Mammographic Breast Density as a General Marker of Breast Cancer Risk

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Abstract

Mammographic breast density is a strong risk factor for breast cancer but whether breast density is a general marker of susceptibility or is specific to the location of the eventual cancer is unknown. A study of 372 incident breast cancer cases and 713 matched controls was conducted within the Mayo Clinic mammography screening practice. Mammograms on average 7 years before breast cancer were digitized, and quantitative measures of percentage density and dense area from each side and view were estimated. A regional density estimate accounting for overall percentage density was calculated from both mammogram views. Location of breast cancer and potential confounders were abstracted from medical records. Conditional logistic regression was used to estimate associations, and C-statistics were used to evaluate the strength of risk prediction. There were increasing trends in breast cancer risk with increasing quartiles of percentage density and dense area, irrespective of the side of the breast with cancer ($P_{trends} < 0.001$). Percentage density from the ipsilateral side [craniocaudal (CC): odds ratios (ORs), 1.0 (ref), 1.7, 3.1, and 3.1; mediolateral oblique (MLO): ORs, 1.0 (ref), 1.5, 2.2, and 2.8] and the contralateral side [CC: ORs, 1.0 (ref), 1.8, 2.2, and 3.7; MLO: ORs, 1.0 (ref), 1.6, 1.9, and 2.5] similarly predicted case-control status (C-statistics, 0.64-65). Accounting for overall percentage density, density in the region where the cancer subsequently developed was not a significant risk factor [CC: 1.0 (ref), 1.3, 1.0, and 1.2; MLO: 1.0 (ref), 1.1, 1.0, and 1.1 for increasing quartiles]. Results did not change when examining mammograms 3 years on average before the cancer. Overall mammographic density seems to represent a general marker of breast cancer risk that is not specific to breast side or location of the eventual cancer. (Cancer Epidemiol Biomarkers Prev 2007;16(1):43–9)

Introduction

Mammographic breast density reflects variation in fat, stromal, and epithelial tissues and is an established risk factor for breast cancer (1-3). Although this risk factor is one of the strongest identified for breast cancer (4), little is understood about its biology. Of particular importance is whether breast cancers arise in regions of highest densities or whether breast density is a general marker of risk.

Mammographic breast density may reflect exposure to hormones and growth factors that stimulate cell division in breast stroma and epithelium (3, 5, 6). This hypothesis is supported by the consistent observation of changes in mammographic density in response to menopausal hormones (7-9), tamoxifen (10-12), and the correlation with serum insulin-like growth factor-I among premenopausal women (5, 13, 14). Additionally, direct studies of mammographically dense tissues suggest that density may represent increased epithelial cellular concentration (15, 16), growth factors (insulin-like growth factor-I; refs. 16, 17), stromal fibrosis, and epithelial hyperplasia (18).

If mammographic density reflects a greater cellular concentration or increased proliferation in either the stroma or epithelium, then areas of increased density may be more susceptible to the initiation and promotion of breast cancers than areas of lower densities. Ursin et al. (19) reported recently that ductal carcinoma *in situ* lesions occurred in mammographically dense regions in a small series of patients. If mammographic density exerts its influence on the microenvironment, one might expect invasive tumors to also arise in the densest regions of the breast. It would also follow that there would be a stronger association of breast density and risk on the side that eventually develops the cancer relative to the contralateral. We address these questions in the context of a well-designed matched case-control study using prediagnostic screening mammograms available on average 7 years before the clinical diagnosis of cancer.

Materials and Methods

Study Population. Subjects were selected from the Mayo Clinic mammography screening practice in Rochester, Minnesota. Patients (3.6%) who did not provide research authorization for medical record studies were not eligible and women with bilateral mastectomies or breast implants before diagnosis were excluded. Breast cancer cases (n = 372) were women 50 years or older diagnosed with primary invasive cancer (n = 301) or ductal carcinoma *in situ* (n = 71) between 1997 and 2001 who had at least two prior screening mammograms 2 years before diagnosis and lived within a 120-mile radius of the clinic. The requirement for multiple mammograms established a population of women having routine screening mammograms and the age requirement provided the opportunity for women to have at least a 10-year experience of routine screening mammography before cancer (because screening is generally recommended starting at age 40 years). The residency requirement was designed to enhance the representativeness of the study population.

