Research Article

Cancer Epidemiology, Biomarkers & Prevention

Common Breast Cancer Susceptibility Variants in *LSP1* and *RAD51L1* Are Associated with Mammographic Density Measures that Predict Breast Cancer Risk

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Abstract

Background: Mammographic density adjusted for age and body mass index (BMI) is a heritable marker of breast cancer susceptibility. Little is known about the biologic mechanisms underlying the association between mammographic density and breast cancer risk. We examined whether common low-penetrance breast cancer susceptibility variants contribute to interindividual differences in mammographic density measures.

Methods: We established an international consortium (DENSNP) of 19 studies from 10 countries, comprising 16,895 Caucasian women, to conduct a pooled cross-sectional analysis of common breast cancer susceptibility variants in 14 independent loci and mammographic density measures. Dense and nondense areas, and percent density, were measured using interactive-thresholding techniques. Mixed linear models were used to assess the association between genetic variants and the square roots of mammographic density measures adjusted for study, age, case status, BMI, and menopausal status.

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Results: Consistent with their breast cancer associations, the C-allele of rs3817198 in *LSP1* was positively associated with both adjusted dense area ($P = 0.00005$) and adjusted percent density ($P = 0.001$), whereas the A-allele of rs10483813 in *RAD51L1* was inversely associated with adjusted percent density ($P = 0.003$), but not with adjusted dense area ($P = 0.07$).

Conclusion: We identified two common breast cancer susceptibility variants associated with mammographic measures of radiodense tissue in the breast gland.

Impact: We examined the association of 14 established breast cancer susceptibility loci with mammographic density phenotypes within a large genetic consortium and identified two breast cancer susceptibility variants, *LSP1*-rs3817198 and *RAD51L1*-rs10483813, associated with mammographic measures and in the same direction as the breast cancer association. *Cancer Epidemiol Biomarkers Prev; 21(7); 1156–66. ©2012 AACR.*

Introduction

Genetic factors play a major role in the pathogenesis of breast cancer (1–3). Recent multistage genome-wide association studies (GWAS) and candidate gene studies conducted by several groups, including the Breast Cancer Association Consortium (BCAC), have successfully identified and replicated associations between over 18 single-nucleotide polymorphisms (SNP) and risk of breast cancer in Caucasians (4–9).

Mammographic density, which reflects variations in the amounts of fat, stromal, and epithelial tissues in the breast, is one of the strongest risk factors for breast cancer with risk being 4- to 6-fold higher for women in the highest relative to lowest density categories after adjusting for age and body mass index (BMI; refs. 10, 11). The biology underlying the mammographic density and breast cancer association is essentially unknown, but twin and family studies suggest that additive genetic factors explain about 60% of variance in the density measures (12, 13). This raises the question of whether breast cancer susceptibility variants identified to date are associated with mammographic density measures. This could lead to new insights into the etiology of breast cancer by revealing the biologic reasons for these associations with breast cancer risk (14).

Five studies have examined the association of breast cancer susceptibility SNPs with age- and BMI-adjusted measures of mammographic density (14–18). The most consistent finding was an association between (lymphocyte-specific protein-1, *LSP-1*)-rs3817198 and adjusted dense area and percent density, in the same direction as the association with breast cancer. The association was observed overall by Odefrey and colleagues (17) but only in specific subgroups by others: in premenopausal women (14), current users of postmenopausal hormones (PMH; ref. 15) or estrogen receptor (ER)+/progesterone receptor (PR)+ cases only (16). Other nominally significant reported SNP–density associations consistent with the association of these SNPs with breast cancer risk include associations of *TOX3*-rs12443621 (14, 15) and rs4666451 (14) with adjusted percent density, in premenopausal women only, and rs13281615 at 8q24 with both adjusted percent density and dense area (17). The largest study to date, a meta-analysis of 5 GWAS of mammographic density involving 4,877 women with and without breast cancer, identified a genome-wide significant association between *ZNF365*-rs10995190, a known breast cancer susceptibility SNP, and adjusted percent density as well as weak evidence of possible associations with *ESR1*-rs2046210 ($P = 0.005$) and *LSP1*-rs3817198 ($P = 0.04$; ref. 18).

Only one previous study (17), however, examined the SNP associations with the components that comprise the percent density phenotype, namely, dense area and nondense area. Dense area has been hypothesized to be the more relevant density phenotype for understanding the etiology of mammographic density (19), as tumors have been shown to arise within the radiodense tissue (20). Whether these SNPs influence dense and/or nondense area could help to interpret the mechanism by which the loci influence density and possibly cancer.

