Published OnlineFirst July 7, 2014; DOI: 10.1158/1055-9965.EPI-13-1212

Cancer Epidemiology, Biomarkers & Prevention

Research Article

Breast Cancer Susceptibility Variants and Mammographic Density Phenotypes in Norwegian Postmenopausal Women

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Abstract

Background: Mammographic density (MD) is one of the strongest known breast cancer risk factors. Twin studies have suggested that a large part of the variation in MD is genetically determined. We hypothesized that breast cancer susceptibility variants may affect MD, and that their effects may be modified by nongenetic factors.

Methods: We assessed MD, using a computer-assisted method, on 2,348 postmenopausal Caucasian women (50–69 years) who participated in the Norwegian Breast Cancer Screening Program (NBCSP) in 2004 or 2006–07. We used linear regression (additive models) to determine the association between each SNP and MD, adjusting for age, body mass index (BMI), and study. We evaluated MD associations with 17 established breast cancer SNPs, overall, and by strata defined by non-genetic factors.

Results: Two variants, 6q25.1-rs9383938 and *TXNRD2*-rs8141691, were statistically significantly associated with percent MD ($P = 0.019$ and 0.03, respectively), with the 6q25.1-rs9383938 association being consistent with the SNP effect on breast cancer risk. The effect of 6q25.1-rs3734805 on percent MD varied between parous and nulliparous women ($P_{interaction} = 0.02$), whereas the effects of 9q31.2-rs865686 and *MRPS30:FGF10*-rs4415084 differed across strata of BMI ($P_{interaction} = 0.01$ and 0.005, respectively). There was no evidence of effect modification by estrogen and progestin therapy use or alcohol consumption.

Conclusion: This study provides novel evidence of shared genetic risk factors between MD and breast cancer and of possible MD genetic–environmental interactions.

Impact: Although the results may be chance findings, they nevertheless highlight the need to investigate interactions with nongenetic factors in studies on the genetics of MD. *Cancer Epidemiol Biomarkers Prev; 23(9); 1752–63. ©2014 AACR.*

Introduction

Mammographic density (MD) is one of the strongest known risk factor for breast cancer (1, 2). Women with a high percentage of MD (≥75%) are at 4- to 5-fold higher breast cancer risk compared with women with a low percentage of MD (≤5%; ref. 2), an association being independent of other breast cancer risk factors. Dense breast tissue is characterized by increased stromal tissue and possibly by increased numbers of breast epithelial cells (3, 4). Studies of twins suggest that genetic factors explain 30% to 60% of the variation in percent MD, adjusted for age and body mass index (BMI; refs. 5–7). Although a few genetic variants have been identified (8–19), the exact genetic determinants of MD are however not clear.

Genome-wide association studies (GWAS) and candidate gene studies have identified more than 75 SNPs that may be associated with risk of breast cancer in Caucasians (20–34). It has been estimated that there is about a 10% overlap in the genetic factors that influence breast cancer risk and those that determine variation in MD (5). One such example is rs10995190 in *ZNF365* that was found to be associated with both percent MD and breast cancer risk. However, this variant explained only 0.5% of the variance in percent MD (18). A better understanding of the role of breast cancer SNPs on MD will help to elucidate the biologic mechanisms through which these variants, or the true causal variants they tag, affect breast carcinogenesis.

Nongenetic factors have also been found to affect MD. Percent MD decreases with older age and menopause, and

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

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doi: 10.1158/1055-9965.EPI-13-1212

Published OnlineFirst July 7, 2014; DOI: 10.1158/1055-9965.EPI-13-1212

increases in postmenopausal women who commence using combined postmenopausal estrogen and progestin therapy (EPT; refs. 35, 36). Parity and possibly early age at first full-term pregnancy are both associated with lower percent MD (37–41). BMI is strongly inversely associated with percent MD (42), and there is some evidence that physical activity (43) and possibly alcohol intake (44–46) are associated with MD phenotypes. We and others have previously suggested the role of possible gene–environmental interactions in explaining variation in the effects of lifestyle factors on MD. One example is the hypothesized interaction between genes involved in hormone metabolism and hormonal factors, such as use of EPT, on MD.

We have previously reported that the effect of a variant (rs10946545) in the *prolactin gene* on percent MD was modified by current EPT use among a large subset of Norwegian women in the current study ($P_{interaction}$ by EPT use was 0.0008; ref. 47). Several other genetic variants have been found to interact with EPT (8, 10, 14). Given that EPT use has strong effects on MD in some, but not all women (35), these gene–environment interactions reported from several studies could represent real effects. Alternatively, some of these results may represent type I errors, i.e., simply be due to chance. However, it seems biologically plausible that genetic factors could modify the effect of EPT. Likewise, it also seems reasonable that environmental factors could modify the effects of established breast cancer genetic variants on MD, with genetic–MD associations being present only in specific subgroups of women defined according to their exposure to nongenetic factors.

We therefore hypothesized that many of the identified breast cancer genetic variants may also affect MD, and that their effect on this phenotype may be modified by environmental or lifestyle factors. Accordingly, our previous study of interactions between hormonal genes and hormonal factors (47) was expanded here to investigate associations between 17 well-established breast cancer SNPs and mammographic MD in a population of Norwegian postmenopausal women. We further examined whether the SNP–MD associations were modified by nongenetic factors such as BMI, EPT use, parity, and alcohol intake at mammography.

Materials and Methods

Participants

The study was nested within the Norwegian Breast Cancer Screening Program (NBCSP), which invites all women ages 50 to 69 years to undergo a mammographic examination every 2 years. The attendance rate is 76.2% (48). In the current study, we used data from subsets of women who attended the NBCSP in either 2004 or 2006–07.