Two controls with no prior history of breast cancer from the screening practice were matched to each case on age (within 5 years), final screening exam date (4 months), menopausal status at final exam date (pre or post), time between baseline and final mammogram (8 months), number of prior screening mammograms (one mammogram), and residence (same

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county). Some controls were ineligible due to unavailability of mammograms or previous breast cancer identified on medical record abstraction. Weight, height, and hormone replacement therapy (HRT) were abstracted from the Mayo Clinic medical record for all serial mammogram dates, including the earliest and latest mammograms used in these analyses. Weight and height are routinely measured and recorded at medical exams associated with the mammogram. HRT was abstracted from patient reported medication use in the medical record over the entire mammogram period.

Height and weight were used to construct body mass index (BMI) in kilograms per meters squared. All remaining patient information was obtained from a clinical database of self-reported information gathered at each visit.

Location of the tumor was abstracted from radiology and pathology reports when available and classified into one of four quadrants (superior-lateral, superior-medial, inferior-lateral, and inferior-medial); if location was not specified on the reports, the original mammogram at time of diagnosis was obtained and the study radiologist (K.R.B.) classified the location based on the diagnostic four-view mammogram. Women with multiple tumors in different quadrants ($n = 9$) were excluded from regional density analyses.

Prediagnostic mammograms were available on all cases and controls. The earliest mammogram during the preceding 10-year period, but at least 2 years before breast cancer or corresponding exam date, was used for primary analyses. These mammograms ranged from 2.1 to 10.4 years before the cancer (or the corresponding exam date for controls), with a mean $\pm$ SD of 7.0 $\pm$ 1.5 years. The earliest mammogram was >5 years before cancer, or index date, for >90% of the participants. We also performed analyses using the mammogram closest to 3 years before the breast cancer (3.0 $\pm$ 1.0 years) to determine whether our findings were similar as you get closer to the date of diagnosis.

For both the earliest mammogram and those on average 3 years before the breast cancer, weight was available within 1 week for 85% of participants, whereas height was available for 68% to 70%. For the remainder, weight and height were used from the closest abstracted date; the median interval was 17 and 22 months for weight and 25 and 27 months for height for the 3-year and earliest mammograms, respectively. HRT information was available over the entire mammogram interval (and consequently at the two mammogram dates evaluated) for 84% of participants; for 16%, this was unknown.

This study was approved by the Mayo Clinic Institutional Review Board.

Breast Density Estimation. Mammograms were digitized on a Lumiscan 75 scanner with 12-bit grayscale depth. The pixel size was 0.130 $\times$ 0.130 mm^2 for both the 18 $\times$ 24 cm^2 and 24 $\times$ 30 cm^2 films. All four views [left and right mediolateral oblique (MLO) and left and right craniocaudal (CC)] were digitized. Batch files were created composed of both cases and controls with randomly assigned views, dates, and sides within a woman to maximize precision of estimates (20). A 5% repeat set of images was included within each batch file for assessment of reliability. Percentage breast density (dense area divided by total area $\times$ 100) and absolute dense area (cm^2) were estimated for each view using a computer-assisted thresholding program that has routinely been used in studies of breast density (1, 2, 21, 22). Briefly, two thresholds are set by a trained programmer; one separates the breast from the background and the second separates dense from nondense tissue (see Fig. 1). In the batch files examined for this study, we consistently showed high reliability ($r > 0.93$) while reading over 500 duplicate images across varying time frames.