Density Twins and Sisters Study (AMDTSS); 43Bavarian Breast Cancer Cases and Controls (BBCC); [44]Determinants of Mammographic Density in Spain (DDM-Spain); [45]EPIC-NL (Dutch part of European Prospective Investigation into Cancer Nutrition; EPIC-NL); [46]European Prospective Investigation into Cancer–Norfolk I and II (EPIC-Norfolk I and II); [47]Women's Learning the Influence of Family and Environment Study (LIFE); [48]Magnetic Resonance Imaging in Breast Screening (MARIBS); [49]Mayo Clinic Breast Cancer Study (MCBCS); [50]Melbourne Collaborative Cohort Study (MCCS); [51]Multiethnic Cohort Study (MEC); [52]Mammography, Oestrogens and Growth Factors Study (MOG); [53]Norwegian Breast Cancer Study (NBCS); [54]Nurses' Health Study (NHS); [55]Ontario Familial Breast Cancer Registry (OFBCR); [56]Polish Breast Cancer Study (PBCS); [57]Polish Nurses and Midwives Study (PNS); [58]Singapore and Sweden Breast Cancer Study (SASBAC); and [59]Sisters in Breast Cancer Screening (SIBS)

Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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We established an international collaboration–the DENSNP consortium-–of studies with data on established breast cancer susceptibility variants and quantitative density measures from film mammography to conduct analyses of breast cancer susceptibility SNPs in relation to the 3 density phenotypes. This article reports the findings for 15 breast cancer SNPs at 14 loci, identified through 2009 when the DENSNP consortium was established.

Materials and Methods

Study samples

The DENSNP consortium comprises 19 studies from Europe, North America, and Australia with the present analyses restricted to Caucasian women. Individual studies, their design, and sample sizes are described in Supplementary Table S1. Covariate data, including age, reproductive variables, and exogenous hormone use, were obtained through self-administered postal questionnaires (12 studies), in-person interviews (6 studies), or telephone interviews (one study; Supplementary Table S2). Participants' weights, heights, and hence BMIs were measured by trained staff (10 studies) and self-reported (9 studies). For 8 studies, there was an average of 6 months or less between mammography and collection of participant information; for 18 studies, the average was 3 years or less.

Each study obtained informed consent and relevant ethics and institutional approvals. Only anonymized data were made available to the DENSNP consortium.

Digitization and density measures

All studies obtained film mammograms–either the mediolateral oblique (MLO; 7 studies) or craniocaudal (CC; 12 studies) views–for participants, including breast cancer cases and/or noncases, except PNS which digitized copies of digital mammograms (Supplementary Table S3). For cases, the film from the unaffected contralateral breast taken at the time of cancer diagnosis was used, except for 3 nested case–control studies for which images obtained before diagnosis were used (2 studies used average measurements of both breasts; 1 study used only the right breast). For noncases, both breasts (averaged), left or right only, or the side that corresponded to the matched case was chosen.

As a requirement for entry, participating studies contributed percent density, dense area, and nondense area measures for cases and/or noncases using 1 of 2 similar semiautomated methods that rely on the interactive threshold technique, Cumulus (21) and Madena (22) softwares. Both require an interactive selection of 2 grayscale thresholds in the image of a digitized mammogram by a trained observer. One threshold separates the breast from the background and the other classifies the breast tissue into dense and nondense areas, from which percent density (100 $\times$ dense area/total breast area) and absolute measures of dense and nondense areas are automatically generated. Images were anonymized and readers were blind to the genotype, case status (if applicable), and risk factor data.

Genotyping and quality control

SNPs confirmed to be associated with breast cancer susceptibility in the 14 regions (loci) of the genes *FGFR2*, *LSP1*, *MAP3K1*, *TOX3*, *SLC4A7/NEK10*, *COX11*, *CASP8*, *TGFB1*, *RAD51L1*, *ESR1*, and *MRPS30/FGF10*, and positions 8q24.21, 2q35 and 1p11.2 were measured (Fig. 1). These loci were identified by GWAS (4–7) except *CASP8* and *TGFB1* which were identified using the candidate gene approach (8). For the *CASP8* locus, there were alternate SNPs (rs1045485 and rs17468277) available in strong linkage disequilibrium (LD; $r^2 = 0.98$). The rs1045485 SNP was used if available; if not, rs17468277 was used. For the 2,275 women with genotypes for both SNPs, these were concordant for all but 9 samples, so were used interchangeably. Two SNPs were also available for each of the *RAD51L1* (rs10483813 and rs999737) and *MRPS30/FGF10* (rs4415048 and rs10941679) loci. The SNPs in *MRPS30/FGF10* were not in strong disequilibrium ($r^2 <$ 0.6 in our data set) and are reported separately. Rs10483813 and rs999737 (*RAD51L1)* were in high LD ($r^2 = 0.98$ in our data set), but studies had either genotyped both SNPs, or only rs10483813; thus, we only report results for rs10483813 for which we had a larger sample size.