Main sample (2004 sample)

The characteristics of the 2004 sample and its participants have been previously described (44, 47, 49, 50). In short, we sent a standardized questionnaire on various breast cancer risk factors (i.e., menstrual and reproductive history, use of oral contraceptives and menopausal hormonal therapy, family history of breast cancer, current weight, and height), together with the NBCSP invitation for screening, to a random sample of 17,050 women living in the counties of Oslo, Akershus and Hordaland in 2004. A total of 12,056 (71%) of the invited women attended the screening program, and 7,941 (66%) returned a completed questionnaire (Fig. 1). A subset (7,174) of the 7,941 women who had completed the questionnaire and agreed to participate in a study of diet, were asked to complete a food frequency questionnaire and provide two buccal swabs. Of these, 3,484 women (49%) returned the dietary questionnaire and 3,728 returned buccal swabs. About 300 women from Oslo had undergone digital mammography and were not included in the current study. We obtained information on risk factors, buccal samples, and analog screening mammograms on 2,876 women. Of these, 130 women were excluded for the following reasons: previous history of cancer ($n = 17$), the breast area could not be determined ($n = 3$), incomplete data on age ($n = 34$), or BMI ($n = 73$, height = 46/weight = 67), use of progesterone only ($n = 3$; Fig. 1). After these exclusions, a total of 2,746 pre- and postmenopausal women were left. Of these, genotype data were available for 2,397, with 2,030 being postmenopausal at mammography (see definition below) and therefore eligible for the present analysis.

2006–07 sample

In 2006–07, all women who underwent a NBCSP mammographic screening were asked, as part of their preexam questionnaire, whether they were willing to participate in an additional study of diet. Information on the same breast cancer risk factors as for the 2004 sample was collected. A food frequency questionnaire was sent to a random sample of 10,000 women living all over Norway who had agreed to participate in the dietary study (Fig. 1). Of these, 6,974 answered the dietary questionnaire and the vast majority (>90%) agreed to provide saliva and a fingerprick blood sample.

We requested analog mammograms from 5 of the 16 participating screening centers, selected to represent the whole country, and received analog mammograms for 632 women. Women from the 2006–07 sample followed the same exclusions criteria as for the 2004 sample. A total of 245 women were excluded because of breast cancer diagnosis ($n = 7$); missing data on BMI ($n = 146$), or age ($n = 22$), improbable height and weight values ($n = 22$, of which 4 with self-reported height < 125 cm, and 18 with self-reported weight <30 kg or >170 kg), age not within the range 50 to 69 years ($n = 5$), and simple hysterectomy without bilateral oophorectomy (unclassifiable regarding menopausal status; $n = 43$; Fig. 1). In addition, we excluded 69 pre- and perimenopausal women, yielding 318 postmenopausal women with risk factor data, analog films, and genetic data for the present analysis.

Mammographic density analysis

Left cranio-caudal analog mammograms were scanned using a high-resolution Kodak Lumisys 85 scanner with

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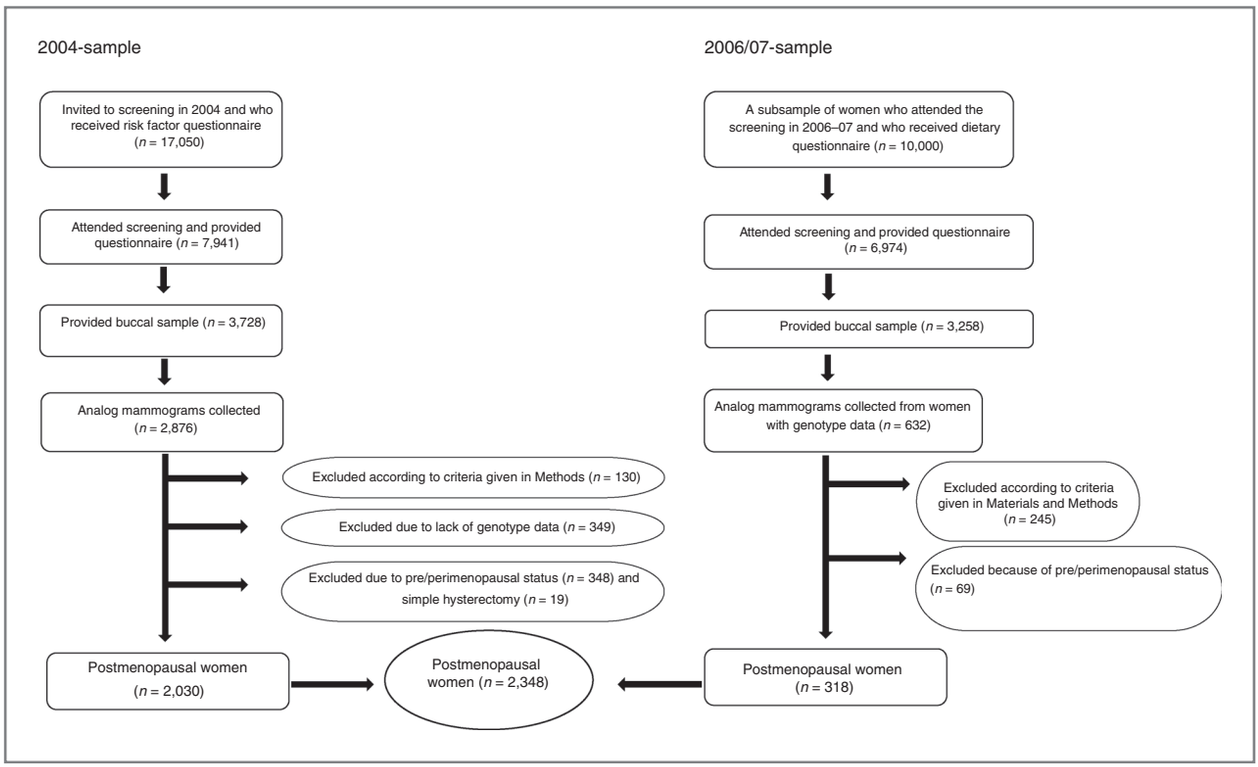