Regional Density. An automated algorithm was used to divide each breast image (CC and MLO) into two distinct regions (Fig. 2). First, the location of the chest wall on the mammogram was defined. For the MLO view, the pectoralis muscle and extension of this muscle (see Fig. 2) is defined as the chest wall; for the CC view, the film edge is the chest wall. The longest line that can be drawn within the breast image that is perpendicular to the chest wall and passes through the nipple bisects the breast to create our regions of interest. For the MLO view, the area above the bisecting line defines the superior region (MLO-S) and the area below the line defines the inferior region (MLO-I). For the CC view, the outer region of the breast is defined as the lateral region (CC-L), and the inner area is defined as the medial region (CC-M).

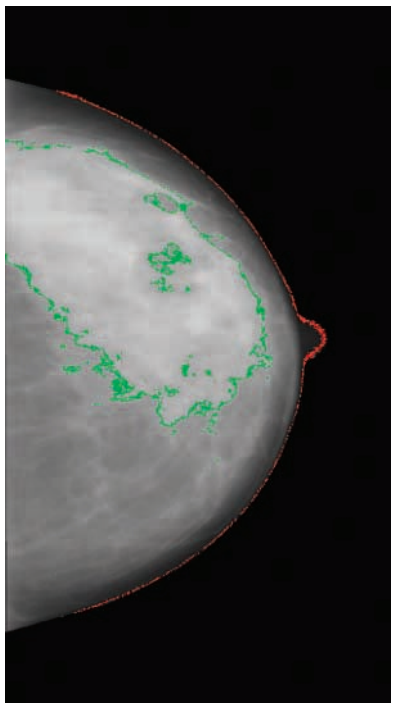

Figure 1. Computer-assisted estimation of breast density. *Red line,* first threshold separating breast from background; *green line,* second threshold separating dense from nondense tissue.

The thresholds and delineations for density of the entire breast using the Cumulus program were saved to a file (a standard feature in Cumulus). The regional density program used these variables to establish the locations of the total breast area, the dense and nondense tissue within the breast image. The regional algorithm counted the number of total breast pixels and dense pixels within each breast region. The proportion of dense tissue within each of the regions was then calculated.

Statistical Analyses. Summaries of the distributions of demographic and other variables are presented as means and SDs or counts and percentages. The baseline risk factors considered were BMI, menopausal status, family history of breast cancer, age at first birth, number of births, and HRT use.

Conditional logistic regression was used to calculate odds ratios (OR) with adjustment for potential confounding factors. Breast density measures were categorized based on quartiles of the control distributions and on modified categories previously published by Boyd et al. (22) for purposes of comparison (modified because we had very few women above 75% density). Associations were examined by laterality: ipsilateral or contralateral to the cancer (with the corresponding side used for controls) and by CC and MLO views. The C-statistic, or the area under the receiver operating characteristic curve from unconditional logistic regression models, was used to summarize the strength of the case-control prediction for the

various models. C-statistics reflect how often a model correctly identifies the case in a random case-control pair as having higher risk and range from of 0.5 (random chance) to 1.0 (perfect prediction). Differences in associations between breast density and breast cancer corresponding to different mammogram sides within a person were assessed using logistic regression models with Generalized Estimating Equations correcting for within-subject correlations (23-25).

To examine the association of regional density and breast cancer, we first performed a case-only analysis, describing the distributions of percentage density in the four regions as well as the location of greatest density by location of the eventual tumor. We next modeled the association of regional density with breast cancer using conditional logistic regression. We could not examine the proportion of density in the region of the eventual cancer with risk because it was highly correlated with overall percentage density ($r = 0.9$). To isolate the effects due to regional density from overall density, we subtracted regional percentage density from overall percentage density; negative values of this difference would indicate that regional density is larger in the area of the eventual cancer relative to the overall breast. This calculation was essentially equivalent to extracting the smallest principal component. To reduce any potential residual confounding, we also included overall percentage density in all models. Analyses were conducted within view (CC versus MLO) on the mammograms used for the primary analyses above. In addition, to explore whether 7 years is too early to detect an association between regional density and breast cancer, we examined mammograms taken closer to the time of diagnosis (on average 3 years before breast cancer or corresponding exam date). Similar to the above, the association of regional percentage density and breast cancer was examined adjusting for overall percentage density.

