Mammographic Density and Candidate Gene Variants: A Twins and Sisters Study

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Abstract

Background: **Mammographic density, the light/white radiographic appearance on a mammogram that represents connective and epithelial tissue, is a strong risk factor for breast cancer which seems to be highly heritable. Little is known about its genetic determinants.**
Methods: **We studied 457 women from 207 sisterhoods (104 monozygotic twins, 182 dizygotic twins, and 171 singletons). Percentage mammographic density (PMD) as well as dense area and nondense area were calculated using a computer-assisted method. We measured six single nucleotide polymorphisms from six candidate genes (*COMT*, *HSD3B1*, *IGFBP3*, *HER2*, *XPD*, and *XRCC3*). Associations between genotypes and mammographic measures were tested (*a*) cross-sectionally using a multivariate normal model fitted using FISHER that allowed separate correlations for monozygotic, dizygotic, and nontwin pairs and (*b*) within sister pairs using paired *t* tests.**
Results: **Cross-sectionally, each additional copy of the HSD3B1 Asn^{367}Thr variant allele was associated with lower PMD (−3.47% per allele; SE = 1.65; *P* = 0.035). Within-pair regression estimates confirmed this association. There was no evidence for an association between the mammographic density measures and any of the other variants studied.**
Conclusion: **We have replicated an association between a variant in the *HSD3B1* gene and PMD, which suggests that *HSD3B1* may be genetic determinant of mammographic density.** (Cancer Epidemiol Biomarkers Prev 2007;16(7):1479–84)

Introduction

The amount of mammographic density, the light/white radiographic areas on a mammogram that represent connective and epithelial tissue, is a strong risk factor for breast cancer (1). Our collaborative study of twin pairs from Australia and North America found that, for all three mammographic measures [percentage mammographic density (PMD), absolute dense area, and absolute nondense area], monozygotic pairs were highly correlated and significantly more correlated than dizygotic pairs. Under the assumptions of the classic twin model, these data suggest that these measures are each highly heritable (2, 3).

Very little is known about the genetic determinants of these mammographic density measures. A handful of studies have examined associations between genetic variants and PMD (4-8). Four of these have considered polymorphisms in the genes that code for enzymes that produce or metabolize the sex hormone estrogen. Of these, for only two polymorphisms has their been nominally significant evidence of an association with PMD, one in each of the *COMT* and *HSD3B1* genes (4, 6, 8). Another pathway that has been investigated has been insulin-like growth factor-I, particularly the gene that controls levels of its principle binding protein, *IGFBP3* (7). Other potential candidates that have not yet been investigated for their association with mammographic density include another growth factor gene, *human epidermal growth factor receptor 2* (*HER2*), and DNA repair genes, *XPD* and *XRCC3*, all of which have a plausible link with breast cancer risk.

In this study, we have measured six polymorphisms selected from six candidate genes: *COMT, HSD3B1, IGFBP3, HER2* (*ERBB2*), *XPD* (*ERCC2*), and *XRCC3*. We have examined potential associations with the three measures of mammographic density—PMD, the absolute area of dense tissue, and the absolute area of nondense tissue. We have used a twins and sisters design and have estimated both cross-sectional and within-sibling pair associations.

Materials and Methods

Subjects. Subjects were women from sisterhoods. These were selected from the Australian Twins and Sisters Breast Density Study, a continuation of our previous twin study designed to recruit a large population-based sample of twin and/or sister pairs to study genetic and environmental determinants of mammographic density (2). Originally, Australian twin pairs aged between 40 and 70 y living in Victoria, New South Wales, and Western Australia were enrolled, but blood samples were not collected. These women were recontacted by the Australian Twin Registry and invited to continue participation. We also asked participating twin pairs for their permission to approach any additional sisters eligible to participate. We similarly approached through the Australian Twin Registry additional twin pairs ages between 40 and 70 y who had not been approached in the original study, irrespective of their state of residence. Finally, we advertised for volunteer sister pairs in a newsletter from the Australian

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Breast Cancer Network[6] and through articles in Melbourne newspapers. Recruitment was designed to maximize the number of sister pairs, regardless of breast cancer status, and eligibility of nontwin sisters was not restricted by age. Women who have had breast cancer or breast augmentation/reduction surgery were eligible only if we could obtain a mammogram taken before diagnosis or before surgery.

