	0.75 (0.30,1.21)
Arms	Age	0.14 (−0.40,0.67)	0.65 (−0.17,1.47)	0.27 (−0.17,0.70)	0.17 (−0.35,0.69)	0.80 (−1.03,2.63)	0.71 (0.05,1.38)	−0.04 (−0.55,0.47)	0.07 (−1.91,2.05)	0.32 (−0.35,1.00)
	Model 1	0.04 (−0.49,0.58)	0.53 (−0.32,1.37)	0.18 (−0.26,0.62)	0.16 (−0.37,0.69)	0.89 (−0.99,2.78)	0.58 (−0.08,1.24)	−0.02 (−0.54,0.50)	0.24 (−1.79,2.28)	0.24 (−0.44,0.92)
	Model 2	−0.57 (−1.03,−0.11)	−0.56 (−1.26,0.13)	−0.55 (−0.92,−0.18)	0.28 (−0.27,0.82)	1.71 (−0.21,3.63)	0.83 (0.16,1.50)	0.43 (−0.05,0.92)	1.87 (−0.09,3.82)	0.93 (0.28,1.57)
Legs	Age	0.92 (0.34,1.50)	0.47 (0.11, 0.84)	0.66 (0.34,0.98)	0.36 (−0.21,0.94)	0.11 (−0.74,0.95)	0.53 (0.03,1.03)	−0.33 (−0.88,0.22)	−0.21 (−1.12,0.69)	0.03 (−0.48,0.54)
	Model 1	0.82 (0.24,1.41)	0.47 (0.09,0.84)	0.61 (0.29,0.93)	0.41 (−0.17,1.00)	0.03 (−0.83,0.89)	0.42 (−0.08,0.91)	−0.29 (−0.85,0.28)	−0.27 (−1.20,0.65)	−0.07 (−0.57,0.44)
	Model 2	−0.30 (−0.82,0.22)	0.01 (−0.30,0.32)	−0.08 (−0.36,0.20)	0.69 (0.08,1.30)	0.35 (−0.53,1.23)	0.69 (0.18,1.20)	0.59 (0.05,1.14)	0.39 (−0.51,1.28)	0.66 (0.16,1.15)

BMD body mineral density

[a] *Beta* coefficients (95% confidence intervals) were assessed by a linear mixed model

[b] Model 1: for premenopausal women, random effect (household, twin pair) and fixed effect (age, smoking habit, alcohol consumption, physical exercise, number of live children, age at menarche, age at birth of first child, duration of breast feeding, and use of oral contraceptives) were adjusted. For postmenopausal women, hormone replacement treatment (fixed effect) was additionally adjusted. For overall participants, menopausal status and hormone replacement treatment were adjusted as a fixed effect

[c] Model 2, body mass index was additionally adjusted

[d] log-transformed

Table 3 Total and additive genetic cross-trait correlations between the mammographic density measures and the bone mineral density (BMD) in the same individual

Sites of BMD	Age-adjusted correlation			Multivariable-adjusted correlation[a]		
	Non-dense area, cm^2	Dense area, cm^2	Percent dense area, %	Non-dense area, cm^2	Dense area, cm^2	Percent dense area, %
Whole body, g/cm^2						
Total[a]	0.11**	0.18**	0.05	−0.04	0.11*	0.10*
Additive genetic[b]	0.14 (0.05)**	0.12 (0.05)*	0.01 (0.06)	−0.02 (0.06)	0.10 (0.06)	0.08 (0.06)
Ribs, g/cm^2						
Total	0.22**	0.20**	−0.002	0.04	0.16**	0.08
Additive genetic	0.26 (0.06)**	0.19 (0.06)**	−0.01 (0.06)	0.03 (0.06)	0.20 (0.06)**	0.13 (0.07)
Spines, g/cm^2						
Total	0.04	0.18**	0.10**	−0.07	0.09	0.11*
Additive genetic	0.09 (0.06)	0.18 (0.06)**	0.09 (0.06)	−0.13 (0.06)	0.18 (0.06)**	0.22 (0.07)
Pelvis, g/cm^2						
Total	0.20**	0.18**	−0.02	0.04	0.10*	0.03
Additive genetic	0.25 (0.05)**	0.13 (0.06)*	−0.06 (0.06)	0.03 (0.06)	0.10 (0.06)	0.04 (0.06)
Arms, g/cm^2						
Total	0.07	0.16**	0.07*	−0.14**	0.15**	0.19**
Additive genetic	0.13 (0.06)*	0.12 (0.06)	−0.01 (0.06)	−0.11 (0.06)	0.12 (0.06)	0.12 (0.06)
Legs, g/cm^2						
Total	0.16**	0.15**	0.01	−0.09	0.12**	0.14**
Additive genetic	0.25 (0.06)**	0.09 (0.06)	−0.09 (0.06)	−0.01 (0.07)	0.10 (0.06)	0.05 (0.07)