Analyses were also conducted among all breast cancers combined and for invasive breast cancers only.

Results

Table 1 presents descriptive information on the 372 cases and 713 controls. The matching algorithm was quite effective, as evidenced by the similarity of cases and controls with regard to the design variables. Case-control differences were apparent for BMI, parity, family history, and menopausal status at earliest mammogram. HRT at earliest mammogram was not available for all cases and controls; among those with information, the proportion that used HRT was higher among controls. The associations of breast cancer risk factors with percentage breast density were examined among controls before building multivariate models. Consistent with the literature (3, 26-28), average percentage density was lower for postmenopausal than premenopausal women [24.8% (SD, 14.1) versus 35.5% (SD, 13.7)] and never versus ever users of HRT [25.5% (SD, 14.8) versus 30.3% (SD, 13.9)]. Average percentage density was inversely associated with age [33.7% (SD, 14.1), 27.3% (SD, 15.9), 23.5% (SD, 13.2), and 21.8% (SD, 12.0) for women <53, 53-60, 61-69, and 20+ years] and positively associated with age at first birth [26.4% (SD, 14.1) for women with an age of first birth >20 years and 23.9% (SD, 15.6) for ≤20 years]. BMI was inversely associated with percentage density [35.5% (SD, 14.9), 27.8% (SD, 12.5), 23.9% (SD, 13.2), and 19.6% (SD, 12.6) for BMI <23.5, 23.5-26.1, 26.1-29.9, and 29.9+]. The associations of these risk factors with dense area were similar in direction, except BMI, which showed a positive association with dense area as seen in other studies to date (data not shown; ref. 29).

Table 2 presents estimated risk of breast cancer for quartiles of percentage density and dense area by ipsilateral and contralateral side and mammogram view (MLO versus CC) at the earliest mammogram. There were increasing trends in risk with increasing quartiles of percentage density and dense area, irrespective of the side (ipsilateral or contralateral) or view (CC or MLO) assessed (all $P_{trends} < 0.001$). The risk estimates were larger for percentage density than dense area and all trends were stronger when the analysis was restricted to invasive cancer (data not shown). When we used modified cut points based on Boyd et al. (22), we observed higher risk estimates than those based on quartiles, especially for women with percentage densities >50%; for the CC contralateral side, these estimated ORs were 3.1 [95% confidence interval (95% CI), 1.7-5.8], 4.8 (95% CI, 2.5-9.1), and 11.3 (95% CI, 5.0-25.9) for women with 10-24%, 25-49%, and 50%+ density, respectively, compared with women with <10% [1.00 (ref)].

The differences in the magnitude of associations of breast density (percentage density and dense area) with breast cancer for ipsilateral versus contralateral mammograms were almost negligible and not statistically significant (P values ranged from 0.43 to 0.68) and there were essentially no differences in C-statistics between these models (see Table 2).