At the time of sample section, 956 subjects from 445 families had provided a blood sample; however, >60% of these subjects were monozygotic twins. Monozygotic twins were included only if an additional sister was participating. A total of 457 subjects from 207 sisterhoods (104 monozygotic twins, 182 dizygotic twins, and 171 singletons) provided a blood sample, completed a questionnaire, and had a (prediagnosis) mammogram measured (see below).

Measurement of Mammographic Density. Mammograms were obtained with the written consent of all subjects. Films were retrieved from state BreastScreen Services (80%), private clinics (12%), and participants who kept their films at home (8%). The craniocaudal views were digitized using a Lumysis 85 scanner in the Australian Mammographic Density Research Facility at the University of Melbourne. For most subjects, the right breast was measured by one reader (J.S.) using a computer-assisted thresholding technique (9). For women with a previous breast cancer in the right breast, the prediagnosis mammogram of the left breast was used, along with the left breast of the subject's sister(s). The interactive thresholding technique involves an operator selecting a gray value as a threshold to separate the image of the breast from the background, which determines breast size. A second threshold is then selected to identify the edge(s) of the mammographically dense tissue. The computer then records the number of pixels in the digitized image that lie within the defined areas. The result is an absolute measure of the total area of the breast and an absolute measure of dense area, respectively, which when subtracted gives the area of nondense tissue and when expressed as a ratio gives a percentage of mammographic density.

The films were randomized first by family into reading sets of ~100, ensuring that all twins and/or sisters of the same family were measured in the same set. Within a set, the films were randomized by subject, so that twins from the original study who contributed two mammograms were viewed one after the other, but the order was unknown to the reader. This procedure has been shown in previous work to be the best method for randomization and viewing of multiple mammograms (10). The reader was blinded to all other identifying information. A 10% random sample of repeats was included in each set and between every fourth set to assess repeatability.

Data Collection. A questionnaire was administered by telephone to each participating subject and included demographic information, weight, height, smoking history, alcohol consumption, reproductive history, cessation of menstruation, use of oral contraceptives and hormone replacement therapy (HRT), and family history of cancer. A woman was defined as postmenopausal if she had a hysterectomy, both ovaries removed, or radiation; was not on HRT at the time of the mammogram and had not menstruated 12 months prior; or was on HRT at the time of the mammogram and had not menstruated 12 months prior and was not menstruating before commencing HRT. All subjects who did not fit the postmenopausal criteria were considered premenopausal.

For twins, zygosity was determined by a standard question that describes the differences between identical and nonidentical pairs. For pairs whose answers contradicted each other, or who were unsure, zygosity was determined using additional questions and methods of classifying responses that have been shown to give 95% agreement with zygosity based on blood typing in middle-aged adults (11-13).

Blood Collection. Participants were posted a blood collection kit containing tubes, a shipping container, and detailed instructions. Collection of a 27 mL blood sample was arranged with a contracted pathology laboratory. Blood samples were couriered to the Genetic Epidemiology Laboratory at the University of Melbourne within 48 h of collection. In the event that participants living within the Melbourne metropolitan area were unable to travel to a designated pathology laboratory, a trained phlebotomist visited the subject(s) at their home.

DNA Extraction and Storage and Genotyping. Blood samples were processed to generate Guthrie cards (stored at room temperature), whole blood, plasma, non lymphocytes (stored at -70°C) and monocytes stored in liquid nitrogen. DNA was extracted from whole blood aliquots using a Corbett Robotics DNA Xtractor and placed in a 96-well plate format. The *XRCC3* single nucleotide polymorphism (SNP; rs861539) was genotyped by Taqman allelic discrimination. Genotyping of the *COMT* SNP (rs17295216) was done by direct sequencing. The other polymorphisms were genotyped using homogenous MassEXTEND matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (*HSD3B1* rs1047303, *IGFBP3* rs2854744, *HER2* rs1801200, and *XPD* rs17359310).