Figure 1. Flowcharts for the 2004 and 2006–07 samples.

automatic feeder. Computer-assisted readings of absolute areas of dense and nondense tissues, as well as percent MD, were performed using the Madena software (51). This method provides a continuous measure of percent MD (calculated as the ratio of absolute dense area to total breast area multiplied by 100) as well as separate estimates of the absolute areas of dense and nondense tissues (both in cm^2). For both the 2004 and 2006–07 samples, the MD assessments were performed by an experienced reader (G. Ursin). The reader was blinded to all subject characteristics. A subset of the images was read twice. The intra-reader correlation coefficient for absolute density was 0.99. Percent MD was our primary endpoint. However, to better understand the biologic mechanisms linking any SNP–MD associations, we also conducted analysis in which the absolute area of dense tissue and the absolute area of nondense tissue were the outcomes of interest.

DNA collection and extraction

DNA was extracted from the 2004 sample buccal swabs using standard modified protocol for the QIAamp Blood DNA Kit (Qiagen). For the 2006–07 sample, we sent out the Oragene DNA Self-Collection Kit (DNA Genotek Inc.), which contains a solution with antibacterial and DNA preserving chemicals that mixes with the saliva, resulting in immediate conservation of the sample (52). Extraction of DNA was performed according to the manufacturer's protocol.

Genotyping of SNPs

For both the 2004 and 2006–07 samples, genotyping was done using an Illumina BeadLab System and GoldenGate Genotyping technology in the University of Southern California Genomics Center. Samples were run in a 96-well format using Illumina Sentrix Array technology on a BeadArray Reader. BeadStudio Software (v.3.0.9) with Genotyping Module (v.3.0.27; Illumina) was used for analyzing scanned samples.

We initially selected 21 SNPs suspected or confirmed to be associated with breast cancer susceptibility in 19 regions (loci) of the genes *MRPS30:FGF10*, *ESR1*, *COX11*, *TOX3*, *FGFR2*, *MAP3K1*, *TGFB1*, *LSP1*, *CASP8*, *CYP1B1*, *SLC4A7*, *RAD51L1*, *TXNRD2*, and positions 10q26.13, 1p11.2, 8q24.21, 6q25.1, 2q35, 9q31, the majority identified through large-scale genotyping studies (20–24, 28, 53, 54).

We included a large number of duplicates in this study, on the order of approximately 7%. We reviewed any SNP with >2 duplicate mismatches for incorrect genotype clusters ($n = 1$). The genotype call regions were adjusted for the one assay in question until the number of duplicate mismatches was <3 (<0.003%).

Out of 21 GWAS SNPs, three (*CASP8*-rs1045485, *ESR1*-rs2046210, and *RAD51L1*-rs10483813) had <80% call rates and one (*FGFR2*-rs2981579) departed from Hardy–Weinberg equilibrium (HWE; $P < 0.001$), leaving 17 SNPs for further analysis.

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Menopausal status at mammography

Mammograms and questionnaires were collected roughly at the same point in time. Women were considered postmenopausal at the time of mammography if they reported (i) a complete cessation of menstruation of at least 6 months (2004 sample) and 12 months (2008 sample), (ii) a previous bilateral oophorectomy, (iii) or used postmenopausal hormone therapy before menopause. We ran a sensitivity analysis excluding the 2004 women with menopause within the past year ($n = 177$), but this yielded essentially unchanged results, and did not alter the order of the most important SNPs (results not shown). We therefore used the 6-month definition of menopause for the 2004 women for consistency with our previous paper (47).

We ran the analysis excluding women under 55 ($n = 630$). We also reran the analysis excluding hormone users and women under 55 ($n = 277$). For both these reanalysis, the 3 most important SNPs remained the same as the analysis including these women.

Our final pooled sample size was 2,348 postmenopausal women ($n = 2{,}030$ from 2004 and 318 from 2006–07).

Alcohol intake, BMI, hormone therapy use, and parity

For both samples, we ascertained postmenopausal hormone therapy use at mammography by asking questions about ever, as well as current, use of hormones using a comprehensive list of preparations. A woman could have used both estrogen-only therapy and combined EPT in her lifetime, but only one of these currently. We divided the ever users into current and past EPT users, and the never EPT users were further subdivided into current and past estrogen-only therapy users.

Current alcohol intake was measured by asking about the type of alcohol (beer, red wine, white wine, liquor) consumed around the time of mammography, and the amounts and frequency of consumption (monthly). Self-reported BMI at the time of mammography was defined as self-reported weight (in kg) divided by height (in m^2).

Ethics committee approval

The study was approved by the relevant regional ethics committees and the Norwegian Data Inspectorate. All participants provided written informed consent.

Statistical analyses

Multivariate linear regression models were used to examine the relation between SNPs and the four MD measures (percent MD, absolute areas of dense and nondense tissues, and total breast area). The MD phenotypes were treated as continuous variables. No transformation of their values was required as the models' residuals satisfied the normality and homoscedasticity assumptions. Furthermore, we repeated the analyses using a square root transformation of the MD values, but as they yielded similar regression coefficient estimates and P values to those obtained with the untransformed (raw) data, we present here only the results from the latter.