Genotyping was conducted on various platforms by the individual studies (Supplementary Table S4). Quality control was conducted at the study level; all SNP call rates were >90%, with few (10 SNPs from 5 studies) <95%. Three SNPs (from 3 studies) with Hardy–Weinberg equilibrium P values <0.001 were excluded. The number of SNPs genotyped by each study varied from all 14 (4 studies) to only 2 (2 studies), with a median of 10 per study.

Statistical methods

Study-specific data were checked to ensure that the coding and scaling of each variable were similar across studies. For the AMTDSS, one twin was selected at random from the 563 monozygous pairs. Examination of the distributions of residuals of density phenotypes adjusted for age, BMI, and menopausal status showed that a square root transformation of all density variables gave a good approximation to a normal distribution and this was used in all analyses.

A test of the null hypothesis of no association between any of the tested SNPs and a given mammographic measure was conducted using Fisher's method (23). As individual-level data were available from all studies, primary analyses used a mixed model approach that included per-study random-effects to capture study-specific differences. When applicable, a repeated measures adjustment within families assuming a compound symmetry correlation structure was used to account for familial correlation. Models were adjusted for the fixed-effects of age (continuous), BMI (1/BMI, was used as it provided a better fit), case status, and menopausal status (pre- and

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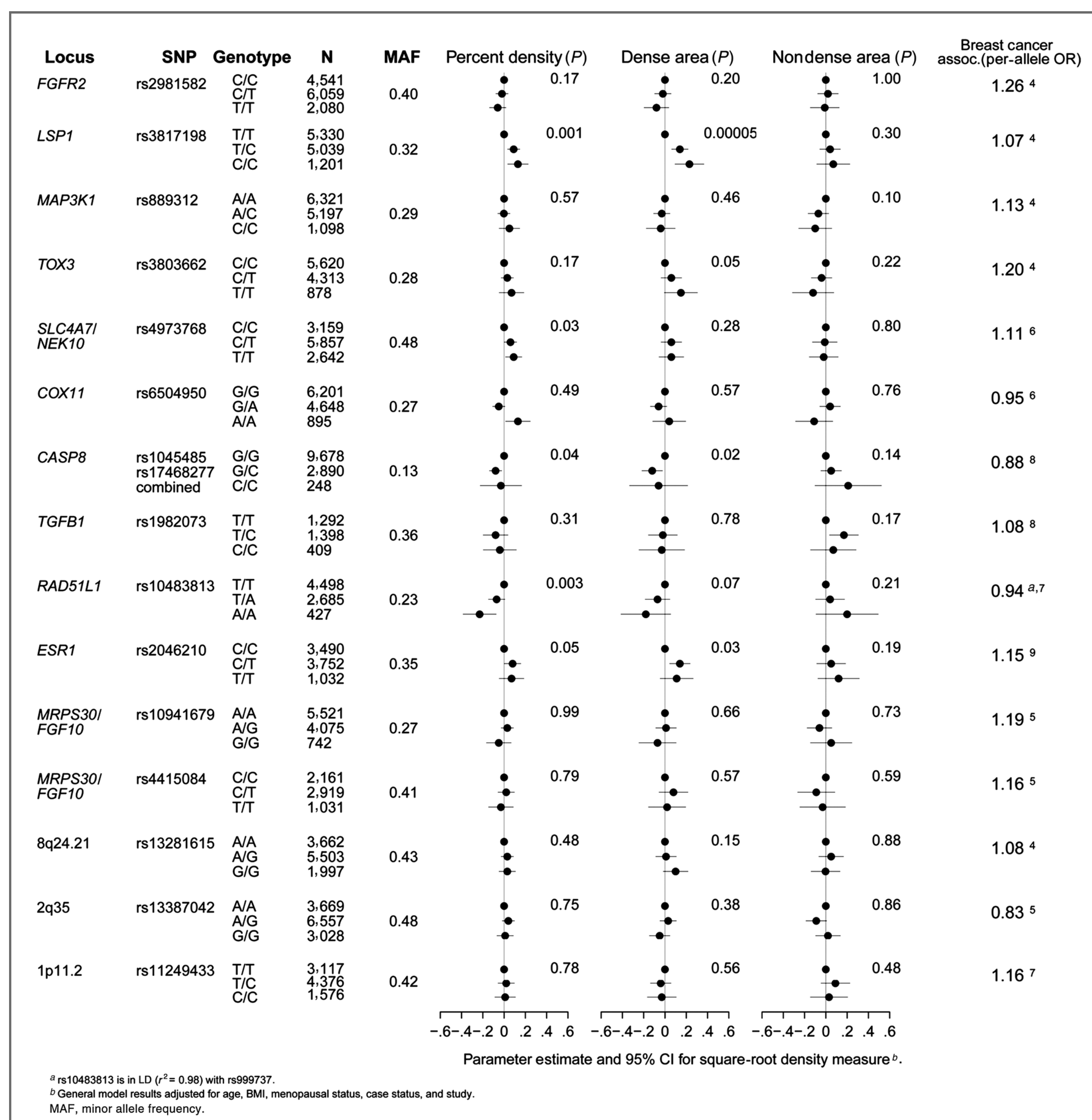