Taqman Allelic Discrimination. The *XRCC3* rs861539 SNP was genotyped using a custom-designed Taqman genotyping assay (Applied Biosystems) according to the instructions of the manufacturer and using fluorescent allele-specific probes. Briefly, 5 ng of template DNA, 2.5 μL 2× Taqman Universal PCR Master Mix, and 0.125 μL 20× SNP Genotyping Assay Mix (including primers and fluorescently labeled probes, available on request) were added to a total reaction volume of 5 μL. PCR cycling was done using an ABI Prism 7900HT Sequence Detection System under the following conditions: 95°C for 10 min followed by 40 cycles of 92°C for 15 s and 60°C for 1 min. Allelic discrimination (via end-point fluorescence) was done in 384-well format using an ABI 7900HT Sequence Detection System and the allelic discrimination analysis module (Applied Biosystems).

Sequencing. The *COMT* rs17295216 (or rs4680) SNP was genotyped via direct sequencing after amplification using the following primers: forward, 5′-CTGTGGCTACTCAGCTGTGC-3′ and reverse, 5′-GCATGCACACCTTGTCCTT-3′ (using standard PCR conditions and annealing temperature of 64°C). Direct sequencing was achieved using the forward primer, ABI Prism BigDye Termination kit, and the ABI Prism 3100 Genetic Analyzer (Applied Biosystems).

Homogenous MassEXTEND Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry. PCR and homogenous MassEXTEND extension primer sequences for the four SNPs were designed using Sequenom RealSNP[7] and multiplexed into three assays (two 9-plex and one 4-plex, other SNPs assessed in these assays do not form the scope of this study). A 5 μL PCR contained 2.5 ng (4-plex assay) or 5 ng (9-plex assay) of template DNA, 10× Qiagen HotStar Taq PCR buffer,[8] 25 mmol/L $MgCl_2$, 25 mmol/L deoxynucleotide triphosphates, 200 nmol/L of each PCR primer, and 0.1 unit Qiagen HotStar Taq polymerase. PCR cycling conditions included 95°C for 15 min, 45 cycles of 95°C for 20 s, 56°C for 30 s, and 72°C for 1 min followed by a final extension step of 3 min at 72°C. After exonuclease cleanup, the MassEXTEND

[6] http://www.bcna.org.au

[7] http://www.RealSNP.com

[8] http://www1.qiagen.com

reaction was done using 1× the appropriate ACT termination mix, 600 nmol/L of each extension primer, and 0.054 unit (5-plex) or 1.25 units (9-plex) of Thermo Sequenase (Sequenom). The cycling conditions were as follows: 94°C for 2 min, 60 (5-plex) or 100 (9-plex) cycles of 94°C for 5 s, 52°C for 5 s, and 72°C for 5 s. Samples were spotted onto SpectroCHIP Bioarrays using the MassARRAY nanodispenser (Sequenom) and run on a MassARRAY Compact System (Sequenom). Results were analyzed using Sequenom MassARRAY RT software.

Statistical Analysis. Deviation of observed genotype frequencies from those expected under Hardy-Weinberg equilibrium was assessed by the usual χ^2 test with one degree of freedom. Polymorphisms from the candidate genes were coded as 0, 1, and 2 for homozygous wild-type, heterozygaus, and homozygous variant genotypes, respectively. Dense and nondense area measurements were log transformed.