The analyses were conducted using both additive and dominant genetic models, but as they yielded similar results, only those from the additive models are presented here. The resulting regression coefficient (β) represents the absolute (i.e., on an arithmetic scale) change in percent MD (or other MD measures) per copy of the minor allele after adjustments for age, BMI and study in an additive model of inheritance. In this article, we describe the beta coefficient as "SNP effects", knowing that a more correct term is "SNP associations" as the former implies causality. Our term "effects" therefore refers to statistical effects rather than biologic causality.

We tested whether there was any evidence of between-sample heterogeneity in the SNP effects on MD, but none of the tests were statistically significant. Therefore, we combined the data from the two samples and present the pooled adjusted estimates. We estimated least-squares means (marginal means) of MD across levels of nongenetic variables. We assessed the effect modification by including a product term of the SNP and potential effect modifier in the linear regression model.

Analyses were conducted using SAS version 9.2 (SAS Institute, Inc.). We used two-sided tests with a $P < 0.05$ considered statistically significant, and estimated a 95% confidence interval (CI) for the regression coefficient (β; Supplementary Tables S1 and S2).

A P value of 0.05 may be considered rather liberal in a study of SNPs. However, we selected SNPs that we had strong prior probability to consider meaningful, as they had been identified in other GWAS studies with substantial stronger power. To ensure that the results were robust, we studied associations with both absolute and percent MD, and with both additive and dominant genetic models.

Results

In this population of 2,348 postmenopausal women, we found that percent MD and absolute dense area were inversely associated with age, BMI, parity, but positively associated with EPT use (Table 1). This is consistent with what we previously described from the 2004 data (47, 51). The strong inverse association of age and BMI with percent MD reflected inverse associations between these two variables and absolute dense area as well as positive associations with nondense area and total breast area. There was also a weaker inverse association of parity with percent MD, which reflected inverse associations with absolute dense area and to a lesser degree, total breast area. In contrast, the positive associations between EPT use and percent MD reflected positive associations with absolute MD as well as inverse associations with nondense and total areas. There was evidence of a weak positive association between ever alcohol use (current and past use) and absolute dense area (Table 1).

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Table 1. MD measurements by nongenetic risk factors in Norwegian postmenopausal women

Risk factors	*N* (2,348)	% MD Mean[a] (SD)	Absolute dense area Mean[a] (SD)	Nondense area Mean[a] (SD)	Total breast area Mean[a] (SD)
Age, y					
50–54	630	21.0 (16.9)	25.2 (19.7)	114.5 (58.7)	140.1 (55.9)
55–59	752	19.8 (15.8)	25.5 (21.9)	122.0 (63.0)	147.5 (61.1)
60–64	605	17.3 (14.9)	22.6 (20.8)	125.6 (60.5)	148.6 (58.7)
65–69	361	13.4 (14.4)	17.7 (18.0)	142.7 (67.4)	160.6 (62.7)
P_{trend}[b]		0.0001	0.0001	0.0001	0.0001
BMI, kg/m[c]					
<20	142	33.9 (20.2)	29.0 (19.0)	60.4 (30.8)	89.4 (32.8)
20–21	163	28.4 (17.0)	27.4 (18.7)	74.6 (38.7)	101.9 (38.0)
22–23	442	23.8 (16.2)	26.0 (18.6)	89.9 (36.2)	115.9 (33.4)
24–25	556	18.8 (13.7)	24.4 (19.3)	112.4 (43.5)	136.8 (42.5)
26–27	423	15.4 (13.9)	22.8 (22.1)	132.2 (47.2)	156.0 (44.0)
28–29	246	11.9 (11.6)	20.1 (22.2)	159.4 (57.1)	179.9 (55.7)
>29	376	9.07 (10.2)	18.2 (22.0)	196.8 (66.0)	215.9 (65.8)
P_{trend}[c]		0.0001	0.0001	0.0001	0.0001
Parity					
0	197	21.3 (17.2)	27.3 (21.8)	123.5 (66.4)	151.0 (61.2)
1	268	21.5 (17.3)	26.5 (20.5)	120.0 (60.7)	146.6 (56.5)
2	1117	18.8 (16.1)	24.1 (21.5)	123.5 (62.0)	147.7 (59.4)
>3	739	16.1 (14.0)	20.3 (18.4)	127.2 (62.5)	147.8 (60.4)
P_{trend}[d]		0.0001	0.0001	0.008	0.6416
HT use					
Never	1039	16.8 (15.1)	21.4 (19.8)	128.2 (63.8)	149.9 (59.7)
Ever	1262	19.9 (16.5)	25.3 (21.3)	121.4 (61.9)	146.8 (59.9)
P df1[d]		0.0001	0.0001	0.0007	0.1050
EPT use					
Never	1318	17.2 (15.7)	22 (20.7)	127.9 (64.1)	150.2 (60.6)
Past	692	19.3 (15.3)	24.7 (20)	120.2 (60.4)	145.0 (58.9)
Current	269	23.1 (17.1)	29.0 (21.7)	116.3 (57.6)	145.5 (56.2)
P_{trend}[d]		0.0001	0.0001	0.0001	0.0207
Alcohol use					
Never	299	18.1 (15.5)	22.4 (19.3)	128 (72.7)	151.2 (68.6)
Ever	2028	18.6 (15.9)	23.7 (20.8)	123.4 (60.7)	147.3 (58.1)
P df1[d]		0.6024	0.0326	0.1195	0.1751

Abbreviation: HT, hormone therapy.

[a]Least-squares means; means adjusted for age, BMI, and study.

[b]Adjusted for BMI and study.

