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Common Genetic Variants Associated with Breast Cancer and Mammographic Density Measures That Predict Disease

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Abstract

Mammographic density for age and body mass index (BMI) is a heritable risk factor for breast cancer. We aimed to determine if recently identified common variants associated with small gradients in breast cancer risk are associated with mammographic density. We genotyped 497 monozygotic and 330 dizygotic twin pairs and 634 of their sisters from 903 families for 12 independent variants. Mammographic dense area, percent dense area, and nondense area were measured by three observers using a computer-thresholding technique. Associations with mammographic density measures adjusted for age, BMI, and other determinants were estimated (a) cross-sectionally using a multivariate normal model for pedigree analysis (P_x), (b) between sibships, and (c) within sibships using orthogonal transformations of outcomes and exposures. A combined test of association (P_c) was derived using the independent estimates from b and c. We tested if the distributions of P values across variants differed from the uniform distribution (P_u). For dense area and percent dense area, the distributions of P_c values were not uniform (both P_u <0.007). Consistent with their breast cancer associations, rs3817198 (*LSP1*) and rs13281615 (8q) were associated with dense area and percent dense area (all P_x and P_c <0.05), and rs889312 (*MAP3K1*), rs2107425 (*H19*), and rs17468277 (*CASP8*) were marginally associated with dense area (some P_x or P_c <0.05). All associations were independent of menopausal status. At least two common breast cancer susceptibility variants are associated with mammographic density measures that predict breast cancer. These findings could help elucidate how those variants and mammographic density measures are associated with breast cancer susceptibility. *Cancer Res; 70(4); 1449–58. ©2010 AACR.*

Introduction

For women of equivalent age, those whose breasts display greater white or bright areas on a mammogram—that is, have greater mammographic density—are at greater risk of developing breast cancer (1). This association has been shown repeatedly by case-control studies nested within large population screening programs in which cases and controls have been matched for age and adjustments have been made for other measured breast cancer risk factors, in particular body mass index (BMI). The association has been shown to persist up to 8 years after initial screening (2) and remains after excluding cases detected within 1 year of the initial screen, indicating that these associations are not simply due to a "masking" effect caused by difficulty in detecting tumors that are in mammographically dense regions of the breast.

Twin and family studies have reported that, under the assumptions of the classic twin model and after adjusting for age, BMI, and other determinants, the patterns of familial correlations for mammographic density measures are consistent with additive genetic factors explaining ~60% of their residual variances (3, 4). This raises the question of whether variants associated with breast cancer risk also explain the genetic component of variation in those mammographic density measures that predict breast cancer.

Recently, genome-wide and candidate gene association studies have identified a number of loci in which there exists one or more common single nucleotide polymorphisms associated with small gradients in breast cancer risk (5–7). Table 1 lists the variants and their corresponding per allele associations. For example, the minor allele of rs2981582, located in

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Table 1. Common variants associated with breast cancer risk

Variant (reference)	Position*	Assigned gene/locus	Change	MAF	Per allele OR (95% CI)	*P*
rs2981582 (6)	10q/123342307	*FGFR2*	G→A	0.38	1.26 (1.23–1.3)	10^{-62}
rs889312 (6)	5q/56067641	*MAP3K1*	A→C	0.31	1.13 (1.09–1.18)	10^{-15}
rs12443621 (6)	16q/51105538	*TNRC9/TOX3*	A→G	0.46	1.11 (1.08–1.14)	10^{-14}
rs2107425 (6)	11p/1977651	*H19*	G→A	0.29	0.96 (0.93–0.99)	0.01
rs3817198 (6)	11p/1865582	*LSP1*	T→C	0.31	1.07 (1.04–1.11)	10^{-5}
rs8051542 (6)	16q/51091668	*TNRC9/TOX3*	C→T	0.41	1.09 (1.06–1.13)	10^{-8}
rs13281615 (6)	8q/128424800	*8q*	A→G	0.43	1.08 (1.05–1.11)	10^{-7}
rs17468277 (5)	21862445	*CASP8*	C→T	0.15	0.88 (0.84–0.92)	10^{-7}
rs13387042 (7)	2q/217614077	*2q*	A→G	0.49	1.20 (1.14–1.26)	10^{-13}
rs981782 (6)	5p/45321475	HCN1	A→C	0.46	0.96 (0.93–0.99)	0.003
rs4973768 (18)	3p/27391017	*NEK10/SLC4A7*	C→T	0.48	1.11 (1.08–1.13)	10^{-18}
rs6504950 (18)	17q/50411470	*STXBP4*	G→A	0.3	0.95 (0.92–0.97)	0.0001

Abbreviations: MAF, minor allele frequency; OR, odds ratio; 95% CI, 95% confidence interval.
*Based on build 36.3.

intron 2 of the gene *FGFR2*, is associated with about a 20% increase in breast cancer risk per allele. It is not known why these variants are associated with breast cancer risk as they are not necessarily "functional," let alone causal. Note also that although a gene has been assigned to a locus-specific disease association, this does not imply a direct role on disease risk of the specific variant or even other variants in the nominated gene.

Finding that one or more breast cancer–associated common genetic variants are associated with mammographic density could help elucidate some of the biological reasons why women of the same age and BMI differ so much in mammographic density. In doing so, it could also help unravel why these variants are associated with breast cancer risk. To date, three studies (8–10) have examined this question but have not provided a convincing answer (see Discussion).

We genotyped a total of 2,288 monozygotic and dizygotic twins and their sisters for 12 variants, one from each locus associated with breast cancer. Taking into account family structure, we analyzed associations with mammographic measures cross-sectionally and between- and within-sibships to determine whether there was any evidence of association between the mammographic density measures and these variants overall, as well as for each variant individually.