Cross-Sectional Analysis. The cross-sectional mean was estimated for each genotype while concurrently adjusting for the potential confounders age, weight, height, number of live births, years of HRT use, pack-years of smoking, menopausal status, and years between time of mammogram and time of interview. The variance was assumed to be a constant and the covariance between sister pairs was allowed to differ according to whether they were both members of a monozygotic pair, both members of a dizygotic pair, or nontwin sisters. The effects of genotype were first parameterized in terms of the mean for the homozygous wild-type genotype and the differences between that mean and the means for each of the other two genotypes. They were also parameterized in terms of an additive genetic model, which assumed that the difference in mean between each genotype was the same, represented by a regression coefficient β. Variables and SEs were estimated under maximum likelihood theory on the assumption that, for each sisterhood, the (adjusted) measures followed a multivariate normal distribution. Models were fitted using the statistical software package FISHER (14).

Within-Pair Analysis. The associations between within-pair differences in mammographic measures and within-pair differences in genotypes were estimated using linear regression. The mean within-pair difference was parameterized first depending on whether sisters in the same pair differed from each other by one or two alleles, and these means were compared with zero. It was also parameterized under the assumption that the mean within-pair difference in mammographic measure was twice that in pairs who differed by two alleles than in pairs who differed by one allele and represented by a regression coefficient β_W. This was achieved by regressing within-pair difference in mammographic measure against within-pair difference in number of alleles and fitting a straight line through the origin. Note that pairs who did not differ in genotype did not contribute to the within-pair analysis of that genotype. All within-pair linear regression models were adjusted for the within-pair differences in other measured covariates. Models were fitted using PROC MIXED in Statistical Analysis System (version 8.2; SAS Institute), with a family indicator term included to take into account that some families contributed multiple pairs.

Results

The characteristics of the 457 subjects from whom we obtained questionnaire, mammographic, and genetic data are given in Table 1. Women were aged between 37 and 78 y at the time of mammogram, and the mean difference between age at interview and age at mammogram was 1.1 y (SD = 2.5).

Details of the investigated polymorphisms are given in Table 2. There was marginal evidence of deviation from Hardy-Weinberg equilibrium for the *XRCC3* polymorphism ($P = 0.02$), but the departure was small in magnitude.

Cross-Sectional Analysis. Table 3 shows the mean and regression estimates for each of the candidate SNPs for each mammographic density measure. The area of nondense tissue was positively associated with number of copies of the variant allele of the *HER2* Val^{655}Ile polymorphism ($P_{trend} = 0.04$). PMD was associated with an average decrease of 3.5 percentage points (representing a change of >10% of mean PMD) in subjects with each increase in the number of copies of the variant allele of the *HSD3B1* Asn^{367}Thr polymorphism ($P_{trend} = 0.035$). This effect was driven by a decrease in dense area and an increase in nondense area, but neither of these separate comparisons generated strong evidence against the null hypothesis of no association. None of the polymorphisms in the *COMT, IGFBP3, XPD*, or *XRCC3* genes was associated cross-sectionally with any of the mammographic measures (all $P_{trend} > 0.1$).

Cross-Sectional Analysis Stratified by Menopausal Status. The magnitude of the association between the HSD3B1 polymorphism and PMD in the 150 premenopausal women was stronger than in the overall sample but only of marginal significance ($\beta = -4.88$; SE = 2.66; $P_{trend} = 0.07$). The association between increased copies of the variant allele of the *HER2* polymorphism and increased log nondense area was stronger in premenopausal women ($\beta = 0.18$; SE = 0.069; $P_{trend} = 0.009$).

Within-Pair Analysis. Table 4 shows the results of the within-pair regression analyses. Estimates replicate the weak association between log dense area and the *HSD3B1* genotype found in the cross-sectional analysis. Sisters with one copy of the variant allele had 0.26 cm^2 less log dense area than those with none (SE = 0.14; $P = 0.06$).

