

# Novel Associations between Common Breast Cancer Susceptibility Variants and Risk-Predicting Mammographic Density Measures

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## Abstract

Mammographic density measures adjusted for age and body mass index (BMI) are heritable predictors of breast cancer risk, but few mammographic density-associated genetic variants have been identified. Using data for 10,727 women from two international consortia, we estimated associations between 77 common breast cancer susceptibility variants and absolute dense area, percent dense area and absolute nondense area adjusted for study, age, and BMI using mixed linear modeling. We found strong support for established associations between rs10995190 (in the region of *ZNF365*), rs2046210 (*ESR1*), and rs3817198 (*LSP1*) and adjusted absolute and percent dense areas (all  $P < 10^{-5}$ ). Of 41 recently discovered breast cancer susceptibility variants, associations were found between rs1432679 (*EBF1*), rs17817449 (*MIR1972-2: FTO*),

rs12710696 (*2p24.1*), and rs3757318 (*ESR1*) and adjusted absolute and percent dense areas, respectively. There were associations between rs6001930 (*MKLI*) and both adjusted absolute dense and nondense areas, and between rs17356907 (*NTN4*) and adjusted absolute nondense area. Trends in all but two associations were consistent with those for breast cancer risk. Results suggested that 18% of breast cancer susceptibility variants were associated with at least one mammographic density measure. Genetic variants at multiple loci were associated with both breast cancer risk and the mammographic density measures. Further understanding of the underlying mechanisms at these loci could help identify etiologic pathways implicated in how mammographic density predicts breast cancer risk. *Cancer Res*; 75(12); 2457–67. ©2015 AACR.

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## Introduction

Mammographic density refers to the white or light areas on a mammogram, which are thought to reflect differing amounts of epithelial and stromal tissue within the breast, as distinct from radiographically lucent fatty tissue. For women of the same age and body mass index (BMI), those with more extensive amounts of either absolute or percent dense area are more likely to develop breast cancer (1). The underlying biologic processes are not clear.

Twin and family studies have shown that a substantial variation in the mammographic density measures could be due to genetic factors (2–4). Moreover, these heritable mammographic density measures are thought to explain about 10% to 20% of the association of family history with breast cancer risk (5, 6).

Finding genetic variants that are associated with both breast cancer risk and the mammographic density measures that predict breast cancer has the potential to reveal underlying biologic pathways that explain the associations between those mammographic measures and cancer, resulting in a better understanding of the etiology of breast cancer. The use of large-scale genotyping projects to discover common genetic variants (single-nucleotide polymorphisms, or SNPs) associated with breast cancer risk has opened up the possibility of achieving this. The international DENSE consortium previously studied the associations of 15 independent breast cancer susceptibility variants with age- and BMI-adjusted mammographic density measures for 17,000 women. This confirmed prior associations found between the variant rs381798 (in the region of *LSP1*; refs. 7, 8) and adjusted absolute and percent dense area and provided evidence for an association between rs10483813 (in the region of *RAD51L1*) and adjusted percent dense area (9). Two genome-wide association studies (GWAS) conducted by the Markers of Density (MODE) consortium found that there was an association between rs10995190 (in the *ZNF365* locus), independently shown to be associated with breast cancer risk (10), and adjusted percent dense area, and weaker evidence for associations with the variants rs2046210 (in the region of *ESR1*) and rs3817198 (see above; ref. 11). More recently, MODE identified novel loci associated with dense area (rs10034692 from *AREG*, rs703556 from *IGF1*,

rs7289126 from *TMEM184B*, rs17001868 from *SGSME/MKL1*, nondense area (rs7816345 from *8p11.23*), and percent density (rs186749 from *PRDM6*, rs7816345 from *8p11.23* and rs7289126 from *TMEM184B*; ref. 11). Furthermore, using a GWAS of both breast cancer and mammographic density, MODE investigators found that adjusted percent dense area and breast cancer risk have a shared genetic basis that is mediated by, at least in theory, a large number of common variants (12).

A further 41 independent breast cancer susceptibility common variants have been discovered by a study of 45,290 cases and 41,880 controls using a custom genotyping array designed, in part, by the Breast Cancer Association Consortium (BCAC; ref. 13). Of these new variants, a recent report from several coauthors found novel associations between breast cancer SNPs in *6q25*: rs9485372 (*TAB2*) and rs9383938 (*ESR1*) with a volumetric measure of mammographic density in approximately 5000 Swedish women (14). They also found novel associations between breast cancer SNPs rs6001930 (*MKL1*) and rs17356907 (*NTN4*) with absolute nondense volume. Here, we provide the largest and most comprehensive report to date of the associations between the current total of 77 known breast cancer susceptibility SNPs and three area-based mammographic density measures using data from over 10,000 women participating in the DENSE and MODE consortia.

## Materials and Methods

### Subjects

Genotypes, mammographic density measures, and information on conventional breast cancer risk factors were available for 10,727 self-reported women of European Ancestry from 13 studies described previously (4, 9, 11, 15). A summary of study design, sample sizes, mammographic, and genotyping characteristics is given in Supplementary Table S1. Each study obtained informed consent and had relevant ethics and institutional approvals. Only anonymized data were used for analyses.