Materials and Methods

Subjects. Between 2004 and 2009, female twin pairs who initially participated in the Australian Twins Study of Mammographic Density between 1995 and 1999, when they were aged 40 to 70 years (3, 4, 11), were asked through the Australian Twin Registry to participate further. This involved completing a questionnaire, giving permission to access their mammograms, donating a small blood sample, and, if they agreed, giving permission to invite their sisters to participate. Those who had breast cancer or breast reduction/augmentation surgery were excluded if a prediagnosis or presurgery mammogram was not available.

Mammographic measurements. Films were retrieved from BreastScreen Services (80%), private clinics (5%), and from women who kept their films at home (15%). Craniocaudal views of the right breast were used for measurement. Films were digitized by the Australian Mammographic Density Research Facility at the University of Melbourne using a Lumysis 85 scanner. Mammographic measurements were performed using Cumulus 3.0, a computer-assisted thresholding technique, by three independent operators (J.S., F.O., and K.N.). Outcomes were the average measures of the total area of the breast and of the area of dense tissue (dense area), which, when subtracted, gave the area of nondense tissue (nondense area). "Percent dense area" is dense area as a proportion of the total area.

Films were randomized (by family) into reading sets of ~100 with twins and/or sisters from the same family measured in the same set. Readers were blind to all other identifying information. A 10% random sample of repeat readings was included in each set and between every fifth set.

Questionnaire data. Telephone-administered questionnaires captured demographic information and self-reported weight, height, smoking history, alcohol consumption, reproductive history, cessation of menstruation, use of oral contraceptives, use of hormone replacement therapy, and familial history of cancer. A woman was defined as postmenopausal if she had had a hysterectomy, with both ovaries removed, or radiation; was not on hormone replacement therapy at the time of the mammogram and had not menstruated 12 months prior; or was on hormone replacement therapy at the time of mammogram and had not menstruated 12 months prior and was not menstruating before commencing hormone replacement therapy. Subjects not fitting these criteria were considered premenopausal. For twin pairs, zygosity was determined by a standard question that describes the

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differences between monozygotic and dizygotic twin pairs. This method has been shown to give 95% agreement with zygosity based on blood typing for middle-aged adults (12).

DNA extraction and genotyping. DNA was extracted from a 180 μL sample of whole blood and normalized in a 96-well plate format for genotyping. TaqMan assays (Applied Biosystem) with fluorescent allele-specific probes were used to genotype the 12 variants, 11 using predesigned assays (rs2981582, rs889312, rs12443621, rs3817198, rs8051542, rs13281615, rs17468277, rs13387042, rs981782, rs4973768, rs6504950) and one using a custom-designed assay (rs2107425). PCR reaction mix contained 4 ng of template DNA, 2.5 μL of 2× genotyping master mix, 0.0625 μL of 40× probes mix, and 0.4375 μL of H_2O for a total volume of 5 μL. PCR cycling and end-point fluorescence measurement were realized on a LightCycler 480 (Roche) in a 384-well plate format. Cycling conditions were 95°C/10 min then 40 cycles of 95°C/15 s and 60°C/1 min. Finally, allelic discrimination was conducted using the LightCycler 480 software. Assays with call rates >95% were deemed successful.

Statistical analysis. Intraclass correlation coefficients were used to assess the repeatability of the mammographic density measures both within and between observers.

For all regression analyses, dense area and nondense area were square root transformed so that the residuals were approximately normally distributed. We tested for departures from Hardy-Weinberg equilibrium using a pseudo-likelihood approach that uses data from multiple siblings within the same family (13).

We performed cross-sectional analyses in which the mean of each mammographic density measure was examined for associations with age, BMI, and other potential covariates. It was then modeled as a linear function of number of minor alleles (implying an additive genetic model). The residual variance was assumed to be constant, and the covariance between sisters was allowed to differ according to whether they were (*a*) both members of a monozygotic pair, (*b*) both members of a dizygotic pair, or (*c*) nontwin sisters by including three parameters: monozygotic correlation, dizygotic correlation, and sister correlation. Parameters and confidence intervals were estimated by maximum likelihood assuming for each sibship that the residuals followed a multivariate normal distribution. Models were fitted using the statistical software package FISHER (14, 15). The percentage of residual variance explained by each variant was estimated empirically by comparing the variance before and after adjusting for the per allele association for that variant.

We also performed between- and within-sibship analyses that accommodated the possibility of familial/population stratification (13). A series of orthogonal transformations were applied to both outcomes and exposures (including the target variant) to allow a decomposition of the exposure-outcome associations into independent between- and within-sibship regression associations; this generalizes the use of within-pair differences. The transformations were standardized to ensure that the already independent residuals (by dint of the orthogonal transformation) were also identically distributed. The details are outlined in Appendix. The magnitude of associations were estimated using linear regression, again assuming an additive genetic model and adjusting for the same covariates (subjected to the same orthogonal transformations) as for the cross-sectional analyses. Models were fitted using PROC MIXED in SAS (version 9.0, SAS Institute). A combined test of the null hypothesis was derived from the independent between- and within-sibship association per allele estimates using inverse variance weighting.

A test of the null hypothesis of no association between any of the variants and a given mammographic measure was conducted by testing whether the distribution of *P* values deviated from the uniform distribution on the interval 0, 1, summing the $-2 \ln P_i$, where P_i is the *P* value for the *i*th variant, across all loci and comparing with the χ^2 distribution with $2n$ degrees of freedom, where n is the number of independent variants (16).