The within-pair regression estimates provided stronger evidence for an association between differences in log nondense area and differences in the *HSD3B1* genotype than that

Table 1. Selected characteristics of subjects (n = 457)

Characteristic	Mean or %	SD
Age (at interview), y	57.1	8.6
Age (at mammogram), y	56.5	8.8
Weight (kg)	69.0	13.4
Height (cm)	162.7	6.8
Body mass index (kg/m^2)	26.1	4.9
Age at menarche (y)	13.1	1.6
Parity status (% parous)	90.4	
Age at first birth (n = 396), y	25.9	4.8
No. live births	2.4	1.5
Use of oral contraceptive ever (% yes)	83.6	
Current use of oral contraceptive (% yes)	5.5	
Years of oral contraceptive use (n = 382)	7.5	7.8
Use of HRT ever (% yes)	43.3	
Use of HRT now (% yes)	17.7	
Years of HRT use (n = 198)	6.1	5.9
Ever smoked (% yes)	35.2	
Current smoker (% yes)	8.3	
Years of smoking (n = 161)	18.8	12.7
Pack-years of smoking (n = 161)	12.6	12.3
Ever drank alcohol (% yes)	62.8	
Current drinker (% yes)	53.2	
Years of drinking (n = 287)	25.5	14.7
Menopausal status (% post)	67.2	
Age at menopause (n = 307), y	46.9	6.35
First-degree relative with breast cancer (% yes)	27.8	
Mammographic density measures		
PMD (%)	37.7	25.7
Dense area (cm^2)	49.2	38.8
Nondense area (cm^2)	94.9	63.6
Log dense area	3.39	1.28
Log nondense area	4.32	0.72

Table 2. Characteristics of genotype data

Gene	Polymorphism	SNP	Variant allele frequency	Function
COMT	G→A Val^{158}Met	rs4680	0.51	"Inactivates catechol (2- and 4-OH) estrogen metabolism/reduced activity, higher levels of catechol metabolites" (8)
HSD3B1	A→C Asn^{367}Thr	rs1047303	0.33	Unknown
HER2	A→G Val^{655}Ile	rs1801200	0.24	Unknown
IGFBP3	A→C A-202C	rs2854744	0.50	Unknown
XPD	A→C Lys^{751}Gln	rs17359310	0.35	May be partially related to overall DNA repair function (20)
*XRCC3**	T→C Thr^{241}Met	rs861539	0.42	May be partially related to overall DNA repair function (21)

*χ^2 test of Hardy-Weinberg equilibrium (P = 0.02).

found by the cross-sectional analysis. On average, sisters with additional copies of the variant allele had 0.16 cm^2 more log nondense area than those with none (β = 0.16; SE = 0.051; P_{trend} = 0.002). Unlike the cross-sectional estimates given in Table 3, there was little evidence of an association between log nondense area and the *HER2* polymorphism from the within-pair regression analysis (β = 0.077; SE = 0.069; P_{trend} = 0.3).

The magnitude of the within-pair regression coefficient between PMD and the *HSD3B1* genotype was stronger than that from the cross-sectional estimates given in Table 3 (β = −5.85; SE = 2.07; P_{trend} = 0.005). There was no nominally significant evidence that any of the polymorphisms in the *COMT, IGFBP3, XPD*, and *XRCC3* genes was associated with any of the mammographic measures (all P_{trend} > 0.2).

Discussion

We have found nominal evidence for an association, both cross-sectionally and within pairs, between a polymorphism in the *HSD3B1* gene and PMD. The Asn^{367}Thr variant allele was associated with lower values of PMD, and this seemed to be driven by both a lower dense area and a higher nondense area.

The association we found between the *HSD3B1* gene and PMD is similar to that previously reported by Haiman et al. (4). They found that Caucasian women (with breast cancer) homozygous for the Thr allele of *HSD3B1* had 5.1 percentage points less PMD than those homozygous for the Asn allele. Interestingly, they found this effect to be in the opposite direction for African-American women (absolute difference +19.7% Thr/Thr versus Asn/Asn; P = 0.02), although there were only four subjects with the Thr/Thr genotype. More than 95% of our study sample indicated their ethnicity as European, of which only seven women indicated that they also came from North African descent. The other 5% of women were of Pacific Island descent. Little is known about the function of this variant and its relationship to steroid hormone levels, let alone breast cancer risk, but our apparent replication of its association with PMD merits further investigation.