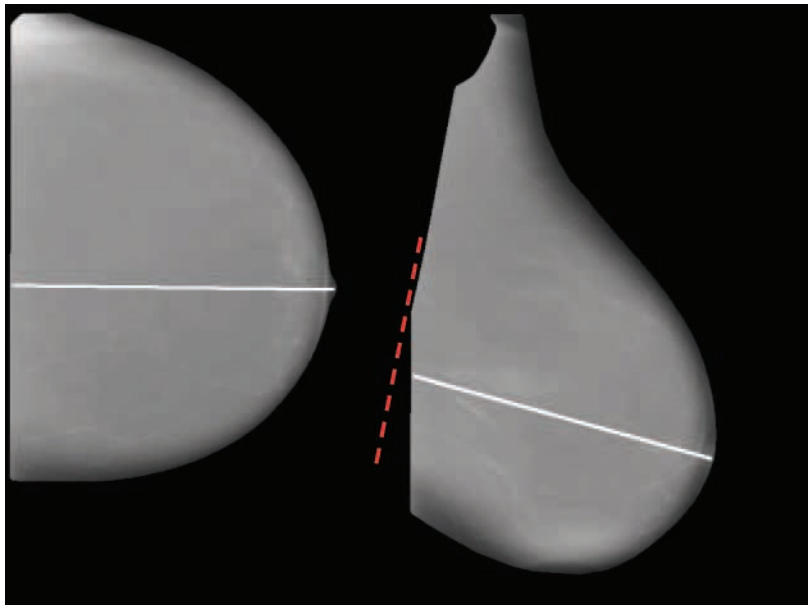

Figure 2. Regions of the breast as pictured on CC and MLO images used for estimation of regional percentage density.

Table 1. Description of matching variables and potential confounders by case-control status

Characteristic	Case, *n*	Case, mean (SD) or %	Control, *n*	Control, mean (SD) or %
Matching variables				
Age at earliest mammogram (y)	372	61.33 (10.36)	713	61.06 (10.03)
Interval between early and late mammograms (y)	372	7.05 (1.51)	713	6.99 (1.48)
No. screening mammograms	372	5.01 (1.45)	713	5.18 (1.79)
Residence (% Olmsted county)	183	49.59%	365	51.19%
Postmenopausal at exam date	363	98.11%	700	99.15%
Percentage density				
Ipsilateral side				
MLO view	371	28.35 (14.27)	709	24.50 (13.74)
CC view	372	30.53 (14.03)	708	26.61 (14.77)
Contralateral side				
MLO view	370	28.02 (14.18)	713	24.39 (13.53)
CC view	368	30.75 (14.38)	710	26.58 (14.59)
Confounding variables				
BMI (kg/m^2)	351	27.63 (5.03)	696	27.06 (5.08)
First-degree family history breast cancer				
No	314	84.41%	625	87.66%
Yes	58	15.59%	88	12.34%
Menopausal status at earliest mammogram				
Premenopausal	58	15.68%	124	17.56%
Postmenopausal	312	84.32%	582	82.44%
HRT at earliest mammogram				
Yes	77	20.7%	204	28.61%
No	256	68.82%	472	66.20%
Unknown	39	10.48%	37	5.19%
Age at fist birth/parity				
Nulliparous	44	11.30%	91	12.82%
AFB ≤20/1 or 2 children	19	5.11%	27	3.80%
AFB >20/1 or 2 children	122	32.80%	204	28.73%
AFB ≤20/3+ children	60	16.13%	118	16.62%
AFB >20/3+ children	127	34.14%	270	38.03%

Abbreviation: AFB, age at first birth.

The next set of analyses was designed to determine whether the region of the breast with the greatest proportion of dense tissue was the one where the tumor was most likely to develop. The CC view and the MLO view were evaluated separately. Among the 363 cases available for these analyses (9 cases had multiple tumors in more than one region), the greatest proportion of density was in the CC-L (versus CC-M) and the MLO-I (versus MLO-S) regions. However, there was no clear association between region with highest density and location of the tumor (Table 3).

At the earliest mammogram, the average percentage density in the region of the cancer was 32.4% and 27.9% for CC and

Table 2. Association of percentage and area density with breast cancer risk by side of cancer (ipsilateral versus contralateral) and mammogram view (MLO and CC)