* $P < 0.05$, ** $P < 0.01$

[a] Spearman correlation

[b] Estimates (standard error) were assessed by bivariate analysis after inverse normal transformation of mammographic density data. For multivariable-adjusted analysis, age (and age^2 for additive genetic correlation estimation), body mass index, alcohol consumption, physical exercise, number of live children, age at menarche, age at birth of first child, duration of breast feeding, use of oral contraceptives, menopausal status, and use of estrogen replacement therapy were considered

estimates for the association between mammographic density measures and BMD did not differ materially between pre- and postmenopausal women, it seems possible that the small number of postmenopausal women ($N = 268$, 57% power to detect medium effect size) in our study have caused adequate statistical power for detecting significant association in postmenopausal women. Finally, it is still possible that the association between BMD and mammographic density measures in postmenopausal women differs from that in premenopausal women. Although statistical testing for the interaction term did not support any modification of the association between BMD and mammographic density as a function of menopausal status, this might be due to the lack of adequate statistical power.

Both mammographic density and BMD are known to be highly heritable [46, 47]. However, Dite and colleagues who have investigated in 134 pairs of twins whether or not the genetic determinants of mammographic density and BMD overlap, did not find any evidence of significant association between percent density and BMD at several sites [34]. In current study, we found a significant additive genetic correlation between the absolute dense area and BMD at the ribs and spines but not for percent dense area. This finding suggests that genetic determinants do play an important role in the association between BMD and absolute dense area of a breast.

Interestingly, we found that the absolute dense area was more strongly associated with BMD and had an association with BMD at more multiple sites compared to the percent dense area, suggesting that the absolute dense area may reflect the effect of exposure to estrogen better than percent dense area. Given the stronger phenotypic and genetic associations of BMD with absolute dense area than that with percent dense area, absolute dense area may reflect the effect of lifetime exposure to estrogen better than percent dense area.

In the current study, the positive association of BMD with the absolute dense area and percent density was strengthened when the analysis was adjusted for body mass index. This is probably because body mass index is positively associated with BMD [48], but inversely associated with mammographic density [49].

This study had several strengths. A wide range of lifestyle factors and reproductive risk factors for breast cancer and osteoporosis were identified and included in the analysis. Also twin and family data allowed for the evaluation of genetic pleiotrophy between BMD and the three different mammographic density measures. However, there were limitations to be considered. We could not measure the relevant hormone levels and could not exclude that hormones other than estrogen may have effects on the BMD and mammographic density. We also did not have information regarding the use of bone medications such as bisphophonates, which could have resulted in an underestimation of positive association in older postmenopausal women who were more likely to be osteoporotic. We could not separately measure the BMD of lumbar spine which is more likely to be hormonally responsive and under greater influence of estrogen deficiency. Finally, although mammographic density and BMD were measured concomitantly in a woman, all premenstrual women did not undergo mammograms and BMD measurements during the same phase of the menstrual cycle.

In conclusion, higher BMD was associated with higher mammographic density in Korean premenopausal women and some part of this association could be explained by genetic effect. This finding suggests that breast density could be influenced by estrogen exposure, if BMD is truly a marker of lifetime estrogen exposure. It also suggests that there could be an underlying common pathway that may link higher mammographic density, greater BMD, and increased breast cancer risk.

Acknowledgements This study was supported by the National Genome Research Institute, Korea, National Institute of Health research contract (budgets 2005-347-2400-2440-215, 2006-347-2400-2440-215, 2007-347-2400-2440-215, 2008-E00255-00, and 2009-E00500-00), Korean Ministry of Education, Science and Technology, (Grant Number M10305030005), and the Samsung Biomedical Research Institute (SBRI C-A9-218-1). The views expressed in this paper are those of the authors and not necessarily any funding body.

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