Figure 1. Associations of common breast cancer susceptibility variants with adjusted percent mammographic density, dense area, and nondense area.

perimenopausal combined vs. postmenopausal, with the latter defined as no menstruation for ≥12 months, not due to pregnancy). A missing category was included, when applicable. Primary analyses considered SNP associations as additive genetic effects, by defining an ordinal covariate as the number of copies of the minor allele carried by the study subjects and fitted a linear association. The resulting estimate of the per-allele effect is reported as the "additive estimate" in the tables. Estimates of the adjusted mean mammographic density measures and their 95% confidence intervals (CI), corresponding to the observed genotypes of each variant, were derived by back-transformation from the square root to the original scale. Additional analyses were conducted within subsets of women defined by menopause categories (pre- and perimenopausal combined vs. postmenopausal), BMI (< vs. ≥ median of 25 kg/m^2), PMH (ever vs. never use), and case status to assess whether SNP–density phenotype associations were modified by these variables.

Between-study heterogeneity was tested by fitting study-by-genotype interactions.

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Analyses were conducted using SAS version 9.2 (SAS Institute, Inc.). Two-sided P values were calculated. A Bonferroni adjustment to account for multiple testing was applied to define the threshold for statistical significance as $P \leq 0.003$ (= 0.05 divided by14 loci).

Results

There were 5,110 breast cancer cases and 11,785 noncases of self-reported Caucasian race/ethnicity with available density phenotypes, risk factors, and at least 1 of the 15 SNPs considered (Table 1). The number of participants varied by SNP with the most comprehensive information for 2q35 ($n = 13{,}254$), *CASP8* ($n = 12{,}816$), and *FGFR2* ($n = 12{,}680$), and least information for *TGFB1* ($n = 3{,}099$), *RAD51L1* ($n = 7{,}610$) and *ESR1* ($n = 8{,}274$).

The majority of the participants were aged ≥40 years (98%) and postmenopausal (77%), and approximately half of those aged ≥55 reported ever using PMH (48%; Table 1). In all, 44% of participants had a BMI < 25 kg/m^2 (Table 1). A small proportion was nulliparous (11%), precluding subgroup analyses by parity. The associations between these variables and the 3 density phenotypes are shown in Table 2 and were similar to those reported in the literature.

The results from our primary analyses of the 15 SNPs in 14 breast cancer loci with the 3 density phenotypes are shown in Fig. 1 and described in Supplementary Tables S5a–S5c. Pictured are the parameter estimates from the mixed linear models corresponding to each genotype. There was strong evidence against the null hypothesis that none of the SNPs were associated with the dense area ($P < 0.001$) and percent density measures ($P = 0.001$), but not with the nondense area measure ($P = 0.5$). This suggests that at least 1 of the 14 breast loci is associated with the density or dense area measures.

The strongest associations were seen with rs3817198 (*LSP1*) and the dense area ($P = 0.00005$) and percent density ($P = 0.001$) phenotypes with little evidence for between-study heterogeneity (Fig. 2). The adjusted mean dense area was 23.7 cm^2 for T/T carriers, 25.1cm^2 for T/C carriers, and 26.0 cm^2 for C/C carriers (Supplementary Tables S5a and S5b). The adjusted mean percent density for T/T carriers was 19.4% compared with 20.1% for T/C and 20.5% for C/C carriers, respectively. These associations were consistent across studies (Fig. 2) and persisted after exclusion of studies that had previously reported on *LSP1* and density, namely NHS, AMDTSS, LIFE, MEC, EPIC-Norfolk I, and SASBAC (refs. 14–18; e.g., $P = 0.004$ for dense area). There was also evidence of an inverse association between rs10483813 (*RAD51L1*) and adjusted percent density ($P = 0.003$), but not with adjusted dense area ($P = 0.07$; Fig. 1). These associations were consistent across studies (Fig. 2) with the adjusted mean percent density for T/T genotype being 21.1%, compared with 20.5% for T/A and 19.0% for A/A.

There were nominal associations of adjusted percent density and dense area with rs2046210 (*ESR1*), rs1045485/rs17468277 (*CASP8*), rs4973768 (*SLC4A7/NEK10*), and rs3803662 (*TOX3*; Supplementary Tables S5a and S5b) which were in the direction of the published corresponding breast cancer associations but not statistically significant after taking into account multiple testing (Fig. 1). None of the investigated SNPs were associated with nondense area (Fig. 1; Supplementary Table S5c).

