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ARTICLE

# Independent Association of Lobular Involution and Mammographic Breast Density With Breast Cancer Risk

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**Background** Lobular involution, or age-related atrophy of breast lobules, is inversely associated with breast cancer risk, and mammographic breast density (MBD) is positively associated with breast cancer risk.

**Methods** To evaluate whether lobular involution and MBD are independently associated with breast cancer risk in women with benign breast disease, we performed a nested cohort study among women (n = 2666) with benign breast disease diagnosed at Mayo Clinic between January 1, 1985, and December 31, 1991 and a mammogram available within 6 months of the diagnosis. Women were followed up for an average of 13.3 years to document any breast cancer incidence. Lobular involution was categorized as none, partial, or complete; parenchymal pattern was classified using the Wolfe classification as N1 (nondense), P1, P2 (ductal prominence occupying <25%, or >25% of the breast, respectively), or DY (extremely dense). Hazard ratios (HRs) and 95% confidence intervals (CIs) to assess associations of lobular involution and MBD with breast cancer risk were estimated using adjusted Cox proportional hazards model. All tests of statistical significance were two-sided.

**Results** After adjustment for MBD, having no or partial lobular involution was associated with a higher risk of breast cancer than having complete involution (none: HR of breast cancer incidence = 2.62, 95% CI = 1.39 to 4.94; partial: HR of breast cancer incidence = 1.61, 95% CI = 1.03 to 2.53; $P_{trend}$ = .002). Similarly, after adjustment for involution, having dense breasts was associated with higher risk of breast cancer than having nondense breasts (for DY: HR of breast cancer incidence = 1.67, 95% CI = 1.03 to 2.73; for P2: HR of breast cancer incidence = 1.96, 95% CI = 1.20 to 3.21; for P1: HR of breast cancer incidence = 1.23, 95% CI = 0.67 to 2.26; $P_{trend}$ = .02). Having a combination of no involution and dense breasts was associated with higher risk of breast cancer than having complete involution and nondense breasts (HR of breast cancer incidence = 4.08, 95% CI = 1.72 to 9.68; $P$ = .006).

**Conclusion** Lobular involution and MBD are independently associated with breast cancer incidence; combined, they are associated with an even greater risk for breast cancer.

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Women are diagnosed with benign breast disease (BBD) when a biopsy of a palpable or a radiological abnormality of the breast reveals benign findings (1). BBD is a common diagnosis given that three to four benign breast biopsies are performed for every breast cancer diagnosed in the United States (2). Women with BBD have an increased risk of future breast cancer that can occur in either breast (3). To counsel women at elevated risk regarding options for breast cancer screening and risk reduction strategies, it is necessary to provide them an estimate of their individual breast cancer risk. To accurately predict a woman's risk of breast cancer, multidisciplinary research including clinical, imaging, and tissue studies is needed to identify factors that contribute to breast cancer risk. In addition to multiple known clinical risk factors for breast cancer such as age, age at menarche, and family history, the two factors that are shown to be strongly associated with breast cancer risk are tissue-based assessment of lobular involution and a radiological marker of mammographic breast density (MBD) (4–7). We also recently reported an inverse association between lobular involution and MBD, leading to the question whether these two factors were independently associated with breast cancer risk (8).

The human breast glandular tissue has 15–20 lobes, each with many lobules that form the anatomical and functional units of the breast tissue. The lobules in turn, contain multiple acini that are the secretory structures of the breast (9). The lobules are surrounded by stroma and fat that vary in amount based on age and lactational status. As women age, the lobules undergo regression or involution (10). Age-related lobular involution has been described as physiological atrophy of the breast, with a decrease in the number and size

of acini per lobule and replacement of the delicate intralobular stroma with dense collagen and fatty tissue (10). In a study of lobular involution among women with BBD, progressive degrees of lobular involution were associated with reduced breast cancer risk (4). However, the mechanism underlying the association between lobular involution and breast cancer risk is yet undefined.