Four studies have investigated the relationship between the *COMT* Val^{158}Met polymorphism and mammographic density, particularly for premenopausal women. Two found little evidence of an association between PMD and the *COMT* gene (4, 5). However, in the 2002 study, all of the subjects had breast cancer, and therefore the distribution of the genotypes may not have been representative of the general population. In the 2003 study, the premenopausal subgroup had only 94 subjects and may not be sufficiently powered to rule out an association. The two remaining studies found that decreased PMD was associated with the low-activity homozygous genotype, Met/Met,

Table 3. Unadjusted mean estimates for homozygous wild-type genotypes (SEs in parentheses), change in mean estimates per variant allele (SEs in parentheses), and linear regression estimates (SEs in parentheses)

	wt/wt	wt/vt vs wt/wt	vt/vt vs wt/wt	β (SE)	P for trend
Log dense area					
COMT: Val^{158}Met	3.25 (0.14)	0.15 (0.16)	0.24 (0.19)	0.084 (0.089)*	0.3
HSD3B1: Asn^{367}Thr	3.51 (0.098)	−0.26 (0.13)	−0.079 (0.21)	−0.14 (0.09)*	0.1
HER2: Val^{655}Ile	3.41 (0.089)	−0.073 (0.14)	0.14 (0.28)	0.026 (0.10)*	0.8
IGFBP3: A-202C	3.32 (0.13)	0.17 (0.16)	−0.040 (0.18)	0.029 (0.086)*	0.7
XPD: Lys^{751}Gln	3.36 (0.10)	0.029 (0.14)	0.15 (0.22)	0.047 (0.094)*	0.6
XRCC3: Thr^{241}Met †	3.47 (0.11)	−0.16 (0.14)	−0.073 (0.18)	−0.051 (0.084)*	0.5
Log nondense area					
COMT: Val^{158}Met	4.39 (0.079)	−0.080 (0.091)	−0.17 (0.11)	−0.058 (0.041) ‡	0.2
HSD3B1: Asn^{367}Thr	4.30 (0.056)	0.018 (0.073)	0.019 (0.12)	0.047 (0.041) ‡	0.3
HER2: Val^{655}Ile	4.25 (0.053)	0.13 (0.080)	0.17 (0.15)	0.095 (0.046) ‡	0.04
IGFBP3: A-202C	4.23 (0.075)	0.063 (0.087)	0.15 (0.10)	0.048 (0.039) ‡	0.2
XPD: Lys^{751}Gln	4.26 (0.060)	0.078 (0.075)	0.037 (0.12)	0.043 (0.043) ‡	0.3
XRCC3: Thr^{241}Met †	4.29 (0.063)	0.050 (0.078)	−0.036 (0.10)	0.027 (0.038) ‡	0.5
PMD					
COMT: Val^{158}Met	35.27 (2.82)	2.62 (3.26)	5.00 (3.80)	1.69 (1.65)§	0.3
HSD3B1: Asn^{367}Thr	39.84 (2.03)	−3.46 (2.66)	−3.94 (4.31)	−3.47 (1.65)§	0.04
HER2: Val^{655}Ile	39.43 (1.88)	−3.73 (2.85)	−3.30 (5.59)	−1.93 (1.86)§	0.3
IGFBP3: A-202C	38.72 (2.70)	0.081 (3.16)	−3.14 (3.70)	−0.41 (1.59)§	0.8
XPD: Lys^{751}Gln	38.42 (2.15)	−1.14 (2.73)	0.52 (4.50)	−0.17 (1.73)§	0.9
XRCC3: Thr^{241}Met †	39.68 (2.27)	−3.71 (2.85)	−0.64 (3.71)	−1.04 (1.55)§	0.5

*Adjusted for age, weight, height, number of live births, years of HRT use, and pack-years of smoking.
† χ^2 test of Hardy-Weinberg equilibrium (P = 0.02).
‡ Adjusted for age, weight, height, parity, age at first birth (nonparous women given mean), years of HRT use, pack-years of smoking, menopausal status, and years between time of mammogram and time of interview.
§ Adjusted for age, weight, height, number of live births, years of HRT use, pack-years of smoking, and years between time of mammogram and time of interview.