### Mammographic density measures

All mammographic density measurements were performed on digitized analogue films using either the Cumulus (16), Madena (17), or MDEST (18) programs. All approaches apply a thresholding

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**Note:** Supplementary data for this article are available at Cancer Research Online (<http://cancerres.aacrjournals.org/>).

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technique to measure total area of the breast and absolute dense area, from which percent dense area and absolute nondense area are derived. Absolute dense and nondense area values were converted to  $\text{cm}^2$  according to the pixel size used in the digitization. All measurements were conducted by observers blind to genotype, case status (if applicable), and breast cancer risk factor data. For cases, mammograms prior to diagnosis were used or, when this was not possible, those from the contralateral breast taken at the time of diagnosis (Table 1).

The mammographic density readings were performed on both craniocaudal (CC) and mediolateral oblique (MLO) views but these have been consistently shown to have high correlation (range, 0.87–0.90; ref. 19).

### Genotyping

The 77 currently known breast cancer susceptibility SNPs were genotyped for the 13 studies either as part of a GWAS (11, 15) or by genotyping of a custom Illumina iSelect genotyping array comprising 211,155 SNPs [described in Michailidou and colleagues (Table 1; ref. 13)]. Quality control was conducted at the study level; for all SNPs in these analyses their call rates were >95%. Five SNPs (from three studies) with Hardy–Weinberg equilibrium  $P < 0.001$  were excluded.

### Statistical methods

Distributions of covariates summarized by frequency and percentages are summarized by breast cancer status (affected/unaffected). Primary analyses used individual level data and included a fixed study effect to adjust for potential differences due to study. Analyses were conducted using the square root of the density measures as the outcome variables, and examination of the distributions of the residuals after adjustment for age and BMI showed an approximately normal distribution.

Primary analyses were conducted using fixed effects ordinary linear regression adjusting for age (continuous), 1/BMI, and study. Analyses considered SNP associations as additive by defining an ordinal covariate as the number of copies of the minor allele (0, 1, or 2) producing per-allele estimates that are reported as  $\beta$  and standard error (SE). (For imputed genotypes from the two GWAS studies, the imputed allelic dosage values were used.) Secondary analyses were performed to evaluate potential confounding with other covariates such as case–control status, menopausal status (pre- and perimenopausal combined vs. postmenopausal), and postmenopausal hormone use (ever vs. never use). To measure the extent to which the mammographic measures mediated the SNP associations with breast cancer risk, we estimated the proportion of change in the regression coefficient for each SNP after adjustment for breast cancer status and calculated 95% confidence intervals based on methods described by Lin and colleagues (20).

We performed a series of analyses to test the robustness of the association between mammographic density measures and the 77 SNPs. First, we performed an overall test of whether there was no association between any of the variants and a given mammographic measure by testing whether the distribution of the 77  $P$  values deviated from the uniform distribution on the interval 0, 1. The Fisher exact test of uniformity tests the sum of the  $-2 \ln P_i$  across all loci where  $P_i$  is the  $P$  value for the  $i^{\text{th}}$  variant, against  $\chi^2$  distribution with  $2n$  degrees of freedom, where  $n$  is the number of independent variants (21). Second, to try to determine the "best" model fit (i.e., the set of independent SNPs that give the

**Table 1.** Design, sample size, data collection, mammographic characteristics, and genotyping information for the 13 studies

Study name (reference)	Study abbreviation	Design <sup>a</sup>	Number cases/controls	Source of covariate data			Breast side <sup>c</sup>			Genotyping (GWAS or iCOGS)
				Reproductive variables	Anthropometry	mammogram and data collection	Film view <sup>b</sup>	Cases	Controls	
Australian Breast Cancer Family Study (38–40)	ABCF5	CC Family	103/0	Questionnaire	Self-report	Within 3 years	CC	Contra	n/a	iCOGS
Bavarian Breast Cancer Cases and Controls (41)	BBCC	CC	512/367	Questionnaire	Self-report	Within 30 days	CC	Contra	Average	iCOGS
European Prospective Investigation into Cancer (42)	EPIC	Cohort	86/968	Questionnaire	Measured	3 years prior	MLO	Contra	Average	iCOGS
Mayo Clinic Breast Cancer Study (43)	MCBCS	CC	677/864	Questionnaire	Measured	Within 30 days	CC	Contra	L	iCOGS
Melbourne Collaborative Cohort Study (44)	MCCS	Nested CC	68/28	Questionnaire	Measured	3 years prior	CC	R	R	iCOGS
Multithnic Cohort Study (45, 46)	MEC	Nested CC	110/101	Questionnaire	Self report	Within 5 years prior	CC	Average <sup>d</sup>	Average	iCOGS
Old Amish Study	OAA	Family	0/400	Questionnaire	Measured	Within 30 days	CC	n/a	L or R	GWAS
Mayo Mammography Health Study (4)	MMHS	Nested CC	456/1,166	Questionnaire	Both	Within 30 days	CC	Average <sup>d</sup>	Average	iCOGS
Norwegian Breast Cancer Study	NBCS	CS	0/38	Questionnaire	Self-report	Within 14 days	CC	n/a	L	iCOGS
Nurses Health Study (47, 48)	NHS	Nested CC	850/849	Questionnaire	Self-report	Within 2 years	CC	Average <sup>d</sup>	Average	GWAS
Ontario Familial Breast Cancer Registry (38)	OFBCR	Family	73/0	Questionnaire	Self-report	2–9 years prior	CC	Contra	n/a	iCOGS
Singapore and Sweden Breast Cancer Study (49)	SASBAC	CC	869/783	Questionnaire	Self-report	Mean 1 year post	MLO	Contra	L or R	iCOGS
Sisters in Breast Cancer Screening (50)	SIBS	Family	0/1,359	Questionnaire	Measured	Within 1 year prior	MLO	n/a	Average	iCOGS