[c]Adjusted for age and study.

[d]Adjusted for age, BMI, and study.

Out of 17 SNPs that we investigated, two SNPs were statistically significantly associated with percent MD. The estimated effects from our study, and how these variants have been associated with either MD or breast cancer risk in previous studies are shown in Table 2, with the results from the current study in the leftmost panel. In adjusted models, the rs9383938 in 6q25.1 was positively associated with percent MD, with each copy of the minor allele being associated with an increase (on an arithmetic scale) of 2.1% ($P = 0.0188$; Table 2). When we included rs9383938 and rs3734805 in the model, the magnitude of the associations with percent MD became weaker (results not shown). In contrast, rs8141691 in *TXNRD2* was inversely associated with percent MD with each copy of the minor allele being associated with a decrease of 1.4% ($P = 0.0333$; Table 2). None of the other variants examined reached statistical significance overall in our study. Eight of the seventeen variants showed associations in the same direction as the associations with breast cancer risk previously reported in other studies (ORs above 1 in the rightmost panel; Table 2).

Table 2. Associations between breast cancer SNPs and percent MD in Norwegian postmenopausal women ($N = 2348$) and comparisons with published data on the associations of these SNPs with MD and breast cancer risk

		Results from the current study						Results from previous studies of genetic variants					
		Number of women with						MD study				Breast cancer studies	
Gene/region	SNP	Common homo zygote	Hetero zygote	Rare homo zygote	% MD per minor allele Beta[a]	SE	P^b	% MD per minor allele Beta[a]	SE	P^c	Per minor allele OR on breast cancer OR	P	References
6q25.1	rs9383938	1594	237	22	2.05	0.87	**0.0188**	—	—	—	1.18	$1.41 \times 10-7$	Fletcher et al. (28)
TXNRD2	rs8141691	925	1105		−1.40	0.66	**0.0333**	—	—	—	1.12	0.046	Cebrian et al. (58)
2q35	rs13387042	972	591	517	0.55	0.31	0.0810	−0.04[e]	0.18[e]	0.75[c]	0.83	1.3×10^{-13}	Stacey et al. (30)
6q25.1	rs3734805	2023	285	11	0.89	0.65	0.1735	—	—	—	1.19	1.35×10^{-7}	Fletcher et al. (28)
10q26.13	rs1051010	1218	636	268	−0.55	0.44	0.2059	—	—	—	1.12	1.58×10^{6}	Fletcher et al. (28)
SLC4A7/3p24	rs4973768	579	964	365	0.52	0.48	0.2755	0.48[e]	0.19[e]	0.03[c]	1.11	4.1×10^{-23}	Ahmed et al. (22)
9q31.2	rs865686	874	803	469	0.39	0.36	0.2767	—	—	—	0.9	2.01×10^{-29}	Warren et al. (54)
TGFB1	rs1800470	830	829	253	−0.35	0.36	0.3328	—	—	—	1.15	0.162	Ma et al. (53)
1p11.2	rs11249433	1061	874	349	0.34	0.38	0.3610	0.10[e]	0.22[e]	0.78[c]	1.16	6.74×10^{-10}	Thomas et al. (23)
8q24.21	rs13281615	1061	874	349	0.34	0.38	0.3610	0.09[e]	0.21[e]	0.48[c]	1.08	5×10^{-12}	Easton et al. (20)
MRPS30:FGF10	rs4415084	614	899	356	−0.43	0.47	0.3647	−0.12[e]	0.28[e]	0.99[c]	1.19	6.4×10^{-10}	Stacey et al. (21)
COX11/17q23.2	rs6504950	1163	826	293	−0.36	0.44	0.4110	0.21[e]	0.22[e]	0.49[c]	0.95	1.4×10^{-8}	Ahmed et al. (22)
CASP8	rs17468277	1450	484	268	0.35	0.45	0.4356	−0.37[e]	0.36[e]	0.04[c]	0.88	5.7×10^{-7}	Cox et al. (24)
MRPS30:FGF10/5p12	rs10941679	1212	861	172	−0.21	0.32	0.5140	0.06[e]	0.23[e]	0.99[c]	1.19	2.9×10^{-11}	Stacey et al. (21)
MAP3K1	rs889312	1070	822	166	−0.23	0.43	0.5932	0.06[e]	0.20[e]	0.57[c]	1.13	7×10^{-20}	Easton et al.(20)
LSP1	rs3817198	977	963	334	0.13	0.41	0.7449	0.70[e]	0.21[e]	0.001[c,d]	1.07	3×10^{-9}	Easton et al. (20)
TOX3	rs3803662	979	720	291	0.06	0.36	0.8669	0.30[e]	0.22[e]	0.17[c]	1.2	10^{-36f}	Easton et al. (20)

NOTE: Bold indicates P values <0.05. rs17468277 and rs17468277 in strong linkage disequilibrium (LD; $r^2 = 0.98$; Vachon et al; ref. 19).

[a]Beta represents the difference (on an arithmetic scale) in percent density per copy of the minor allele of each SNP after adjustments for age, BMI, and study assuming an additive model of inheritance.

[b]Linear regression adjusted for age, BMI, and study, untransformed.

[c]Root squared transformed, adjusted for age, BMI, menopausal status, case status, and study (Vachon et al; ref. 19).

[d]Positive association with percent MD.

[e]Untransformed betas and SEs, unpublished data.

[f]rs3803662 was not part of the initial tag SNP set but identified as a result of fine-scale mapping and typed in the stage 2 and stage 3 sets (but not the stage 1 set).