The *P* values were written as P_x, from the cross-sectional analyses; P_w, from the within-sibship analyses; P_b, from the between-sibship analyses; P_c, from the combined analyses; and P_u, for the test of the uniform distribution. Following convention, all reported tests were two-sided and $P = 0.05$ was the threshold for claiming nominal significance for rejecting hypotheses specified *a priori*.

Results

Subjects missing information on BMI, menopausal status, benign biopsy status, or any of the genotyped variants were excluded. Genotyping revealed that 35 of the 532 twin pairs originally reported to be monozygotic were dizygotic. Genotypes of duplicate samples were 100% identical. The final sample comprised 2,288 subjects: 497 monozygotic pairs, 330 dizygotic pairs, and 634 nontwin sisters. There was no evidence of deviation from Hardy-Weinberg equilibrium for any variant (all $P > 0.15$). The intraclass correlation coefficient was >0.90 within readers and 0.87 between readers for measurements of percent dense area. Similarly, the intraclass correlation coefficient was >0.82 within readers and 0.84 between readers for measurements of dense area.

Table 2 shows the characteristics of subjects. Although the majority (70%) were postmenopausal, there was a wide range in age at mammogram from 31 to 79 years and the SD was 8.38 years. There were also wide variations in the mammographic density measures, with the coefficients of variation (SD/mean) being 67%, 39%, and 28% for percent dense area, dense area, and nondense area, respectively. Table 3 shows the means for each mammographic measure and all three genotypes for each variant.

After adjusting both dense area and percent dense area for age and BMI, their correlation was 0.8. The monozygotic, dizygotic, and sister correlations (95% confidence intervals in parentheses) for the residual measures were 0.73 (0.69–0.77), 0.30 (0.20–0.30), and 0.17 (0.09–0.25) for dense area; 0.67 (0.63–0.71), 0.37 (0.27–0.47), and 0.22 (0.014–0.30) for percent dense area; and 0.64 (0.58–0.70), 0.20 (0.10–0.30), and 0.13 (0.05–0.21) for nondense area.

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Table 2. Characteristics of subjects

Characteristics	Mean or % (95% CI)
Age at mammogram (y)	55.3 (54.9–55.6)
Physical	
Weight (kg)	69.1 (68.5–69.7)
Height (cm)	162.6 (162.4–162.9)
BMI (kg/m^2)	26.2 (25.9–26.4)
Reproductive variables	
Age at menarche (y)	13.07 (13.01–13.14)
Parous (%)	86.8 (85.3–88.2)
No. of live births	2.33 (2.28–2.39)
Postmenopausal (%)	69.7 (67.7–71.5)
Oral contraceptives	
Ever use of oral contraceptive (%)	86.6 (85.1–88.0)
Current use of oral contraceptive (%)	5.6 (4.7–6.7)
Years of oral contraceptive use (n = 2,050)	7.6 (7.3–7.9)
HRT	
Ever use of HRT (%)	39.6 (37.6–41.6)
Current use of HRT (%)	14.2 (12.7–15.6)
Years of HRT use (n = 906)	6.3 (5.9–6.7)
Smoking	
Ever smoked (%)	36.1 (34.2–38.2)
Current smoker (%)	9.1 (7.9–10.3)
Years of smoking (n = 827)	17.8 (16.9–18.7)
Alcohol	
Ever drank alcohol (%)	59.4 (57.4–61.5)
Years of drinking (n = 1,417)	22.7 (22.1–23.3)
Mammographic density measures	
Percent dense area (%)	25.26 (24.56–25.95)
Square root dense area (cm)	4.98 (4.90–5.06)
Square root nondense area (cm)	9.60 (9.49–9.71)

Abbreviation: HRT, hormone replacement therapy.

Table 4 shows the nominal P values for *a priori* hypothesis testing based on cross-sectional, between-sibships, within-sibships, and combined estimates. These were inconsistent with the uniform distribution for transformed and adjusted dense area (P_u = 0.0005 and 0.001 for combined and cross-sectional analyses, respectively) and for adjusted percent dense area (P_u = 0.007 for combined analyses), but not for transformed and adjusted nondense area (P_u = 0.7 and 0.8 for combined and cross-sectional analyses, respectively). That is, for each of the dense area and percent dense area measures, there is evidence that at least one variant is associated with these predictors of breast cancer risk.

For transformed and adjusted dense area, there were highly significant associations with the number of minor alleles of rs3817198/*LSP1* and rs13281615/8q (P_c = 0.001 and 0.003, and P_x = 0.0002 and 0.05, respectively). There was marginal evidence of an association with the number of minor alleles of rs889312/*MAP3K1* (P_c = 0.04), rs2107425/*H19* (P_x = 0.03), and rs17468277/*CASP8* (P_x = 0.04). Examination of Table 3 shows that the means increase or decrease monotonically, consistent with the fitted linear associations (except perhaps rs2107425/*H19*).

For each of the above five variants, the associations were in the same direction as their associations with breast cancer risk (see Table 1). The cross-sectional residual variance explained by these variants individually were 0.71%, 0.26%, 0.16%, 0.27%, and 0.24%, in the order discussed. When fitted together, they explained 1.5% of variance and the associations with rs2107425/*H19* and rs889312/*MAP3K1* and were no longer nominally significant. The variants rs3817198/*LSP1* and rs13281615/8q together explained 1.0% of variance.

For adjusted percent dense area, there were associations with the number of minor alleles for both rs3817198/*LSP1* and rs13281615/8q (all P_x and P_c <0.05). For rs889312/*MAP3K1,* the combined estimate was marginal (P_c = 0.03). All associations were in the same direction as for dense area, as described above. Examination of Table 3 shows that the means increase or decrease monotonically, consistent with the fitted linear associations (except perhaps rs13281615/8q).