Category	View	Quartiles	Cases, *n*	Cancer side, OR (95% CI)	Quartiles	Cases, *n*	Control side, OR (95% CI)
Percentage density (%)	CC	0<PD≤14.8	55	1.00 (Reference)	0<PD≤15.8	56	1.00 (Reference)
		14.8<PD≤25.7	83	1.71 (1.09-2.68)	15.8<PD≤25.8	90	1.76 (1.14-2.71)
		25.7<PD≤36.7	122	3.10 (2.00-4.82)	25.8<PD≤35.7	92	2.22 (1.41-3.49)
		36.7<PD	111	3.06 (1.88-4.98)	35.7<PD	129	3.66 (2.25-5.94)
C-statistic				0.65			0.65
	MLO	0<PD≤14.3	61	1.00 (Reference)	0<PD≤14.5	61	1.00 (Reference)
		14.3<PD≤23.5	85	1.54 (1.00-2.39)	14.5<PD≤24.0	95	1.58 (1.04-2.41)
		23.5<PD≤33.2	110	2.24 (1.45-3.44)	24.0<PD≤32.7	101	1.94 (1.27-2.96)
		33.2<PD	114	2.77 (1.74-4.42)	32.7<PD	112	2.47 (1.56-3.91)
C-statistic				0.64			0.64
Dense area (cm^2)	CC	0<DA≤17.9	52	1.00 (Reference)	0<DA≤18.2	45	1.00 (Reference)
		17.9<DA≤27.9	79	1.45 (0.93-2.28)	18.2<DA≤28.0	91	2.04 (1.30-3.20)
		27.9<DA≤39.8	104	2.17 (1.38-3.40)	28.0<DA≤41.3	115	2.66 (1.70-4.15)
		39.8<DA	126	2.45 (1.59-3.78)	41.3<DA	106	2.63 (1.64-4.23)
C-statistic				0.65			0.65
	MLO	0<DA≤19.9	57	1.00 (Reference)	0<DA≤19.3	48	1.00 (Reference)
		19.9<DA≤30.6	83	1.58 (1.02-2.45)	19.3<DA≤30.2	78	1.66 (1.02-2.49)
		30.6<DA≤42.7	90	1.57 (1.01-2.45)	30.2<DA≤43.7	116	2.40 (1.56-3.69)
		42.7<DA	130	2.43 (1.58-3.73)	43.7<DA	117	2.33 (1.50-3.60)
C-statistic				0.64			0.65

NOTE: Mammograms on average 7 years before breast cancer (or exam date). Analyses were adjusted for age, family history, menopausal status at mammogram, HRT at mammogram, BMI at mammogram, age at first birth, and number of births.
Abbreviations: PD, percentage density; DA, dense area.

Table 3. Location of tumor in the breast by location of highest percentage of density in the breast for cases (CC and MLO view)

Region where cancer occurred	Region with highest percentage density (*n* and %)		*P* value*
CC view	Medial	Lateral	
Medial	31 (8.5%)	92 (25.3%)	
Lateral	66 (18.2%)	174 (47.9%)	0.60
MLO	Inferior	Superior	
Inferior	65 (17.9%)	24 (6.6%)	
Superior	173 (47.7%)	101 (27.8%)	0.10

*P value from χ^2 test.

MLO views compared with 27.6% and 23.4% for the same regions among the matched controls. However, the results from the conditional logistic models showed that for both mammogram views, there was no trend in risk for increasing categories of cancer region density corrected for overall percentage density; this was also true for models with invasive cancers alone. After adjusting cancer-region densities for overall percentage density, their estimates of breast cancer risk were nearly all equal to 1.0, and when the overall percentage density and the regional percentage density were modeled simultaneously, the area under the receiver operating characteristic curve was not higher than for the models that did not include regional density (Tables 2 and 4).

We also performed analyses of regional density using the mammogram nearest to 3 years before the breast cancer. The average regional densities for cases and controls were similar to those from the earliest mammogram described above (30.3% and 27.6% for CC and MLO view for cases and 26.3% and 24.0% for controls). The associations between regional density and breast cancer were similar to what was observed for analyses based on the earlier mammograms (Table 4).