Published OnlineFirst July 7, 2014; DOI: 10.1158/1055-9965.EPI-13-1212

Table 3. Associations between the two top SNPs for percent density and its components (absolute dense area, absolute nondense area, and total breast area) among Norwegian postmenopausal women ($N = 2,348$)

					% density		Absolute dense area		Absolute non-dense area		Total breast area	
Gene	SNP	WW[a]	WV[b]	VV[c]	Beta	*P*	Beta	*P*	Beta	*P*	Beta	*P*
6q25.1	rs9383938	1,594	237	22	2.05	**0.0188**	1.84	0.1443	−2.07	0.4686	−0.24	0.9322
TXNRD2	rs8141691	925	1105		−1.40	**0.0333**	−2.14	**0.0210**	3.64	0.0871	1.50	0.4684

NOTE: Bold indicates *P* values <0.05.

[a]WW = number of women homozygotes for the wild-type genotype.

[b]WV = number of women heterozygotes for the wild-type and variant genotype.

[c]VV = number of women homozygotes for the variant genotype.

The directions of the associations of rs9383938- 6q25.1 and rs8141691-*TXNRD2* with absolute MD paralleled those observed for percent MD (Table 3).

We examined possible gene–environment interactions for BMI, parity, EPT use, and alcohol (Table 4). There was no evidence of effect modification by any of these variables for the rs9383938- 6q25.1 or the rs8141691-*TXNRD2*. Out of a total 68 ($17 \times 4 = 68$, gene × environment tests) statistical tests, three were statistically significant at the significance level of 0.05, i.e., no more than what would be expected by chance. Thus although likely type I errors, all three interactions had interesting features, and are therefore commented on. Two of the SNPs showed significant interactions with BMI, and one with parity (Table 4). Interestingly, for both SNPs that interacted with BMI the direction of their associations with percent MD was consistent with their previously reported effects on breast cancer risk only among heavy women (BMI ≥ 25 kg/m^2). The 9q31.2-rs865686 was inversely associated with percent MD among heavy women (BMI $\geq$ 25 kg/m^2), but positively associated with percent MD among lean women (BMI < 25 kg/m^2; per minor allele change in percent MD: −0.67% and 1.43%, respectively; $P_{\text{interaction}} = 0.0105$). Similarly, the *MRPS30:FGF10*-rs4415084 was positively associated with percent MD among heavy women, as expected given its reported effect on breast cancer risk (Table 2), but inversely associated with percent MD among lean women (per minor allele change: 1.01% and −1.50%, respectively; $P_{\text{interaction}} = 0.0051$).

The magnitude of the positive association of 6q25.1-rs3734805 with percent MD was modified by parity with this SNP being more strongly associated with this phenotype among nulliparous women than among parous women (per minor allele change in percent MD: 5.03%, and 0.80%, respectively; $P_{\text{interaction}} = 0.0225$; Table 4). The direction of the association between 6q25.1-rs3734805 and percent MD was consistent with the previously reported effect of this SNP on breast cancer risk (Table 2).

Discussion

Out of the 17 breast cancer susceptibility variants investigated, we found that, overall, two SNPs (6q25.1-rs9383938 and *TXNRD2*-rs8141916) were statistically significantly associated with percent MD. Eight SNPs showed associations with percent MD that were in the same direction as those previously reported for breast cancer risk. Our hypothesis was that these variants could interact with various environmental or lifestyle factors. Two SNPs interacted with BMI and one with parity, and although interesting, these may have been chance findings.

Consistency and inconsistency of overall findings with previous MD and breast cancer studies

Our findings with 6q25.1-rs3734805 and 6q25.1-rs9383938 are consistent with the GWAS study on breast cancer where these variants were first reported (28) and with a subsequent case–control study of 6q25.1 (55). A meta-analysis of five GWAS of MD within the Marker Of DEnsity (MODE) consortium, examined 23 of the established breast cancer variants with MD and reported a significant association of rs2046210 -*ESR1* and MD (18). The genetic variants rs2046210 and rs9383938 are not in LD (r2 = 0.12) in whites of European ancestry in 1000 Genome dataset (56). However rs9383938-6q25.1 is intergenic, 5′ to *ESR1* (57), suggesting indirectly an association between these two genetic variants and MD.

However, no previous studies have reported on the effect of these SNPs on MD. Our results therefore suggest that this represents another example of a shared genetic determinant between MD and breast cancer risk. Previously, large GWAS on MD showed that a variant in *ZNF365* was associated with both breast cancer risk and MD (18) and a large international consortium on the genetics of MD (DENSNP) reported associations of MD with variants in *LSP1* and *RAD51L1* (19). The *LSP1* variant was examined in the current study. Although not statistically significant, our finding was consistent with previous studies on MD and breast cancer (19, 20). One possible reason for the lack of a statistically significant association in our study might be its small sample size relative to those of the previous GWAS on MD and the DENSNP study (19).