<sup>a</sup>CC, case-control study; CS, cross-sectional study.

<sup>b</sup>CC, craniocaudal view; MLO, mediolateral oblique view.

<sup>c</sup>Average, average from left and right breasts; contra, unaffected contra-lateral breast; L, left breast; n/a, not applicable; R, right breast.

<sup>d</sup>Prediagnostic films.

best-fitting model for adjusted breast density), we used Lasso (least absolute shrinkage and selection operator) regression, a method that combines estimation and model selection that limits overestimation of associations when there are a large number of covariates (22). The final model was chosen by the minimum Schwarz Bayesian Information Criterion (SBC), which combines goodness of fit with a penalty based on the number of parameters in the model. Finally, we tried to quantify whether there was information in the other variants that did not reach our *P* value threshold (see below for details) but that could help further explain some of the missing heritability. For each mammographic density measure, we removed the most significant variants ( $P < 0.00065$ , selected by 0.05/77) associated with that measure and tested whether the distribution of the remaining *P* values was different from zero. The least informative variant was removed sequentially until there was no evidence to reject the null hypothesis.

Analyses were performed using SAS version 9.3 (SAS Institute, Inc.). Two-sided *P* values were calculated. We used a conservative threshold of  $0.05/77 = 0.00065$  to define statistical significance, while presenting the results for all tested variants.

## Results

Table 2 shows summary characteristics for each study. The majority of women were older than 60 years, more than 80% were postmenopausal, 55% had BMI  $\geq 25$  kg/m<sup>2</sup>, and 35% were breast cancer cases.

Percent and absolute dense area were negatively associated with age, BMI, parity, and postmenopausal status and positively associated with postmenopausal hormone therapy use (Supplementary Table S1). Conversely, absolute nondense area was positively associated with age, BMI, and parity and negatively associated with hormone therapy use. All of the above associations were similar in direction and magnitude for cases and controls (data not shown). None of the density measures were statistically significantly different by mammogram view (Supplementary Table S1).

Of the 77 variants, nine were associated with at least one adjusted mammographic density measure, using the threshold of 0.00065 (Table 3, results for all SNPs in Supplementary Table S1). Figure 1 is a forest plot of all 77 breast cancer susceptibility

**Table 2.** Summary characteristics at time of mammogram and by case status for the participating studies

Characteristic	Category	Breast cancer	
		cases N (%)	Noncases N (%)
Age, y	<50	542 (14.3)	776 (11.2)
	50–59	1,197 (31.5)	2,307 (33.3)
	$\geq 60$	2,065 (54.3)	3,840 (55.5)
Parity	Nulliparous	466 (12.3)	775 (11.2)
	Parous	3,283 (86.3)	6,063 (87.6)
	Unknown	55 (1.5)	85 (1.2)
Menopausal status	Premenopausal	611 (16.1)	1,268 (18.3)
	Postmenopause	3,152 (82.9)	5,575 (80.5)
	Unknown	41 (1.1)	80 (1.2)
Postmenopausal hormone therapy use	Ever	1,848 (48.6)	2,942 (42.5)
	Never	1,710 (45.0)	2,885 (41.7)
BMI, kg/m <sup>2</sup>	Unknown	39 (1.0)	50 (0.7)
	<25	1,644 (43.2)	2,751 (39.7)
	$\geq 25$	2,121 (55.8)	4,122 (59.5)
Mammographic side, view	L - CC	426 (11.2)	1,102 (15.9)
	R - CC	511 (13.4)	296 (4.3)
	LR average - CC	1,911 (50.2)	2,415 (34.9)
	L - MLO	446 (11.7)	415 (6.0)
	R - MLO	510 (13.4)	409 (5.9)
	LR average - MLO	0 (0)	2,286 (33.0)

Abbreviations: CC, cranio-caudal; L, left; MLO, mediolateral oblique; R, right.