For transformed and adjusted nondense area, the only association was for rs889312/*MAP3K1,* marginal, and from the combined between- and within-sibship analyses only (P_c = 0.04). The negative within-pair association with rs6504950 (P_w < 0.0001) was in the opposite direction to that predicted by the association between mammographic density measures and breast cancer, and to the marginal between-pair association (P_b = 0.02).

None of the associations above differed by menopausal status (all P > 0.1).

Discussion

Twin and family studies have shown that genetic factors could explain a considerable proportion of variance in the mammographic density measures that predict breast cancer risk; dense area and percent dense area both adjusted for age, BMI, and determinants. Based on a theoretical model of multiplicative risk (17) and the assumptions of the classic twin model, it has been estimated that mammographic density genes might explain ~10% of the familial aggregation of breast cancer (3, 4). In this article, we found that some of the common breast cancer genetic susceptibility variants recently identified and validated by large-scale case-control studies are also associated with the mammographic density measures that predict breast cancer risk, in particular age- and BMI-adjusted dense area. There was overwhelming evidence that there were too many small P values than would be expected under the null hypothesis that none of the variants are associated with these mammographic density measures (Table 4).

We found strong evidence that two variants, one on chromosome 11p in the region of *LSP1* and another on chromosome 8, are associated with the mammographic density

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measures that predict disease. There was some marginal evidence that another 3 of the 12 established breast cancer–associated common genetic variants might be associated with dense area. These common variants explained 1.5% of residual variance of dense area. Given that all these variants are prespecified candidates, and that the direction of their mammographic density associations are consistent with their breast cancer associations, they warrant genotyping by other large studies to determine more definitively if they are associated with this heritable breast cancer risk factor. The nominally significant associations were in general more apparent for dense area than percent dense area, and the test of uniformity was more significant for dense area.

The variants rs2107425 (*H19*) and rs3817198 (*LSP1*) are at the same locus (only 100 kb apart) and the association of rs2107425 (*H19*) with breast cancer risk was originally posited to be due to its linkage disequilibrium with rs3817198 (*LSP1*; ref. 6). We found that the cross-sectional association of rs2107425 (*H19*) with dense area was no longer evident after adjustment for rs3817198 (*LSP1*). Moreover, within sibships, there was evidence of an association with rs3817198 (*LSP1*; $P_w = 0.02$) but not with rs2107425 (*H19*; $P_w = 0.9$). This study of a disease-associated genetic marker

Table 3. Mean of each mammographic density measure by genotype, with 95% confidence intervals in parentheses for each variant

Variant	Genotype	Dense area*	Percent dense area	Nondense area*
rs2981582	GG (*n* = 866)	4.99 (4.86–5.11)	25.70 (24.56–26.84)	9.51 (9.33–9.69)
	GA (*n* = 1,083)	4.99 (4.87–5.10)	25.08 (24.07–26.08)	9.64 (9.48–9.80)
	AA (*n* = 339)	4.91 (4.70–5.12)	24.70 (22.88–26.53)	9.69 (9.39–10.00)
rs889312	AA (*n* = 1,059)	4.91 (4.80–5.02)	24.74 (23.73–25.75)	9.67 (9.50–9.83)
	AC (*n* = 1,024)	5.00 (4.88–5.12)	25.43 (24.38–26.49)	9.56 (9.4–9.73)
	CC (*n* = 205)	5.20 (4.92–5.47)	27.05 (24.64–29.46)	9.47 (9.10–9.83)
rs12443621	AA (*n* = 666)	5.11 (4.96–5.26)	26.49 (25.15–27.83)	9.46 (9.26–9.67)
	AG (*n* = 1140)	4.90 (4.79–5.00)	24.34 (23.38–25.29)	9.73 (9.57–9.89)
	GG (*n* = 482)	4.98 (4.80–5.15)	25.74 (24.20–27.28)	9.49 (9.25–9.73)
rs2107425	GG (*n* = 1,169)	5.10 (4.99–5.21)	26.08 (25.12–27.05)	9.53 (9.38–9.68)
	GA (*n* = 918)	4.83 (4.71–4.96)	24.29 (23.19–25.40)	9.68 (9.50–9.86)
	AA (*n* = 201)	4.90 (4.64–5.17)	24.85 (22.42–27.27)	9.63 (9.25–10.02)
rs3817198	TT (*n* = 1,059)	4.85 (4.73–4.96)	24.83 (23.78–25.88)	9.56 (9.39–9.73)
	TC (*n* = 993)	5.05 (4.93–5.17)	25.42 (24.39–26.44)	9.62 (9.46–9.79)
	CC (*n* = 236)	5.23 (4.98–5.48)	26.50 (24.31–28.68)	9.67 (9.32–10.01)
rs8051542	CC (*n* = 774)	5.02 (4.88–5.15)	25.15 (23.98–26.32)	9.66 (9.46–9.86)
	CT (*n* = 1,081)	4.99 (4.87–5.10)	25.43 (24.41–26.45)	9.55 (9.39–9.71)
	TT (*n* = 433)	4.87 (4.70–5.05)	25.02 (23.37–26.68)	9.62 (9.36–9.88)
rs13281615	AA (*n* = 739)	4.85 (4.71–4.98)	23.72 (22.57–24.87)	9.70 (9.52–9.89)
	AG (*n* = 1,140)	5.02 (4.90–5.13)	26.12 (25.10–27.13)	9.49 (9.33–9.66)
	GG (*n* = 409)	5.09 (4.9–5.28)	25.65 (23.97–27.32)	9.71 (9.46–9.96)
rs17468277	CC (*n* = 1,647)	5.03 (4.93–5.12)	25.40 (24.58–26.22)	9.63 (9.50–9.76)
	CT (*n* = 579)	4.85 (4.70–5.00)	25.06 (23.66–26.45)	9.48 (9.25–9.70)
	TT (*n* = 62)	4.88 (4.35–5.40)	23.31 (18.85–27.76)	10.02 (9.25–10.79)
rs13387042	GG (*n* = 569)	5.03 (4.87–5.19)	25.3 (23.92–26.68)	9.62 (9.40–9.84)
	GA (*n* = 1,117)	4.96 (4.84–5.07)	25.39 (24.38–26.41)	9.60 (9.44–9.77)
	AA (*n* = 602)	4.96 (4.81–5.11)	24.96 (23.64–26.29)	9.58 (9.37–9.79)
rs981782	AA (*n* = 646)	5.12 (4.97–5.27)	25.91 (24.63–27.19)	9.56 (9.36–9.76)
	AC (*n* = 1,155)	4.92 (4.81–5.04)	24.93 (23.94–25.92)	9.62 (9.47–9.78)
	CC (*n* = 487)	4.92 (4.75–5.08)	25.17 (23.66–26.69)	9.61 (9.35–9.86)
rs4973768	CC (*n* = 632)	4.90 (4.75–5.04)	24.95 (23.62–26.27)	9.54 (9.33–9.76)
	CT (*n* = 1,115)	5.02 (4.90–5.13)	25.56 (24.55–26.58)	9.61 (9.45–9.77)
	TT (*n* = 541)	4.98 (4.82–5.15)	24.99 (23.60–26.37)	9.65 (9.42–9.87)
rs6504950	GG (*n* = 1,116)	4.96 (4.84–5.08)	25.08 (24.10–26.06)	9.61 (9.45–9.77)
	GA (*n* = 927)	4.92 (4.79–5.04)	24.85 (23.73–25.97)	9.66 (9.48–9.83)
	AA (*n* = 245)	5.26 (5.02–5.50)	27.61 (25.47–29.76)	9.35 (9.04–9.66)