Table 4. Within-pair analysis of associations using 334 sister pairs from 180 families

Polymorphism	Difference of one allele (SE)		Difference of two alleles (SE)		β_W (SE)	
Log dense area*						
COMT: Val^{158}Met	0.038 (0.16)	$P = 0.8$	−0.56 (0.30)	$P = 0.07$	−0.089 (0.14)	$P = 0.5$
HSD3B1: Asn^{367}Thr	−0.26 (0.14)	$P = 0.06$	0.069 (0.23)	$P = 0.8$	−0.19 (0.12)	$P = 0.1$
HER2: Val^{655}Ile	0.37 (0.19)	$P = 0.05$	−0.012 (0.39)	$P = 0.98$	0.29 (0.16)	$P = 0.08$
IGFBP3: A-202C	−0.0042 (0.14)	$P = 0.98$	−0.0081 (0.26)	$P = 0.98$	0.022 (0.13)	$P = 0.9$
XPD: Lys^{751}Gln	0.25 (0.15)	$P = 0.09$	−0.12 (0.28)	$P = 0.7$	0.16 (0.13)	$P = 0.2$
XRCC3: Thr^{241}Met †	0.0052 (0.14)	$P = 0.97$	−0.31 (0.20)	$P = 0.1$	−0.099 (0.12)	$P = 0.4$
Log nondense area ‡						
COMT: Val^{158}Met	−0.016 (0.066)	$P = 0.8$	0.023 (0.12)	$P = 0.9$	−0.015 (0.058)	$P = 0.8$
HSD3B1: Asn^{367}Thr	0.18 (0.059)	$P = 0.003$	0.14 (0.089)	$P = 0.1$	0.16 (0.051)	$P = 0.002$
HER2: Val^{655}Ile	0.067 (0.078)	$P = 0.4$	0.12 (0.15)	$P = 0.4$	0.077 (0.069)	$P = 0.3$
IGFBP3: A-202C	−0.040 (0.061)	$P = 0.5$	0.13 (0.11)	$P = 0.2$	−0.013 (0.053)	$P = 0.8$
XPD: Lys^{751}Gln	−0.070 (0.061)	$P = 0.2$	−0.030 (0.11)	$P = 0.8$	−0.063 (0.054)	$P = 0.2$
XRCC3: Thr^{241}Met †	0.036 (0.059)	$P = 0.5$	0.070 (0.083)	$P = 0.4$	0.053 (0.050)	$P = 0.3$
PMD§						
COMT: Val^{158}Met	0.70 (2.69)	$P = 0.8$	−3.34 (5.18)	$P = 0.5$	−0.15 (2.38)	$P = 0.95$
HSD3B1: Asn^{367}Thr	−5.53 (2.41)	$P = 0.008$	−3.78 (3.74)	$P = 0.3$	−5.85 (2.07)	$P = 0.005$
HER2: Val^{655}Ile	2.55 (3.18)	$P = 0.4$	−2.35 (6.48)	$P = 0.7$	1.81 (2.82)	$P = 0.5$
IGFBP3: A-202C	1.03 (2.47)	$P = 0.7$	−2.06 (4.55)	$P = 0.7$	0.83 (2.17)	$P = 0.7$
XPD: Lys^{751}Gln	4.09 (2.48)	$P = 0.1$	−0.42 (4.60)	$P = 0.9$	3.05 (2.19)	$P = 0.2$
XRCC3: Thr^{241}Met †	0.75 (2.43)	$P = 0.8$	−4.96 (3.34)	$P = 0.1$	−1.31 (2.02)	$P = 0.5$