variants sorted by magnitude of association with breast cancer risk in these studies; the nine variants are highlighted in bold. The findings confirm previously identified associations with both adjusted percent and absolute dense areas for rs10995190 in the *ZNF365* gene ( $\beta = 0.16$ , SE = 0.028,  $P = 8.5 \times 10^{-9}$  and  $\beta = 0.25$ , SE = 0.038,  $P = 4.7 \times 10^{-11}$ , respectively), rs2046210 in the region of *ESR1* ( $\beta = 0.098$ , SE = 0.021,  $P = 2.4 \times 10^{-6}$  and  $\beta = 0.14$ , SE = 0.029,  $P = 1.7 \times 10^{-6}$ , respectively) and rs3817198 in the region of *LSP1* ( $\beta = 0.087$ , SE = 0.021,  $P = 4.4 \times 10^{-5}$  and  $\beta = 0.16$ , SE = 0.029,  $P = 1.3 \times 10^{-7}$ , respectively). None of these three variants showed evidence of association with adjusted nondense area (Table 3). There were marginal associations between two independent variants ( $r^2 = 0.003$ ) in the region of *RAD51L1*; rs999737 ( $P = 0.003$  and  $P = 0.01$ ) with both adjusted percent and absolute dense area [reported in our previous DENSNP study (9)] and rs2588809 ( $P = 0.002$ ,  $P = 0.04$ , and

**Table 3.** Top associations between common breast cancer susceptibility variants and each of the mammographic measures

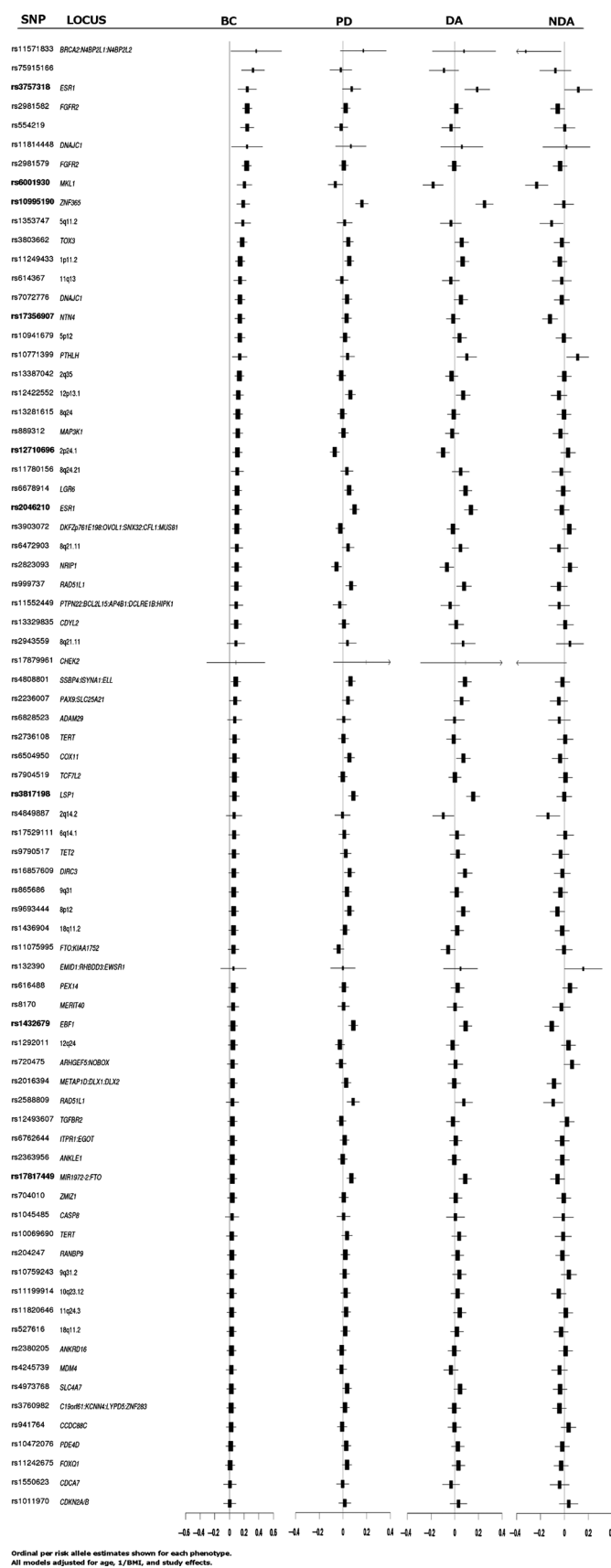
SNP	Locus	Alleles <sup>a</sup>	Percent dense area			Dense area			Nondense area		
			Beta estimate <sup>b</sup>	Standard error	<i>P</i>	Beta estimate <sup>b</sup>	Standard error	<i>P</i>	Beta Estimate <sup>b</sup>	Standard error	<i>P</i>
Previously reported											
rs10995190	<i>ZNF365</i>	A/G	<b>0.16</b>	<b>0.03</b>	<b>8.5E-09</b>	<b>0.25</b>	<b>0.04</b>	<b>4.7E-11</b>	-0.005	0.04	0.91
rs3817198	<i>LSP1</i>	A/G	<b>0.09</b>	<b>0.02</b>	<b>4.4E-05</b>	<b>0.16</b>	<b>0.03</b>	<b>1.3E-07</b>	-0.001	0.03	0.97
rs2046210	<i>ESR1</i>	G/A	<b>0.10</b>	<b>0.02</b>	<b>2.4E-06</b>	<b>0.14</b>	<b>0.03</b>	<b>1.7E-06</b>	-0.02	0.03	0.50
Novel associations											
rs6001930	<i>MKL1</i>	A/G	-0.06	0.03	0.044	<b>-0.18</b>	<b>0.04</b>	<b>3.2E-05</b>	<b>-0.23</b>	<b>0.05</b>	<b>1.7E-06</b>
rs1432679	<i>EBF1</i>	A/G	<b>0.09</b>	<b>0.02</b>	<b>1.1E-05</b>	0.09	0.03	7.1E-04	<b>-0.11</b>	<b>0.03</b>	<b>4.5E-04</b>
rs17356907	<i>NTN4</i>	G/A	0.03	0.02	0.16	-0.01	0.03	0.68	<b>-0.12</b>	<b>0.03</b>	<b>2.4E-04</b>
rs17817449	<i>MIR1972-2-FTO</i>	C/A	<b>0.07</b>	<b>0.02</b>	<b>4.4E-04</b>	0.09	0.03	0.001	-0.06	0.03	0.06
rs3757318	<i>ESR1</i>	G/A	0.07	0.04	0.066	<b>0.19</b>	<b>0.05</b>	<b>4.6E-04</b>	0.12	0.06	5.0E-02
rs12710696	<i>2p24.1</i>	G/A	-0.07	0.02	8.7E-04	<b>-0.10</b>	<b>0.03</b>	<b>5.9E-04</b>	0.03	0.03	0.32