*Square-root transformed.

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Table 4. Per allele regression estimate, with 95% confidence intervals and associated *P* values from cross-sectional, within-sibship, between-sibship, and combined analyses for each variant and each adjusted mammographic density measure

Variant	Cross-sectional (95% CI)	P_x	Within sibships (95% CI)	P_w
Dense area*				
rs2981582	0.02 (–0.11–0.14)	0.8	0.18 (0.00–0.36)	0.06
rs889312	0.11 (–0.03–0.24)	0.1	0.17 (–0.06–0.39)	0.1
rs12443621	–0.02 (–0.14–0.10)	0.7	0.07 (–0.12–0.26)	0.5
rs2107425	–0.14 (–0.27–0.01)	0.03	–0.02 (–0.23–0.20)	0.9
rs3817198	0.24 (0.11–0.37)	0.0002	0.23 (0.03–0.42)	0.02
rs8051542	–0.05 (–0.17–0.07)	0.4	–0.04 (–0.22–0.15)	0.7
rs13281615	0.12 (0.00–0.24)	0.05	0.36 (0.16–0.56)	0.0005
rs17468277	–0.18 (–0.34–0.01)	0.04	0.01 (–0.27–0.28)	0.96
rs13387042	0.00 (–0.12–0.11)	0.9	0.12 (–0.05–0.30)	0.2
rs981782	–0.11 (–0.24–0.01)	0.06	–0.11 (–0.30–0.09)	0.3
rs4973768	0.02 (–0.10–0.14)	0.7	–0.04 (–0.24–0.15)	0.7
rs6504950	0.02 (–0.11–0.15)	0.8	0.10 (–0.11–0.31)	0.3
P_u		0.0010		0.007
Percent dense area†				
rs2981582	0.12 (–0.82–1.05)	0.8	0.34 (–1.10–1.78)	0.6
rs889312	0.82 (–0.20–1.84)	0.1	1.71 (–0.04–3.46)	0.06
rs12443621	–0.05 (–0.98–0.87)	0.9	0.19 (–1.30–1.68)	0.8
rs2107425	–0.71 (–1.71–0.3)	0.2	0.40 (–1.28–2.08)	0.6
rs3817198	0.98 (0.00–1.95)	0.05	1.75 (0.23–3.28)	0.03
rs8051542	–0.04 (–0.95–0.87)	0.9	0.22 (–1.25–1.68)	0.8
rs13281615	1.02 (0.09–1.96)	0.03	2.57 (1.01–4.13)	0.001
rs17468277	–0.89 (–0.20–0.21)	0.1	1.59 (–0.58–3.76)	0.2
rs13387042	0.05 (–0.83–0.94)	0.9	0.74 (–0.61–2.1)	0.3
rs981782	–0.40 (–1.33–0.53)	0.4	–0.27 (–1.76–1.23)	0.7
rs4973768	0.01 (–0.91–0.93)	1.0	0.25 (–1.29–1.78)	0.8
rs6504950	0.05 (–0.93–1.02)	0.9	2.35 (0.74–3.96)	0.005
P_u		0.2		0.004
Nondense area‡				
rs2981582	–0.05 (–0.19–0.10)	0.5	0.02 (–0.17–0.21)	0.8
rs889312	–0.12 (–0.28–0.04)	0.1	–0.24 (–0.48–0.01)	0.04
rs12443621	–0.01 (–0.15–0.13)	0.9	0.25 (0.05–0.45)	0.01
rs2107425	0.08 (–0.07–0.23)	0.3	0.01 (–0.21–0.23)	0.9
rs3817198	0.10 (–0.05–0.25)	0.2	–0.17 (–0.38–0.03)	0.09
rs8051542	–0.02 (–0.16–0.12)	0.8	0.04 (–0.15–0.24)	0.7
rs13281615	–0.02 (–0.16–0.12)	0.8	–0.07 (–0.28–0.13)	0.5
rs17468277	0.08 (–0.11–0.28)	0.4	–0.25 (–0.54–0.04)	0.09
rs13387042	–0.03 (–0.16–0.11)	0.7	–0.12 (–0.3–0.06)	0.2
rs981782	–0.04 (–0.19–0.1)	0.6	0.14 (–0.05–0.34)	0.2
rs4973768	0.06 (–0.08–0.20)	0.4	–0.04 (–0.24–0.17)	0.7
rs6504950	–0.05 (–0.20–0.10)	0.5	–0.46 (–0.67–0.24)	<0.0001
P_u		0.8		0.0005