In contrast, the inverse association we observed between *TXNRD2* -rs8141691 and percent MD was in the opposite direction of what would have been expected

Published OnlineFirst July 7, 2014; DOI: 10.1158/1055-9965.EPI-13-1212

Table 4. The association between the breast cancer SNPs and percent density stratified by selected nongenetic variables among Norwegian postmenopausal women ($N = 2,348$)

			BMI (kg/m^2)[a]			Parity[a]			EPT use[a]				Alcohol use[a]			
			<25 $n = 1,303$	≥25 $n = 1,045$		Nulliparous $n = 215$	Parous $n = 2,119$		Never $n = 1,318$	Past $n = 692$	Current $n = 269$		Users $n = 2,028$	Nonusers $n = 299$		
Locus	SNP	Alleles	Beta[b]		$P_{interaction}$	Beta		$P_{interaction}$	Beta			$P_{interaction}$	Beta			$P_{interaction}$
6q25.1	rs9383938	G/T	2.13	1.20	0.3026	5.62	1.97	0.1475	0.97	4.07	−0.19	0.5459	2.30	0.51		0.6016
TXNRD2	rs8141691	A/C	−0.77	−2.16	0.2702	0.25	−1.45	0.4471	−1.90	−0.85	−0.19	0.3951	−1.54	−0.52		0.8190
2q35	rs1338704	A/G	0.88	0.09	0.1603	−0.31	0.67	0.8887	0.85	0.06	0.04	0.3397	0.68	−0.39		0.8783
6q25.1	rs3734805	A/C	2.00	−0.16	0.0588	5.03	0.80	**0.0225**	0.86	0.00	4.76	0.5492	0.81	2.31		0.8067
10q26.13	rs1051010	A/T	−0.84	−0.04	0.3450	0.16	−0.65	0.5924	−0.13	−1.37	−1.01	0.3567	−0.79	1.58		0.7477
SLC4A7	rs4973768	C/T	0.89	−0.02	0.3978	−2.08	0.76	0.1628	0.44	−0.29	1.99	0.4980	0.43	1.31		0.6346
9q31.2	rs865686	G/T	1.43	−0.67	**0.0105**	0.06	0.47	0.8479	0.59	−0.10	1.01	0.9208	0.47	−0.08		0.8257
TGFB1	rs1800470	C/T	−0.17	−0.57	0.5220	−0.23	−0.37	0.9188	−0.20	−0.55	0.69	0.7580	−0.45	0.90		0.6592
1p11.2	rs1124943	C/T	0.53	0.03	0.5633	−0.51	0.42	0.6471	0.05	0.67	0.83	0.3906	0.35	0.92		0.5300
8q24.21	rs1328161	A/G	0.53	0.03	0.563	−0.51	0.42	0.6471	0,05	0,67	0,83	0,3906	0,35	0,92		0,5300
MRPS30:FGF10	rs4415084	A/G	−1.50	1.01	**0.0051**	−1.15	−0.36	0.7589	−1,00	0,79	0,47	0,1042	−0,39	−0,90		0,4273
COX11	rs6504950	A/G	0.23	−1.00	0.2804	3.24	−0.52	0.3319	−0.58	−0.68	−0.44	0.7980	−0.54	1.23		0.3283
CASP8	rs1746827	C/T	0.38	0.22	0.8554	−1.13	0.52	0.4996	1.36	−0.59	−0.16	0.1980	0.35	0.31		0.4842
MRPS30:FGF10	rs1094167	A/G	−0.14	−0.25	0.7742	−0.04	−0.26	0.5640	−0.26	−0.35	1.93	0.2003	−0.23	0.21		0.6336
MAP3K1	rs889312	A/C	−0.51	0.11	0.4119	−2.10	−0.09	0.0883	−0.56	0.62	−0.81	0.5780	0.02	−2.10		0.5520
LSP1	rs3817198	T/C	0.11	0.20	0.7383	−0.04	0.12	0.6409	−0.05	0.41	1.19	0.5885	−0.01	1.37		0.2054
TOX3	rs3803662	C/T	−0.07	−0.21	0.5095	−1.07	0.21	0.5126	−0.43	−0.24	1.32	0.2527	0.24	−1.11		0.3553

NOTE: Bold indicates P values <0.05.

[a]As ascertained at the time of mammography.

[b]For each SNP the beta coefficient represents the change (on an arithmetic scale) in percent MD associated with each copy of the minor allele after adjustment for age, BMI, and study assuming an additive model of inheritance.

Published OnlineFirst July 7, 2014; DOI: 10.1158/1055-9965.EPI-13-1212

given its reported effect on breast cancer risk. A British case–control study based on cases from the Studies of Epidemiology and Risk Factors in Cancer Heredity (SEARCH) breast cancer study, and controls from the Norfolk component of European Prospective Investigation of Cancer found a positive association between the variant in *TXNRD2* and risk of breast cancer (58). The reasons for this discrepancy are unclear. It is possible that our finding arose simply by chance. Alternatively, this variant may exert its effect on breast cancer risk through a biologic pathway which is unrelated to MD.

The directions of most other SNP–MD associations were as expected given their reported effects on breast cancer risk (20, 22, 23, 28), but none was statistically significant in our analysis.

Two nonsignificant associations overall are worth mentioning. Our finding pertaining to 9q31.2-rs865686 and percent MD is novel, but in the opposite direction of what was reported in the breast cancer GWAS (28) as well as a replication study comprising 37 case–control studies within the Breast Cancer Association Consortium (54). Our inverse association with *MRPS30:FGF10*-rs4415084 on percent MD was also in the opposite direction of what was found in a large breast cancer GWAS from Iceland, Sweden, the Netherlands, Spain, and the United States (21). However, both genetic variants showed a statistically significant effect modification by BMI. Specifically, the effect observed among the heavier women (BMI $\geq$ 25 kg/m^2), was consistent with the effect that has previously been reported for these variants and breast cancer risk (21, 54). This could have been a chance finding, but could also imply that the biologic mechanisms of these genetic variants might involve BMI, or that the effect on MD is dependent of BMI. As far as we are aware, no one has published results on whether the effect of 9q31.2- rs865686 on breast cancer risk varies by BMI.