The genetic associations above did not diminish after further adjustment for parity or view (data not shown) and, in general, did not appear to differ by case status, BMI, menopausal status, or PMH use (Supplementary Tables S6a–S6c) but the study had low power to examine interactions.

We also examined the association of these SNPs with breast cancer risk before and after adjustment for the density measures by pooling data from studies that recruited both cases and noncases (identified in Supplementary Table S1). Using 3,175 cases and 6,504 noncases from 8 studies, the per C-allele OR for rs3817198 (*LSP1*) was 1.04 (95% CI, 0.97–1.12) without adjustment for either density measure. When including dense area as a covariate, the OR was 1.03 (95% CI, 0.96–1.10), and after adjustment for percent density instead, the OR was 1.02 (95% CI, 0.95–1.11). Similarly, using 2,765 cases and 3,022 noncases from 4 studies, the per A-allele OR for rs10483813 (*RAD51L1*) was 0.92 (95% CI, 0.84–1.00) without adjustment for either density measure, 0.93 (95% CI, 0.85–1.01) after adjustment for dense area, and 0.94 (95% CI, 0.86–1.03) after adjustment for percent density.

Discussion

There is wide interindividual variability in mammographic density measures, but known epidemiologic risk factors account for only 20% to 30% variability in percent density (13, 24, 25). We hypothesized that common low-penetrance breast cancer susceptibility variants contribute to the remaining interindividual differences in the density phenotypes and examined this within a large international consortium (DENSNP). Here, we report the first findings from this collaborative effort and identify associations between adjusted measures of density and 2 breast cancer susceptibility SNPs, rs3817198 (*LSP1*) and rs10483813 (*RAD51L1*), which were in the same direction as the corresponding SNP associations with cancer risk.

The most marked association with density was with rs3817198 (*LSP1*). We also confirmed this association using the 10 studies that had not previously published on the *LSP1* variant and density association, providing consistent evidence for this mammographic density locus. The mechanisms through which this SNP (or more likely the causal allele(s) it tags) may affect density and cancer risk are unclear. The *LSP1* gene encodes an intracellular F-actin–binding protein, which is expressed in lymphocytes, neutrophils, and endothelium and might regulate neutrophil motility, adhesion to fibrinogen matrix proteins, and transendothelial migration (26).

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Table 1. Summary characteristics of the 19 DENSNP studies

Characteristic	Category	No. of studies	BC cases N (%)	Noncases N (%)	Overall N (%)
Overall		19	5,110 (30)	11,785 (70)	16,895 (100)
Study design	Cohort	3	16 (0.3)	1,582 (13)	1,598 (9)
	Cross-sectional	5	38 (1)	3,064 (26)	3,102 (18)
	Case–control	5	3,280 (64)	2,217 (19)	5,497 (33)
	Nested case–control	3	1,599 (31)	2,099 (18)	3,698 (22)
	Family-based	3	177 (3)	2,823 (24)	3,000 (18)
Source of demographic and reproductive data	In-person interview	6	1,631 (32)	1,276 (11)	2,907 (17)
	Postal questionnaire	12	3,378 (66)	8,831 (75)	12,209 (72)
	Telephone interview	1	101 (2)	1,678 (14)	1,779 (11)
Age,[a] y	<40	9	221 (4)	145 (1)	366 (2)
	40–49	17	937 (18)	1,857 (16)	2,794 (17)
	50–59	18	1,643 (32)	4,843 (41)	6,486 (38)
	60–69	16	1,659 (32)	4,011 (34)	5,670 (34)
	≥70	13	650 (13)	929 (8)	1,579 (9)
Parity[a]	Nulliparous	19	614 (12)	1,167 (10)	1,781 (11)
	Parous	19	4,329 (85)	10,479 (89)	14,808 (88)
	Unknown	8	167 (3)	139 (1)	306 (2)
Menopausal status[a]	Premenopausal	16	1,185 (23)	2,241 (19)	3,426 (20)
	Perimenopausal	5	13 (0.2)	251 (2)	264 (2)
	Postmenopausal	18	3,769 (74)	9,195 (78)	12,694 (77)
	Unknown	6	143 (3)	98 (1)	241 (1)
PMH use (at age ≥55)	Ever	16	1,703 (53)	3,364 (46)	5,067 (48)
	Never	16	1,326 (41)	3,474 (47)	4,800 (45)
	Unknown	8	178 (6)	537 (7)	715 (7)
Source of anthropometric data	Self-reported	9	3,784 (74)	5,909 (50)	9,693 (57)
	Measurements by trained staff	10	1,326 (26)	5,876 (50)	7,202 (43)
BMI,[a] kg/m^2	<25	19	2,284 (45)	5,071 (43)	7,355 (44)
	≥ 25	19	2,737 (54)	6,597 (56)	9,334 (55)
	Unknown	10	89 (2)	117 (1)	206 (1)
Average time interval between mammography and data collection (months),[b]	≤6	8	2,129 (42)	4,330 (37)	6,459 (38)
	>6	11	2,981 (58)	7,455 (63)	10,436 (62)
Mammographic side, view	L–CC	8	831 (16)	2,547 (22)	3,378 (20)
	R–CC	6	949 (19)	1,830 (16)	2,779 (16)
	LR average–CC	3	2,402 (47)	2,285 (19)	4,687 (28)
	L–MLO	3	465 (9)	1,978 (17)	2,443 (14)
	R–MLO	1	447 (9)	418 (4)	865 (5)
	LR average–MLO	4	16 (0.3)	2,727 (23)	2,743 (16)
Density reading software	Cumulus	15	3,814 (75)	10,213 (87)	14,027 (83)
	Madena	4	1,296 (25)	1,572 (13)	2,868 (17)