NOTE: P_u is significance of *P* values against the uniform distribution.

*Square-root transformed and adjusted for age, difference of time between mammogram and interview, menopausal status, number of live birth, BMI, number of years drinking alcohol, number of years using hormone replacement therapy, number of years using oral contraceptives and begin breast dysplasia removed.

†Untransformed and adjusted for as in the dense area, plus number of years smoking.

‡Square-root transformed and adjusted for age, menopausal status, number of live birth, BMI, number of years drinking alcohol, number of years on hormone replacement therapy and number of years smoking.

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Table 4. Per allele regression estimate, with 95% confidence intervals and associated P values from cross-sectional, within-sibship, between-sibship, and combined analyses for each variant and each adjusted mammographic density measure (Cont'd)

Between sibships (95% CI)	P_b	Combined (95% CI)	P_c
−0.13 (−0.3–0.02)	0.09	0.00 (−0.12–0.12)	1.0
0.12 (0.04–0.29)	0.1	0.14 (0.01–0.27)	0.04
−0.10 (−0.25–0.05)	0.2	−0.03 (−0.15–0.09)	0.6
−0.12 (−0.30–0.05)	0.2	−0.08 (−0.21–0.05)	0.2
0.19 (0.03–0.35)	0.02	0.21 (0.08–0.33)	0.001
0.02 (−0.13–0.17)	0.8	0.00 (−0.12–0.11)	0.9
0.15 (−0.007–0.3)	0.06	0.22 (0.10–0.35)	0.0003
−0.19 (−0.40–0.01)	0.07	−0.12 (−0.29–0.04)	0.2
−0.05 (−0.20–0.1)	0.5	0.03 (−0.09–0.14)	0.7
−0.08 (−0.23–0.07)	0.3	−0.09 (−0.21–0.03)	0.1
0.09 (−0.06–0.23)	0.2	0.04 (−0.08–0.16)	0.5
0.07 (−0.09–0.22)	0.4	0.08 (−0.05–0.21)	0.2
	0.008		0.0005
−0.69 (−1.9–0.55)	0.3	−0.25 (−1.19–0.69)	0.6
0.89 (−0.4–2.18)	0.2	1.18 (0.14–2.22)	0.03
−0.30 (1.5–0.90)	0.6	−0.11 (−1.04–0.82)	0.8
−0.37 (−1.67–0.93)	0.6	−0.08 (−1.11–0.94)	0.9
0.41 (−0.87–1.70)	0.5	0.97 (−0.01–1.95)	0.05
0.54 (−0.65–1.73)	0.4	0.41 (−0.51–1.34)	0.4
1.67 (0.47–2.87)	0.006	2.01 (1.06–2.96)	0.00004
−1.28 (−2.88–0.33)	0.1	−0.26 (−1.55–1.03)	0.7
−0.16 (−1.36–1.04)	0.8	0.24 (−0.66–1.14)	0.6
0.000 (−1.20–1.20)	1.0	−0.11 (−1.04–0.83)	0.8
0.30 (−0.86–1.46)	0.6	0.28 (−0.65–1.21)	0.6
−0.29 (−1.53–0.95)	0.6	0.69 (−0.29–1.67)	0.2
	0.25		0.007
0.00 (−0.18–0.17)	1.0	0.01 (−0.12–0.13)	0.9
−0.09 (−0.27–0.09)	0.3	−0.15 (−0.29–0.01)	0.04
−0.08 (−0.24–0.09)	0.4	0.06 (−0.07–0.18)	0.4
−0.04 (−0.22–0.14)	0.4	−0.02 (−0.16–0.12)	0.8
0.18 (0.00–0.36)	0.04	0.03 (−0.11–0.16)	0.7
−0.05 (−0.21–0.12)	0.6	−0.01 (−0.14–0.12)	0.9
−0.07 (−0.24–0.10)	0.4	−0.07 (−0.2–0.06)	0.3
0.07 (−0.15–0.29)	0.5	−0.05 (−0.23–0.13)	0.6
−0.03 (−0.20–0.13)	0.7	−0.07 (0.20, 0.05)	0.2
−0.05 (−0.22–0.11)	0.5	0.03 (−0.10, 0.16)	0.7
0.01 (−0.15–0.17)	0.9	−0.010 (−0.13–0.12)	0.9
0.20 (0.03–0.37)	0.02	−0.06 (−0.19–0.08)	0.4
	0.3		0.7

has provided information that could help tease apart the roles of common variants in linkage disequilibrium on the disease itself.