NOTE: Bold type denotes SNPs with  $P < 6.5 \times 10^{-4}$  for that association. Study heterogeneity  $P > 0.05$  for all SNPs apart from the association between rs2046210 and adjusted dense area ( $P = 0.03$ ).

<sup>a</sup>Second allele is modeled allele (breast cancer risk allele).

<sup>b</sup>Ordinal per risk allele estimate, age, 1/BMI, study adjusted.





**Figure 1.** Associations between the 77 common breast cancer susceptibility SNPs and breast cancer (BC), adjusted percent dense area (PD), adjusted dense area (DA), and adjusted nondense area (NDA), ordered by the magnitude of the association with breast cancer.

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$P = 0.02$ ) with adjusted percent dense area, dense area, and nondense area respectively (Supplementary Table S2).

Of the 41 recently identified breast cancer loci, we found evidence of novel associations between at least one of the adjusted density measures and six variants (Table 3). The minor G allele of rs1432679 (*EBF1*) was positively associated with adjusted dense area and negatively associated with adjusted nondense area, and hence was positively associated with adjusted percent density ( $\beta = 0.087$ ,  $SE = 0.020$ ,  $P = 1.1 \times 10^{-5}$ ). The minor G allele of rs6001930 in the region of *MKL1* was negatively associated with both adjusted absolute dense and nondense areas ( $\beta = -0.18$ ,  $SE = 0.044$ ,  $P = 3.2 \times 10^{-5}$  and  $\beta = -0.23$ ,  $SE = 0.048$ ,  $P = 1.7 \times 10^{-6}$ , respectively), but was not associated with adjusted percent density ( $P = 0.04$ ). The A allele of rs17356907 in the region of *NTN4* was negatively associated with adjusted nondense area ( $\beta = -0.12$ ,  $SE = 0.033$ ,  $P = 2.4 \times 10^{-4}$ ), but not with adjusted dense area or percent density. The A allele of rs3757318 (close to *ESR1*) was positively associated with adjusted dense area ( $\beta = 0.19$ ,  $SE = 0.054$ ,  $P = 4.6 \times 10^{-4}$ ), but not with either of the other density phenotypes. Both rs17817449 (*MIR1972-2:FTO*) and rs12710696 (*2p24.1*) were negatively associated with adjusted percent and absolute dense area. Although sample sizes were substantially reduced ( $n < 7000$ ), these associations were similar when analyses were restricted to images from controls only, CC mammogram views, and mammograms within a year of covariate information (data not shown).

Further adjustment for case-control status showed evidence that percent dense area and dense area mediated the associations of rs10995190 (*ZNF365*), rs2046210 (*ESR1*), rs1432679 (*EBF1*), and rs3817198 (*LSP1*) with breast cancer risk (Supplementary Table S3). There was also evidence that dense area mediated the association of rs3757318 (*ESR1*) and breast cancer, and nondense area mediated the association of rs1432679 (*EBF1*) and rs6001930 (*MKL1*) with breast cancer. These estimates ranged from 4% to 18% of the SNP and breast cancer association being explained by density phenotypes (Supplementary Table S3). However, adjustment for other additional covariates did not substantially influence the regression estimates (data not shown). The between-study test of heterogeneity  $P$  value was  $>0.05$  for all the variants in Table 3, except for the association between rs2046210 (*ESR1*) and adjusted dense area ( $P = 0.03$ ).