Abbreviations: BC, breast cancer; CC, craniocaudal; L, left; MLO, mediolateral oblique; R, right.
[a]At time of mammography and/or data collection.
[b]Average time interval for each study given in Supplementary Table S2 (range, 0–5 years).

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The SNP rs10483813 in *RAD51L1*, a gene on chromosome 14q24.1 involved in the double-strand DNA repair and homologous recombination pathway, may also be associated with the adjusted density measures, although the evidence is less compelling than for rs3817198 (*LSP1*). The biologic mechanisms underlying the possible association of this variant with density and cancer risk are unknown. *RAD51L1* interacts with *RAD51*, and a SNP in the 5′ untranslated region of *RAD51* has been found to be associated with breast cancer risk for *BRCA2* mutation

Table 2. Mammographic density measurements by known breast cancer risk factors, mammographic view, and case status at time of mammography

Risk factor Categories	N (%)	PD (%) Mean (95% CI)	Dense area, cm^2 Mean (95% CI)	Nondense area, cm^2 Mean (95% CI)
Age,[a] y				
<40	366 (2.2)	34.2 (30.3–38.3)	36.8 (31.9–42.1)	75.1 (66.8–83.8)
40–49	2,794 (16.5)	28.2 (25.3–31.4)	33.0 (29.1–37.1)	89.7 (82.9–96.8)
50–59	6,486 (38.4)	20.3 (17.9–22.9)	26.4 (23.0–30.0)	112.2 (104.8–119.8)
60–69	5,670 (33.6)	14.9 (12.8–17.2)	21.3 (18.2–24.6)	130.2 (122.2–138.4)
≥70	1,579 (9.3)	13.0 (11.0–15.2)	17.3 (14.5–20.4)	143.0 (134.1–152.3)
P		<0.001	<0.001	<0.001
BMI,[b] kg/m^2				
<25	7,355 (44.1)	25.8 (23.2–28.6)	27.0 (23.6–30.7)	82.9 (77.1–89.0)
≥25	9,334 (55.9)	14.8 (12.8–16.9)	23.3 (20.1–26.7)	144.3 (136.6–152.3)
P		<0.001	<0.001	<0.001
Menopausal status[c]				
Pre- or perimenopausal	3,690 (22.2)	21.5 (19.1–24.1)	27.1 (23.6–30.8)	113.5 (106.4–120.9)
Postmenopausal	12,964 (77.8)	18.4 (16.2–20.7)	24.1 (20.9–27.5)	116.3 (109.3–123.5)
P		<0.001	<0.001	0.05
PMH use (at ages ≥ 55)[c]				
Never	4,800 (48.6)	14.6 (12.5–16.9)	20.2 (16.7–23.9)	129.1 (120.4–138.2)
Ever	5,067 (51.4)	17.8 (15.5–20.4)	23.6 (19.9–27.7)	122.7 (114.2–131.6)
P		<0.001	<0.001	<0.001
Parity[c]				
Nulliparous	1,781 (10.7)	22.6 (20.1–25.2)	29.0 (25.4–32.9)	109.2 (102.2–116.4)
Parous	14,808 (89.3)	18.7 (16.5–21.0)	24.3 (21.1–27.7)	116.7 (109.8–123.8)
P		<0.001	<0.001	<0.001
Mammographic view[c]				
CC	6,051 (35.8)	17.7 (14.2–21.5)	25.1 (19.7–31.1)	122.4 (111.1–134.2)
MLO	10,844 (64.2)	20.1 (17.3–23.2)	24.8 (20.6–29.4)	111.5 (103.2–120.2)
P		0.3	0.9	0.1
Case status[d]				
BC case	4,530 (37.8)	24.5 (20.8–28.4)	30.0 (24.1–36.4)	108.2 (95.6–121.5)
Noncase	7,439 (62.2)	19.3 (16.0–22.8)	24.2 (19.0–30.1)	117.9 (104.9–131.7)
P		<0.001	<0.001	<0.001