Discussion

This study contributes to the growing literature that mammographic breast density is an important risk factor for breast cancer (3, 4). Our results indicate strong associations of dense area and percentage density with breast cancer risk in a matched case-control study with mammograms obtained on average 7 years before diagnosis. Importantly, the subsequent risk of breast cancer was predicted equally well regardless of which breast was used for density estimation. Further, breast density in the region of the eventual cancer was not a significantly stronger risk factor than overall density; this was true for mammograms on average 7 and 3 years before diagnosis. Thus, overall breast density seems to represent a general marker of breast cancer susceptibility, regardless of side or location of density.

We attempted to address the question of whether breast density in the location of the eventual tumor enhanced risk prediction using two approaches. We first examined breast density from the ipsilateral versus contralateral mammograms with risk. Although the mammographic appearances of both breasts are relatively symmetric, the mammographic densities are not identical (27, 30). Our results showed that breast density predicted breast cancer similarly for the two sides. These findings are consistent with the only study known that assessed breast density from both mammogram sides in relation to risk (26). We next focused on the region of the breast where the cancer eventually developed. There was no association of percentage density in the region of the cancer with subsequent risk while correcting for the overall measure of percentage density in the breast. This correction enabled us to look at the contribution of density that was independent of overall percentage density and assured that the originally observed association between overall percentage density and risk was retained. Hypothesizing that 7 years might be too early in the disease process, we examined mammograms 3 years before diagnosis, but the results were unchanged.

These findings provide provocative evidence for overall breast density as a general predisposing marker for future breast cancer and not specifically in the breast with the cancer or at the location of the greatest density. Two other well-established markers for invasive breast cancer, including benign breast disease and lobular carcinoma *in situ*, also seem to confer a general susceptibility of risk to both breasts. A recent publication from the largest cohort of benign breast disease to date (n = 9,087) with a median 15-year follow-up showed that 45% of incident breast cancers occurred in the contralateral breast to the benign biopsy (31). Similarly, lobular carcinoma *in situ* is considered a strong risk marker of future breast cancer in both breasts (32, 33), and there is recent evidence that ductal

Table 4. Association of regional PD and overall PD with breast cancer risk at 3 and 7 years before breast cancer diagnosis

Time before diagnosis	PD measure	Cases, *n*	MLO, OR (95% CI)	C-statistics	Cases, *n*	CC, OR (95% CI)	C-statistics
3-yrs*	Regional PD†						
	Q1	85	1.00 (Reference)	0.62	90	1.00 (Reference)	0.64
	Q2	89	1.00 (0.67-1.48)		81	1.26 (0.82-1.95)	
	Q3	77	0.99 (0.64-1.52)		77	1.02 (0.65-1.62)	
	Q4	98	1.05 (0.68-1.63)		109	1.28 (0.81-2.03)	
	Overall PD						
	Q1	57	1.00 (Reference)		55	1.00 (Reference)	
	Q2	82	1.53 (0.96-2.44)		78	1.57 (0.99-2.47)	
	Q3	98	1.93 (1.22-3.06)		115	2.69 (1.68-4.31)	
	Q4	112	2.50 (1.54-4.08)		109	2.72 (1.66-4.43)	
7-yrs*	Regional PD†			0.64			0.65
	Q1	86	1.00 (Reference)		90	1.00 (Reference)	
	Q2	83	1.09 (0.73-1.63)		90	1.27 (0.83-1.95)	
	Q3	81	1.05 (0.69-1.61)		74	0.97 (0.62-1.54)	
	Q4	100	1.12 (0.72-1.74)		104	1.23 (0.78-1.95)	
	Overall PD						
	Q1	52	1.00 (Reference)		52	1.00 (Reference)	
	Q2	83	1.77 (1.10-2.85)		81	1.82 (1.12-2.96)	
	Q3	105	2.46 (1.52-3.97)		111	3.16 (1.96-5.09)	
	Q4	110	3.22 (1.93-5.37)		114	3.71 (2.22-6.20)	

NOTE: ORs (95% CIs) for quartiles of regional percentage density variable and overall percentage density.
*Analyses include both overall PD and regional PD in the same model.
† Regional PD is defined as the difference between overall PD and regional PD.

carcinoma *in situ* may also be associated with ipsilateral (OR, 1.9) and contralateral (OR, 2.0) breast cancer (34).