When taking a global, as distinct from individual SNP, view we found that of the 77 variants examined, the nominal  $P$  value was  $<0.05$  for 20 associations with adjusted dense area, 18 associations with adjusted percent dense area, and 10 associations with adjusted nondense area comprising in total 25 separate variants (Supplementary Table S2). For any one density measure, by chance alone we would have expected 3.9 (95% CI, 1–7) associations to be nominally significant at  $P = 0.05$ . The distributions of the 77  $P$  values for each of the mammographic measures were not consistent with the uniform distribution ( $P_{\text{uniform}} < 2 \times 10^{-6}$  for each density measure), suggesting the existence of true associations between at least some breast cancer susceptibility variants and the mammographic density measures that predict breast cancer. From Lasso regression, the "best fitting" model for adjusted percent dense area included the bolded variants in Table 3 plus three others, including rs12710696 (*2p24.1*), rs4808801 (*SSBP4:ISYNA1:ELL*), and rs12422552 (*12p13.1*), which, when combined,

explained 1.2% of the variation in the adjusted percent dense area trait. Similarly, the best fitting model from Lasso regression of adjusted dense area included the six bolded variants in Table 3 and rs1432679 (*EBF1*), rs17817449 (*MIR1972-2:FTO*), and rs4808801 (*SSBP4:ISYNA1:ELL*). When combined, they explained 1.4% of the variation in adjusted dense area. Finally, the best fitting model for adjusted nondense area included only the three bolded variants in Table 3, which, when combined, explained 0.4% of the variation.

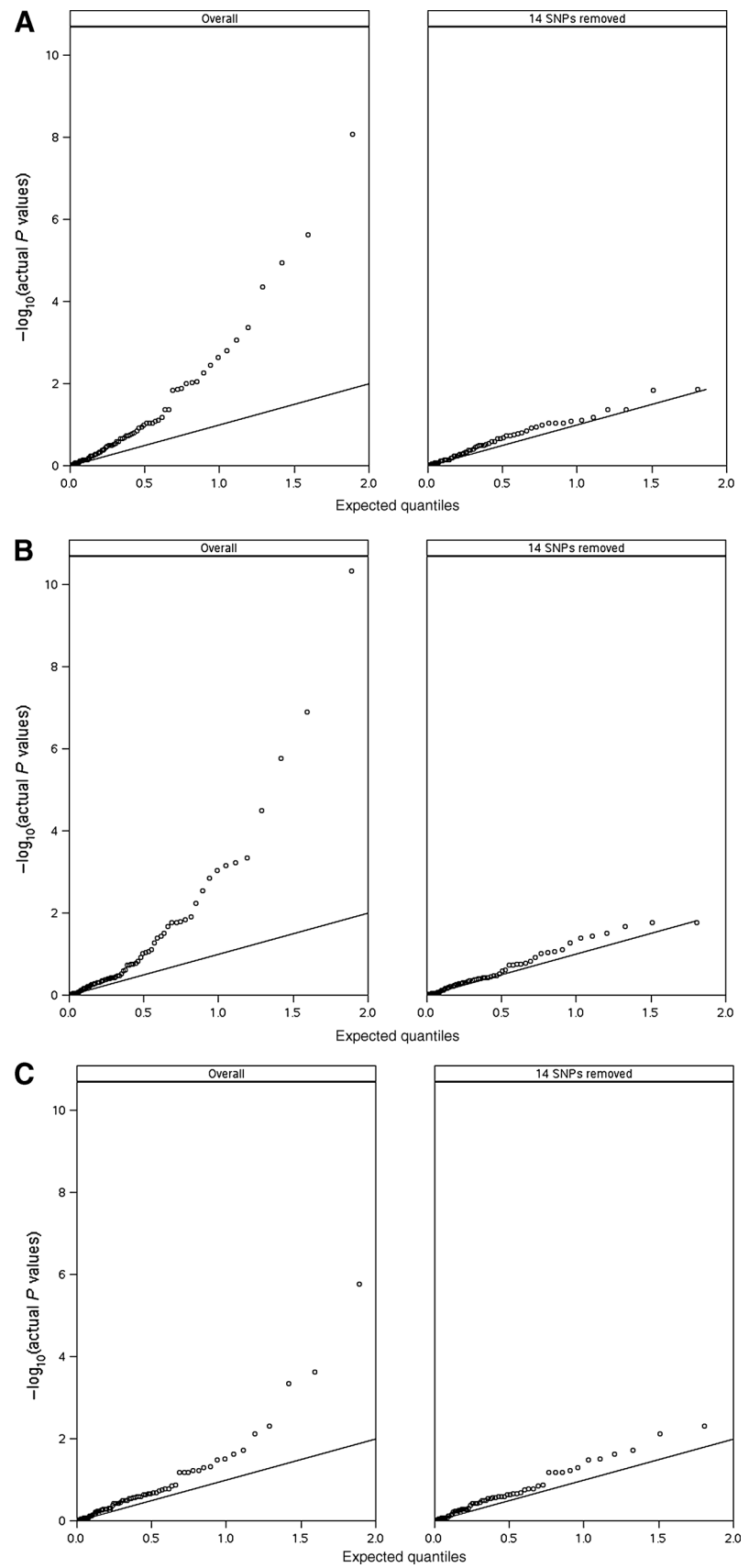
When we removed the variants resulting from the best fitting Lasso models for each phenotype noted above, the distribution of the remaining  $P$  values still deviated from the null hypothesis of no association between the genetic variants for both percent and absolute dense area ( $P = 0.00005$  for percent density,  $P = 0.00006$  for dense area) but not nondense area ( $P = 0.01$ ). Sequentially removing the variants most strongly associated with the mammographic density measure until the test no longer found deviation from the null using a  $P$  value threshold of 0.00065, we found evidence of one more SNP associated with each percent dense and absolute dense area (rs999737 and rs6678914, respectively).