Abbreviations: BC, breast cancer; CC, craniocaudal; MLO, mediolateral oblique.
[a]Adjusted for study.
[b]Adjusted for study and age.
[c]Adjusted for study, age, and BMI.
[d]Restricted to 9 studies that recruited both cases and noncases and adjusted for study, age, and BMI.

carriers (27). However, mutations in *BRCA1* and *BRCA2* have not been found to be associated with the density phenotypes (28, 29).

Several breast cancer GWAS have consistently identified polymorphisms in intron 2 of fibroblast growth factor receptor 2 (*FGFR2*), with each copy of the T-allele of rs2981582 being associated with about a 26% increased breast cancer risk (30). Our study had 90% power to detect an average difference in percent density of less than 1% between homozygote carriers and noncarriers of this SNP, if such a difference truly exists, and therefore the lack of finding an association suggests that density is unlikely to mediate the association between *FGFR2* and breast cancer risk. Similar considerations apply to SNPs in several other breast cancer loci, including *TOX3*-rs3803662, 2q35-rs13387042 and *MAP3K1*-rs889312. These loci are likely to contribute independently of density to risk prediction. In fact, when we added *LSP1*-rs3817198 and *RAD51L1*-rs10483813 to a risk model with age, BMI, menopause, study, and percent density, the inclusion of these 2 SNPS did not affect the area under the curve whereas the addition of the remaining 12 SNPs increased the area under the curve from 0.62 to 0.65 ($P < 0.001$).

Previous studies were based on smaller sample sizes [ranging from 578 (ref. 16) to 4,877 (ref. 18)], which could have precluded the detection of small effects. Our study is

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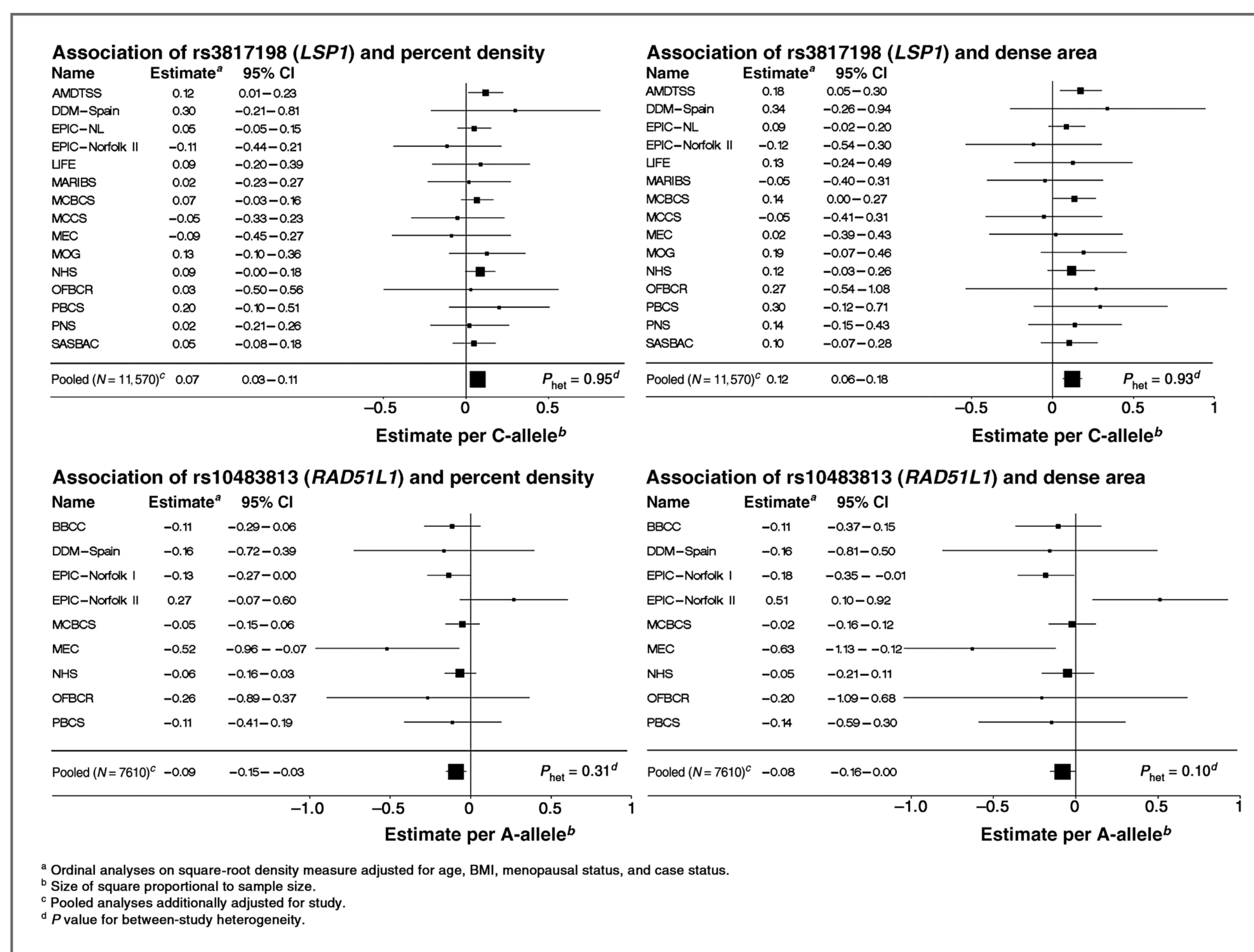