Lee and colleagues (8) genotyped 516 breast cancer cases for six variants [rs889312 (*MAP3K1*), rs2981582 (*FGFR2*), rs3803662 (*TOX9*), rs3817198 (*LSP1*), rs13281615 (8q), and rs13387042 (2q)] and found no evidence that any was associated with percent dense area. From subanalyses, they reported marginal evidence that rs3817198 (*LSP1*) was positively associated with percent dense area when analyzing 221 steroid receptor–positive cases only. Tamimi and colleagues (9) genotyped 1,121 women for 11 variants [rs889312 (*MAP3K1*), rs2981582 (*FGFR2*), rs12443621 (*TOX9*), rs3803662 (*TOX9*), rs3817198 (*LSP1*), rs13281615 (8q), rs4666451, rs2107425 (*H19*), rs981782 (*HCN1*), rs8051542 (*TOX3*), and rs30099 (5)] and also found no evidence that any was associated with

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percent dense area. From subanalyses, they reported marginal evidence of associations with rs12443621 (*TOX3)*, rs3817198 (*LSP1*), and rs4666451 when analyzing premenopausal women only. Woolcott and colleagues (10) genotyped 825 women (361 with breast cancer) for eight variants [rs2981582 (*FGFR2*), rs3803662 (*TOX3*), rs12443621 (*TOX3*), rs3817198 in *LSP1*, rs981782 (*HCN1/MRPS30*), rs10941679 (*HCN1/MRPS30*), rs889312 (*MAP3K1*), and rs13387042 (2q)] and found marginal evidence that percent dense area was positively associated with rs12443621/*TOX3* and negatively associated with rs10941679 near *HCN1/MRPS30*. Subanalyses showed that rs3817198/*LSP1* was associated with percent dense area when analyzing current users of combined hormone replacement therapy only. These nominally significant subgroup associations with rs3817198/*LSP1* support our overall finding.

Based on our cross-sectional findings for percent dense area, for which the strongest associations were ~1% per allele with a SE of ~0.5%, and that our effective sample size was ~2,000 after taking into account the nonindependence of some subjects, a cross-sectional study of 1,000 women would have 30% power to detect the associations we found. Therefore, if our results represent reality, none of the previous studies had more than a 50:50 chance of finding a nominally significant association. It would be of interest to pool raw data from published studies.

The strengths of our study include its size, although larger studies are warranted especially for the variants with equivocal findings, and averaging measurements taken by trained observers with demonstrated high repeatability. Collecting, and analyzing as we have, data from twins and their sisters was another strength. The within-sibship associations are naturally adjusted not only for age and other measured covariates but also for unmeasured factors shared by twins and sisters. The between-sibship analyses summarize data from groups of similar ages and familial, including genetic, background. Finally, the combined test statistic for association makes use of the independence of the between- and within-sibship estimates to gain more power to test the null hypothesis of no association.

Little is known about how the variants we have studied convey susceptibility to breast cancer. They could be in linkage disequilibrium with other, perhaps rarer and as yet unidentified, "causal" variants. These findings suggest that whatever the underlying mechanisms, for at least two loci they are also likely associated with mammographically dense area. This lead could help elucidate why those variants are associated with breast cancer risk.

In addition, unraveling the mechanisms behind the associations of variants with breast cancer risk could help understand what it is about mammographically dense area that makes it a risk factor for breast cancer. Understanding the function of the genes, and in particular the change in function due to the causal variant(s), could lead to new insights about mechanisms that cause breast cancer. Furthermore, the discovery of these causal variant(s) could be aided by studying the continuously distributed risk factor (mammographic density) rather than the disease itself. That is, understanding the genetic link between mammographic density and breast cancer risk could help unravel the etiology of the disease.

Appendix: Decomposition of Exposure-Outcome Association into Independent between- and within-Sibship Regression Associations Using Orthogonal Transformations

Suppose we have data from sibships, with outcome Y_{ij} and exposure X_{ij} recorded on the jth member of the ith sibship. Extension to multiple exposures is straightforward and we omit it only to avoid complicating the notation. The outcome and exposure are related through the linear regression equation

$$Y_{ij} = \alpha_i + \beta X_{ij} + \epsilon_{ij} \quad (5)$$

where α_i incorporates a shared familial effect. We therefore assume that the residual error terms ε_{ij} are i.i.d. normally distributed with mean 0 and variance σ^2. For a given sibship of n members, the series of n regression equations can be written in vector form

$$\boldsymbol{Y} = \boldsymbol{\alpha} + \beta \boldsymbol{X} + \boldsymbol{\epsilon} \quad (6)$$

where $\boldsymbol{Y}$, $\boldsymbol{X}$, and $\boldsymbol{\varepsilon}$ are all vectors of length n. In addition, $\boldsymbol{\alpha}$ is a constant vector and β is a scalar. If $\boldsymbol{A}$ is an orthogonal matrix of full rank, the estimability of parameters is preserved if we premultiply both sides of Eq. 6 by $\boldsymbol{A}$,

$$\boldsymbol{AY} = \boldsymbol{A\alpha} + \beta \boldsymbol{AX} + \boldsymbol{A\epsilon} \quad (7)$$

which we rewrite as

$$\boldsymbol{Y}^* = \boldsymbol{\alpha}^* + \beta \boldsymbol{X}^* + \boldsymbol{\epsilon}^* \quad (8)$$