We also compared the quantile-quantile plots before and after exclusion of the top 14 breast cancer variants (nine identified via ordinary linear regression and five others identified via LASSO regression) most strongly associated with the mammographic density measures (see Fig. 2). On the basis of the analysis described above and the lack of departure from the 45° line once the top 14 variants have been removed (indicative of a probable threshold of commonly shared variants), we estimate that there is an approximate 18% overlap between breast cancer- and mammographic density-associated variants.

## Discussion

This is the largest and most comprehensive study to date of the associations between breast cancer risk-predicting mammographic density measures and the 77 independent established breast cancer susceptibility common variants, 41 of which were recently identified by large-scale genotyping (13). In addition to previously reported associations between common breast cancer susceptibility SNPs in the regions of *ZNF365*, *ESR1*, and *LSP1* and age- and BMI-adjusted absolute and percent dense area, we found strong evidence of novel associations between SNPs with all three adjusted mammographic density measures (in the region of *EBF1*); with adjusted dense area or percent dense area (*MIR1972-2:FTO*, *2p24.1*, and another in the region of *ESR1* independent of the initially reported SNP, rs2046210); adjusted dense and nondense area (*MKL1*) and nondense area only (*NTN4*). The directions of these associations were consistent with that of their associations with breast cancer risk, with the exception of *MKL1* and *2p24.1* that were both negatively associated with dense area (and percent dense area) but are reportedly positively associated with breast cancer risk. Furthermore, these mammographic measures show evidence for mediating the association of several of these SNPs and breast cancer risk.

These findings are consistent with those recently reported by our coauthors' Swedish study (14). Despite differences in phenotypes (area vs. volume), both studies independently showed novel associations between absolute measures of dense tissue with rs2046210 (*ESR1*) and between absolute measures of



**Figure 2.** Quantile-quantile plots before and after exclusion of the top 14 breast cancer susceptibility SNPs most strongly associated with the mammographic density measures. A, percent dense area; B, dense area; C, nondense area.

nondense tissue with rs17356907 (*NTN4*). Both studies also reported strong negative associations between and absolute measures of dense and nondense tissue with rs6001930 (*MKL1*). The other novel association reported in Brand and colleagues (14) between percent dense volume and rs9485372 (*TAB2*), a variant associated with breast cancer risk in Asian women, was not investigated in this study. The Swedish study did not replicate the previously reported association with rs3817198 (*LSP1*) nor our novel associations with rs12710696 (2p24.1) and rs17817449 (*MIR1972-2: FTO*), underscoring the differences in the volumetric and area phenotypes. Of note, both volumetric and area-based density measures have been shown associated with breast cancer risk, with similar magnitude of association (23).

Although the standard approach using linear regression identified nine variants associated with mammographic density, the nonuniform distributions of the remaining *P* values suggest that there are additional genetic variants associated with both breast cancer risk and the mammographic density measures that predict risk. In total, there is evidence of at least 14 breast cancer susceptibility variants (18%) associated with at least one mammographic density measure; approximately 10%, 12%, and 4% of the breast cancer susceptibility SNPs were associated with percent dense area, dense area and nondense area, respectively. Our estimate of 18% is consistent with empirical estimates that the percentage of overlap between genetic determinants of breast cancer and the risk-predicting mammographic density measures is 14% (95% CI, 4–39%; refs. 5, 12).

The nine density-associated variants identified here (using the standard approach) account for only a small proportion of the between-woman variation in the three risk-predicting mammographic density phenotypes (<1.5% for each), but the contribution of the true causal variants could be larger. Also, it has been estimated that there are more than 1,000 loci involved with breast cancer susceptibility (13) and therefore it is possible that a considerable subset of these will also be associated with the mammographic density measures. Importantly, several of the SNP associations with breast cancer appear to be mediated by the mammographic density phenotypes. In fact, 15% to 20% of the associations of variants at *ESR1* and *EBF1* with breast cancer were mediated by percent dense area or dense area. Understanding which susceptibility loci exert their influence on breast cancer risk partially through mammographic density measures could be important for identifying subgroups of women who are at a high "genetic" risk for both breast cancer and mammographic density. There is increasing demand for the evidence-base to support stratified breast screening programs instead of the "one-size-fits-all" approaches that are currently recommended in most countries. Discriminating between genetic risk due to mammographic density and/or breast cancer risk could identify which women may have a greater benefit from density reduction strategies and/or additional breast screening measures.