Breast density is associated with several established risk factors for breast cancer, including positive associations with HRT, nulliparity, late age at first birth, alcohol intake, and inverse associations with age, postmenopausal status, and BMI (26, 30, 35-40). These risk factors account for only 20% to 30% of variation in breast density (26, 28, 29) and there is evidence that genetics and their interaction with environment likely account for the remaining variation (37, 41). Genes for breast density may predispose women to high densities in both breasts, translating to increased breast cancer risk.

In addition, mammographic density has been hypothesized to parallel age-related involution in the breast, which involves the reduction in size and number of acini per lobule as well as the replacement of the extralobular stroma by fat (42-45). Both mammographic density and involution are influenced by reproductive factors and change with age, with the greatest changes after menopause. Recent data from a large benign breast disease cohort study (31) suggest that involution as assessed on biopsy specimen is associated with breast cancer risk reduction (46). Involution status at the site of the benign biopsy was associated with a reduction of both ipsilateral and contralateral events. Thus, one could speculate that breast densities mirror involution of the breast and both represent a general marker of breast cancer risk.

Our negative findings of an association between cancer-region densities with subsequent breast cancer risk are in apparent contrast with the only other published study that has examined density in the location of the cancer. Ursin et al. (19) examined a small series of ductal carcinoma *in situ* patients and found 21 of 22 ductal carcinoma *in situ* examined occurred in dense areas of the breast and 61% of these lesions also occurred in the upper-outer quadrant, which also corresponded to the highest density. Although the reason for the discrepant results is not immediately obvious, it may relate in part to differences in timing of the mammograms evaluated (which were at the time or immediately preceding the cancer in the Ursin study versus either an average of 3 or 7 years in ours), the focus: invasive versus *in situ* lesions, or the inclusion of a control group. Unlike the study by Ursin et al. (19), our study was able to compare the cancer-region density with that in the corresponding region of controls.

Both studies, however, suffer from several limitations, including the use of a crude four-quadrant approach for examining locations of percentage density and using a two-dimensional film to address volume. The use of magnetic resonance imaging or other imaging modalities may provide more precision to the location of the densities (and the tumor) than film mammography. In addition, densities can be diffuse or focal in nature, and an overall estimate of regional percentage density would not capture the distribution of densities in the region that may be important to the development of breast cancer. The capability to coregister the exact location of the tumor on serial mammograms would greatly improve the precision with which this question could be answered.

Prior studies have suggested that the association of breast density with subsequent breast cancer may in part be due to bias wherein increased density masks subsequently detected breast cancer (47). Because we did not see an association between cancer-region density and subsequent risk, this would imply that other areas in the breast are also prone to developing breast cancer. As such, we provide suggestive evidence that masking bias is not responsible for the breast density and breast cancer association. If masking bias was at play, we would also have expected a stronger association with regional density closer to diagnosis.

Our case-control study had several strengths, including the close matching on pertinent factors, high participation rates due to the retrospective design, mammograms from at least 5 years before breast cancer for >90% of cases and controls, medical record–abstracted weight, height, and risk factor data on all cases and controls generally at time of mammogram, and importantly, the quantitative assessment of density. Further, this is the first study to examine the location of density with breast cancer risk and comprehensively examine density in the breast that becomes cancerous. Limitations include the minimal ethnic diversity of this Midwest population and the clinic-based design. Although this was not a population-based study, by restricting to the 120-mile radius and requiring serial mammograms on all women, the study population was closer to a community-based population rather than a referral or high-risk population.

In conclusion, percentage density and dense area are both measures of breast density that are strongly associated with breast cancer risk. The association is not limited to mammograms from the ipsilateral breast or to the regions that develop the breast cancer. We therefore conclude that density is a marker of risk for both breasts and continued research to understand the biology of breast density is warranted.

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