Figure 2. Study-specific associations of *LSP1*-rs3817198 and *RAD51L1*-rs10483813 with adjusted percent mammographic density and dense area.

the largest conducted so far with sample sizes greater than 6,000 for all but one SNP and greater than 10,000 for all but 5 SNPs. We had more than 90% power to detect per-allele differences in adjusted percent density of 1% or less for all but 3 SNPs (rs17468277, rs10483813, and rs4415084), and even for these SNPs, we were similarly powered to detect per-allele differences of less than 2%. However, limited power precluded a more detailed examination of interactions with BMI (e.g., differential SNP effects in BMI-defined quartiles) and PMH use (e.g., different SNP effects by type of PMH, recency of use). The study also had low power to assess the mediation of the SNP and breast cancer associations by density.

The mammographic density readings were conducted in different sets of films (e.g., left, right, or both breasts; CC or MLO views), but it is unlikely that this may have substantially affected our findings because there is a high correlation between a woman's density measurements taken from the various breast view combinations (31). For cases, both prediagnostic films and films from the unaffected breast at the time of diagnosis, but before treatment, were used—an approach used by others (10); furthermore, our findings were not modified by case status. One small study (PNS) used digitized copies of digital mammograms, but its exclusion did not affect the results shown here. Although mammographic density readings were not standardized, all studies used a similar interactive threshold approach and had very high within- and between-observer repeatability (typically >90%; ref. 32). Also, all analyses were adjusted for study hence minimizing the impact of any between-study differences on density measurements which would have likely reduced our power to detect real associations. Reassuringly, we were able to reproduce the well-established influences of age, BMI, parity, menopausal status, and PMH on density phenotypes within each one of the participating studies as well as in joint analyses.

Our findings suggest that 2 of 14 well-established breast cancer loci may contribute to the large between-woman differences in risk-predicting density phenotypes, consistent with estimates of 5% to 10% genetic overlap between this biomarker and breast cancer (33). The 2 common variants in *LSP1* and *RAD51L1* explained 0.2% (combined, 0.1% for each) of the variance in adjusted percent density and dense area, although the overall contribution could be larger if the true causal variants are more strongly

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associated with density than the tagging SNPs we examined here. At the individual level, these SNPs were associated with a 0.6% absolute increase in percent density per allele for *LSP1* and 0.8% absolute decrease in percent density per allele for *RAD51L1*. These magnitudes can be compared with, for example, the change in density measures of 1% decrease per year of ageing (34), 2% increase with use of PMH, and 2% decrease over the menopausal transition (35). Our findings are consistent with the hypothesis that mammographic density is likely a polygenic trait, influenced by many common low-penetrance variants, and/or rarer variants with larger effects which cannot be identified through current GWAS. Identification of such variants, and clarification of their role and function, is likely to improve our understanding of the biology of mammographic density and how this phenotype is associated with breast cancer risk.

Disclosure of Potential Conflicts of Interest

M. Pollan is the principal investigator of one of the studies included in this analysis (DDM-Spain). M.O. Leach has employment (other than primary affiliation; e.g., consulting) from Specialty Scanners PLC as Director and Ownership Interest (including patents) and is named on patents that relate to breast analysis and density measurements. If these are commercialized by the Institute of Cancer Research, then he may receive compensation under the rewards for inventors scheme. D.F. Easton is a Principal Research Fellow of Cancer Research UK. J.L. Hopper is an Australian Fellow of the NHMRC and a Victorian Breast Cancer Research Consortium (VBCRC) Group Leader. No potential conflicts of interest were disclosed by other authors.

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