The DENSNP study examined interactions of several SNPs examined in the current study with BMI and postmenopausal hormone therapy. It reported an interaction between rs4415084- *MPRS30:FGF10* and postmenopausal hormone therapy (never/ever use), but no interaction between this SNP and BMI (19). In contrast, we found no interaction between this SNP and EPT use, but there was an interaction with BMI. Despite the apparent lack of consistency of these findings, the biologic mechanisms underlying the observed interactions may be similar as both hormone therapy and BMI are predominantly associated with estrogen receptor (ER)-positive breast cancer in postmenopausal women (59).

We also found effect modification by parity for 6q25.1-rs3734805, in which a stronger effect between this SNP and percent MD was found in nulliparous women. Interestingly, the other 6q25.1 variant, the rs9383938, which was found to be associated with percent MD overall, also showed a stronger association among nulliparous women. The estimated effect among nulliparous women is strong (a per minor allele change in percent MD of about 5% for each one of these two SNPs), suggesting there may be a linked causal variant that is affected by parity status.

Possible biologic mechanisms

6q25.1-rs3734805 and rs9383938 maps 72 kb from the 5′ untranslated region of *ESR1* and, given the prior evidence that *ESR1* plays a role in breast cancer etiology, it seems likely that this SNP correlates with a causal variant that exerts an effect on *ESR1* levels of expression (28). We have no explanation as to why this variant yields higher effect on percent MD among nulliparous women.

Thioredoxin reductase 2 (TXNRD2) is a protein responsible for mediating numerous cytoplasmic functions and is implicated in control of cell growth in which the redox function is essential for growth stimulation and apoptosis (60, 61).

9q31.2-rs865686 is a noncoding SNP whose role in the disease process is unclear (54). This SNP lies more than 600 kb from the nearest gene and is not in LD with any genomic elements that suggest a possible causal mechanism to breast cancer. The mechanism as to why it could be involved in mammary gland development or carcinogenesis is not clear, nor how it is related to BMI. It may be involved in the synthesis of microRNAs; i.e., small noncoding RNAs that are frequently located in genomic regions involved in cancers, and are important in the coordination of cell proliferation and cell death during development and in stress resistance and fat metabolism (62).

MRPS30:FGF10-rs4415084 is a genetic variant on chromosome 5p12 and confers a risk of ER-positive breast cancer (21). *MRPS30:FGF10* is not expressed in normal breast luminal epithelial cells, but is upregulated in ductal carcinomas (63). If the interaction with BMI is real, this could be explained by the role this gene plays for ER-positive cancer, for which BMI is a risk factor (59).

Mechanisms, through dense or nondense areas

It has been argued that absolute dense area is the more relevant phenotype when studying etiology of MD (64), as tumors arise predominantly within the radio-dense tissue (65, 66). Whether these SNPs influence dense, nondense, or total breast area could help elucidate the mechanisms by which the loci influence MD and possibly breast cancer risk. In this study, the statistically significant positive association between 6q25.1-rs9383938 and percent MD reflected a positive association with absolute dense area and an inverse association with the nondense area, although none were statistically significant. Another large study (DENSNP) found that rs3817198 in *LSP1* was positively associated with both adjusted dense area and adjusted percent MD (19), which is consistent with our finding, although ours did not reach statistically significance, possibly due to our much smaller sample size.

Strengths and weaknesses

A strength of this cross-sectional study is that it was population-based and from a relatively homogenous population. The original analog films from the two samples (2004 and 2006–07) were all digitized using the same scanning machine, and read by the same reader. Similar

Published OnlineFirst July 7, 2014; DOI: 10.1158/1055-9965.EPI-13-1212

detailed risk factor information was also available for both samples. Although the two samples of women were recruited at two different time points, they were both nested within the same long-term cohort of screened women. Furthermore, tests for heterogeneity showed no statistically significant differences between the two samples and the results did not change materially when the analyses were restricted only to the largest (2004) sample (results not shown). A weakness of the study is that although it was of a reasonable size, its power to detect weak SNP–MD associations, and effect modifications by nongenetic variables was limited. On the other hand, our findings may have arisen by chance as none would have been statistically significant after adjustment for multiple testing; however, the SNPs examined here were selected *a priori* as strong candidates given their established, or putative, associations with breast cancer risk. Thus, while our significant findings may have arisen by chance, it is also possible that the study may have been underpowered to detect weak SNP effects and SNP–environment interactions.

It has been argued that the false positive report probability, would give an indication as to whether any reported significant result is likely to be false positive (67). This probability depends both on the power of the study, the observed significance level of the variant-disease association, but also on the prior probability that an association between variant and the disease is real. We did not estimate this probability. As we selected variants that had been identified by prior GWAS studies, our prior probability that these variants are important would be above average. However, our findings were marginally significant, and our power was limited, which increases the chance that a result is false positive (67). Our results should therefore be interpreted with caution.

Conclusion

The study found that rs9383938 in 6q25.1 and rs8141691 in *TXNRD2* were statistically significantly associated with age-BMI study adjusted percent MD, thus providing novel evidence of shared genetics between this phenotype and breast cancer risk. The results also suggest that the effect of a variant in 6q25.1 may be modified by parity status and variants in 9q31.2 locus and *MRPS30:FGF10* may be modified by BMI. Our data suggest that gene–environment interactions should be investigated when studying the etiology of MD.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M. Ellingjord-Dale, D.J. Van Den Berg, G. Ursin
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Grant Support

This work was supported by grants from the Norwegian Cancer Society (171743 - PR-2009-0210).

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Received November 20, 2013; revised May 12, 2014; accepted June 4, 2014; published OnlineFirst July 7, 2014.

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Published OnlineFirst July 7, 2014; DOI: 10.1158/1055-9965.EPI-13-1212

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Cancer Epidemiol Biomarkers Prev 2014;23:1752-1763. Published OnlineFirst July 7, 2014.