The biologic reasons why mammographic density measures predict breast cancer risk are not understood. There is evidence to suggest that extensive mammographic density is causally related to breast cancer rather than a simple correlate of its determinants (24). Breast cancer arises from epithelial cells lining the ducts or lobules of the breast and mammographic density might represent areas of the breast in which there are higher rates of epithelial proliferation, which are likely to increase the risk of somatic mutations, epigenetic

alterations, and carcinogenesis, and/or slower rates of involution (25, 26).

This is consistent with our findings that most of the associations between breast cancer susceptibility variants and risk-predicting mammographic density phenotypes are driven by associations with absolute or percent dense area. However, the recent discovery that at least one breast cancer susceptibility variant (at *NTN4*) appears to be solely associated with adjusted nondense tissue provides evidence for the hypothesis that nondense fatty tissue may play an independent role in the pathogenesis of breast cancer (27); but we did not find mediation of the *NTN4*-breast cancer association by nondense area in our study. Furthermore, the fact that the association of adjusted dense area with risk is in the opposite direction to that for adjusted nondense area, and these two measures are negatively correlated, raises the possibility that the risk associations with adjusted nondense area and adjusted dense area are just the "opposite sides of the same coin" (6). In this regard, it is interesting that the familial correlations are very similar for nondense area, as they are for adjusted percent dense area (28). Studies that have examined whether adjusted absolute nondense area is independently associated with breast cancer risk have produced contradictory results (6, 29–32).

Variants in the regions of *MKL1*, *ESR1*, and *ZNF365* were among nine variants previously reported to be associated with bra cup size, although none of the variants overlap those examined in this study (33). The *ESR1* variant reported by Eriksson and colleagues (34) is in moderate LD with both the *ESR1* variants reported on here ( $r^2 = 0.23$  with rs2046210 and with rs375318, although the mutual LD between these is just 0.07), and Eriksson's *MKL1* variant (rs73167017) is also in moderate LD with *MKL1* rs6001930 reported here ( $r^2 = 0.33$ ), but the *ZNF365* variant, rs7089814, is independent of rs10995190 ( $r^2 = 0.04$ ). Within *MKL1*, rs6001930 is correlated with rs5995871 ( $r^2 = 0.75$ ), which has been recently reported to be associated with mammographically measured female breast size, which correlates strongly with nondense area. Little is known about the functionality of any of the density-associated variants identified in this study.

Our study benefited from its large sample size and genotyping performed using the same custom Illumina iSelect genotyping array in 11 of the 13 studies. We also used the same strict quality controls for all studies except the two GWAS, and exclusion of data from these two GWAS-based studies did not substantially affect the findings reported here (data not shown). Mammographic density measurements were performed using well-established methods shown to have high repeatability by trained observers, with all analyses being adjusted for study to reduce the impact of any between-study differences in the type of films available, digitizer used, quality of the density readings, and source of covariate data and other unmeasured confounders. We reproduced established associations between the three risk-predicting density phenotypes and measured breast cancer risk factors as well as prior genetic associations with breast cancer variants. Because over 80% of our sample population were postmenopausal, these results are generally applicable to postmenopausal women. However, based on previous work using longitudinal twin data, we have shown that the familial/genetic component of mammographic density is established before mid-life (35) and therefore, we believe that the direction of the associations reported in this study would be the same for premenopausal women.



More than 40 studies have found an association between mammographic density and breast cancer risk, many using different qualitative or quantitative methods of measuring mammographic density (19, 36). This suggests that mammographic density, as currently measured, is a useful biomarker. Our previous collaborations (9, 37) have demonstrated that data from multiple mammographic density studies can be combined to produce internally consistent results. One reason for this is the very wide variation in mammographic density measures within populations, even for women of the same age and BMI.

In summary, our findings provide further support for shared genetic determinants of breast cancer risk and the mammographic density measures that predict risk, presumably representing shared etiologic pathways. Although the contributions of the genetic risk markers identified to date explain little of the phenotypic variance, uncovering the cause of familial aggregation (the so-called "missing heritability") of the mammographic density measures that predict breast cancer could substantially increase understanding of the biologic pathways involved in the development of the disease.

### Disclosure of Potential Conflicts of Interest

P.A. Fasching has received speakers bureau honoraria from Novartis, Pfizer, Roche, Amgen, and Genomic Health. No potential conflicts of interest were disclosed by the other authors.

### Disclaimer

The content of this article does not necessarily reflect the views or policies of the National Cancer Institute or any of the collaborating centers in the Breast Cancer Family Registry (BCFR), nor does mention of trade names, commercial products, or organizations imply endorsement by the USA Government or the BCFR.

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