*Adjusted for age, weight, height, number of live births, years of HRT use, and pack-years of smoking.
† χ^2 test of Hardy-Weinberg equilibrium ($P = 0.02$).
‡ Adjusted for age, weight, height, parity, age at first birth (nonparous women given mean), years of HRT use, pack-years of smoking, menopausal status, and years between time of mammogram and time of interview.
§Adjusted for age, weight, height, number of live births, years of HRT use, pack-years of smoking, and years between time of mammogram and time of interview.

in premenopausal women (6, 8). However, the direction of these associations is opposite to expectation, as *COMT* is involved in the detoxification of harmful estrogen metabolites thought to increase breast cancer risk. It was therefore hypothesized that the low activity of the *COMT* gene results in increased mammographic density and increased breast cancer risk (8). We found little evidence of an association between the *COMT* gene and PMD in the cross-sectional analysis (including separate examination of the premenopausal subgroup) or the within-pair analysis. We pooled the mean estimates across all five studies for each *COMT* genotype, weighted by the inverse of the variance of each estimate. The overall pooled estimates of mean PMD for each of the genotypes were 40.8 (SE = 2.1), 40.3 (SE = 3.2), and 37.4 (SE = 4.0) for Val/Val, Val/Met, and Met/Met genotypes, respectively, showing little evidence of an association.

To date, the commonly reported Val^{655}Ile polymorphism in the *HER2* gene has not previously been investigated for an association with mammographic density. We found, at best, only weak evidence of a positive association between nondense area and the variant allele. One of the activities of the *HER2* gene is the control of cell amplification and it may play a role in the development of breast cancer, as overexpression of this gene is found in >20% of human breast tumors (15). This would not explain why the variant allele would be associated with increased nondense or fatty tissue.

Insulin-like growth factor-I is a growth hormone that is produced in the breast stroma and promotes the proliferation of normal and malignant breast epithelial cells. Its principal binding protein, IGFBP3, slows breast cell proliferation and promotes apoptosis. Lai et al. (7) found a positive association of the A allele of the single base pair polymorphism at position −202 relative to the CAP site in the *IGFBP3* gene (A-202C) with serum levels of IGFBP3 and with PMD in premenopausal women. Whereas they found no association between serum levels of IGFBP3 and PMD, three other studies reported a negative association between levels of IGFBP3 and PMD in premenopausal women (16-18). This is consistent with the biological theory that IGFBP3 slows breast cell proliferation and promotes apoptosis, inferring that increased levels of IGFBP3 should be protective against breast cancer. We found no evidence that the *IGFBP3* A-202C polymorphism, which has recently been shown to be associated with both levels of IGFBP3 and breast cancer risk (19), was associated with any of the mammographic measures.

Two coding DNA repair gene polymorphisms were genotyped because there is a plausible link with breast cancer risk. There was no evidence that the Lys^{751}Gln polymorphism from the *XPD* gene or the Thr^{241}Met polymorphism from the *XRCC3* gene was associated with any of the mammographic measures.

A potential limitation of this study is its modest sample size so that small real effects would not have been detectable. For example, for PMD, the SE of the linear effect term (β) ranged from ~1.5 to <3 across the cross-sectional and within-pair analysis, so only effects of about 4 to 7 units per allele or more would have been detectable with 80% power. Similar post hoc power calculations can be calculated for the other mammographic measures, and the size of these compared with the means of these measures presented in Table 1. Therefore, in general, we cannot exclude effects of <10% of the mean per allele.

In conclusion, we have replicated an association between the *HSD3B1* gene and PMD. The discovery of genetic determinants of mammographic density is of considerable importance as it is a strong and highly heritable risk factor for breast cancer and may identify genetic variants associated with the disease or, at least, may lead to the discovery of new genetic pathways of relevance to breast cancer control. Clearly, much larger studies of mammographic density using candidate genes or genome-wide scans are needed.

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