where $\boldsymbol{Y}^* = \boldsymbol{AY}$, $\boldsymbol{\alpha}^* = \boldsymbol{A\alpha}$, $\boldsymbol{X}^* = \boldsymbol{AX}$, and $\boldsymbol{\varepsilon}^* = \boldsymbol{A\varepsilon}$. The variance-covariance matrix of $\boldsymbol{\varepsilon}$ being $\sigma^2\boldsymbol{I}$, the corresponding matrix for $\boldsymbol{\varepsilon}^*$ is $\boldsymbol{A}\sigma^2\boldsymbol{I}\boldsymbol{A}^{\mathrm{T}} = \sigma^2\boldsymbol{I}$, by the definition of an orthogonal matrix. Our proposed approach constrains the matrix $\boldsymbol{A}$ to have one row of the form (1,...,1) so that any terms common to all members of the sibship are captured in the corresponding transformed outcome and exposure variables, with the relevant regression coefficient representing a cross-sectional or between-sibship effect. The remaining rows of $\boldsymbol{A}$ will then be orthogonal to (1,...,1) and hence to $\boldsymbol{\alpha}$, implying that only the first component of $\boldsymbol{\alpha}^*$ is nonzero. These other rows represent within-sibship contrasts, with $n - 1$ transformed (X_j, Y_j) pairs contributing to estimating the within-sibship regression effect. In the simplest case of sib-pairs, we use

$$A = \frac{1}{\sqrt{2}} \begin{bmatrix} 1 & 1 \\ 1 & -1 \end{bmatrix}$$

in which case the pair of equations

$$Y_1 = \alpha + \beta X_1 + \epsilon_1 \quad (9)$$

$$Y_2 = \alpha + \beta X_2 + \epsilon_2 \quad (10)$$

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transforms to

$$\frac{1}{\sqrt{2}}(Y_{i1}+Y_{i2}) = \beta\frac{1}{\sqrt{2}}(X_{i1}+X_{i2}) + \eta_i \qquad (11)$$

$$\frac{1}{\sqrt{2}}(Y_{i1}+Y_{i2}) = \beta\frac{1}{\sqrt{2}}(X_{i1}+X_{i2}) + \epsilon_i \qquad (12)$$

If we use only the latter formula, we obtain the standard paired difference regression. Comparing estimates β_b and β_w from the first and second sets, we can test the hypothesis that the effect of X is the same within and between pairs. For sibships of $n > 2$ members, the process is similar, with each sibship providing $n - 1$ data points to the "within" analysis and one to the "between." Incorporating sibships of different sizes into a single regression analysis is straightforward provided a suitable matrix $\boldsymbol{A}_k$ can be found for sibships of size k that represents an orthogonal transformation and can be standardized (the factors of $1/\sqrt{2}$ in the $n = 2$ case above) to ensure that the residual error terms are identically distributed for sibships of different sizes, allowing the estimation to proceed using a single linear regression. The mathematical details, including a systematic method for obtaining $\boldsymbol{A}_k$ for arbitrary k, are given in a separate forthcoming article.

For the purposes of this article, the following orthogonal transformations were used for pairs, triples, quartets, quintets, and sextets.

$$A_2 = \frac{1}{\sqrt{2}}\begin{bmatrix} 1 & 1 \\ -1 & 1 \end{bmatrix};$$

$$A_3 = \frac{1}{\sqrt{3}}\begin{bmatrix} 1 & 1 & 1 \\ -1 & \frac{1}{2}(1+\sqrt{3}) & \frac{1}{2}(1-\sqrt{3}) \\ -1 & \frac{1}{2}(1-\sqrt{3}) & \frac{1}{2}(1+\sqrt{3}) \end{bmatrix};$$

$$A_4 = \frac{1}{2}\begin{bmatrix} 1 & 1 & 1 & 1 \\ -1 & 5/3 & -1/3 & -1/3 \\ -1 & -1/3 & 5/3 & -1/3 \\ -1 & -1/3 & -1/3 & 5/3 \end{bmatrix};$$

$$A_5 = \frac{1}{\sqrt{5}}\begin{bmatrix} 1 & 1 & 1 & 1 & 1 \\ -1 & \frac{1}{4}(3\sqrt{5}+1) & \frac{1}{4}(1-\sqrt{5}) & \frac{1}{4}(1-\sqrt{5}) & \frac{1}{4}(1-\sqrt{5}) \\ -1 & \frac{1}{4}(1-\sqrt{5}) & \frac{1}{4}(3\sqrt{5}+1) & \frac{1}{4}(1-\sqrt{5}) & \frac{1}{4}(1-\sqrt{5}) \\ -1 & \frac{1}{4}(1-\sqrt{5}) & \frac{1}{4}(1-\sqrt{5}) & \frac{1}{4}(3\sqrt{5}+1) & \frac{1}{4}(1-\sqrt{5}) \\ -1 & \frac{1}{4}(1-\sqrt{5}) & \frac{1}{4}(1-\sqrt{5}) & \frac{1}{4}(1-\sqrt{5}) & \frac{1}{4}(3\sqrt{5}+1) \end{bmatrix}.$$

$$A_5 = \frac{1}{\sqrt{5}}\begin{bmatrix} 1 & 1 & 1 & 1 & 1 \\ -1 & \frac{1}{5}(4\sqrt{6}+1) & \frac{1}{5}(1-\sqrt{6}) & \frac{1}{5}(1-\sqrt{6}) & \frac{1}{5}(1-\sqrt{6}) \\ -1 & \frac{1}{5}(1-\sqrt{6}) & \frac{1}{5}(4\sqrt{6}+1) & \frac{1}{5}(1-\sqrt{6}) & \frac{1}{5}(1-\sqrt{6}) \\ -1 & \frac{1}{5}(1-\sqrt{6}) & \frac{1}{5}(1-\sqrt{6}) & \frac{1}{5}(4\sqrt{6}+1) & \frac{1}{5}(1-\sqrt{6}) \\ -1 & \frac{1}{5}(1-\sqrt{6}) & \frac{1}{5}(1-\sqrt{6}) & \frac{1}{5}(1-\sqrt{6}) & \frac{1}{5}(4\sqrt{6}+1) \end{bmatrix}.$$

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Common Genetic Variants Associated with Breast Cancer and Mammographic Density Measures That Predict Disease

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