MBD, which represents the proportion of tissues that appear white or dense on a mammogram, is a strong and consistent risk factor for breast cancer (5–7). MBD decreases as women age (11). It has been hypothesized that the association between mammographic density and increased breast cancer risk may be related to reduced lobular involution of breast tissue in dense breasts (5,12,13). In addition to age, MBD is associated with a variety of factors including body mass index (BMI), family history, and postmenopausal hormone (PMH) use (5,14).

Although lobular involution and MBD are both associated with breast cancer risk, it is not known whether they represent independent risk factors for breast cancer. The extent of association of MBD and lobular involution with breast cancer risk can contribute to efforts to improve breast cancer risk prediction and may provide further insight into the biological mechanisms underlying the development of breast cancer. In this report, we examined the associations of lobular involution and MBD with breast cancer risk in a large well-characterized cohort of women with BBD with long-term follow-up for breast cancer events.

## CONTEXT AND CAVEATS

**Prior knowledge**

The milk-forming lobules in human breasts undergo natural involution or regression with age. The lobules are believed to be the anatomic sites from which breast cancer originates. Increased lobular involution is associated with reduced breast cancer risk. Increased mammographic breast density is associated with increased breast cancer risk and may be related to less involution.

**Study design**

The study examined whether lobular involution and mammographic breast density, independently and combined, were associated with breast cancer risk in a cohort of women whose breast biopsy revealed benign findings and were followed up for an average of 13.3 years to document any breast cancer incidence.

**Contribution**

Lobular involution and mammographic breast density were independently associated with breast cancer risk. Extremely dense breasts and no lobular involution were associated with the highest risk of breast cancer compared with nondense breasts and complete lobular involution.

**Implications**

A combination of the two independent risk factors, lobular involution, and mammographic breast density, may be used in developing breast cancer risk prediction models to estimate a woman's risk of breast cancer.

**Limitations**

The different categories of lobular involution and mammographic breast density used in this study were subjective measures.

*From the Editors*

## Participants and Methods

### Study Setting and Population

The Mayo Benign Breast Disease cohort included 9376 women, aged 18–85 years, with no history of breast cancer, who were diagnosed with BBD at Mayo Clinic between January 1, 1967, and December 31, 1991 (1). A woman was diagnosed with BBD when she had a biopsy (surgical excision) of a palpable or radiographic abnormality in the breast that revealed benign findings. The extent of lobular involution was assessed in this benign breast tissue (described below). We studied a subcohort of women (n = 2666), nested in the Mayo BBD cohort, who were diagnosed with BBD between January 1, 1985, and December 31, 1991 (an era during which MBD was clinically assessed and recorded as the parenchymal pattern), and had a mammogram within 6 months of the BBD diagnosis. Breast cancer events were assessed through the comprehensive Mayo medical records (inpatient and outpatient) and a study-specific questionnaire (previously described) (1). Women who subsequently developed breast cancer in this subcohort were considered case subjects.

All study protocol procedures and patient contact materials were reviewed and approved by the Institutional Review Board of Mayo Clinic.

### Assessment of Lobular Involution

To assess the extent of lobular involution, the study pathologists (D. W. Visscher and C. Reynolds) performed histopathological review of hematoxylin- and eosin-stained slides of breast tissue of all study participants, without knowledge of the original histology report or MBD pattern. This measure was assessed in the normal lobules on the slide containing the benign breast finding on the hematoxylin- and eosin-stained slide. The extent of lobular involution was classified into three categories as none (0% lobules involuted), partial (1%–74% lobules involuted), or complete (≥75% lobules involuted) (6). In addition, the histological type of BBD was categorized as nonproliferative disease, proliferative disease without atypia, and atypical hyperplasia (1).

### Assessment of MBD

MBD was measured clinically from all four views of the mammogram—the craniocaudal and mediolateral oblique views of the right and left breasts. Wolfe's parenchymal pattern was used to classify MBD into four parenchymal patterns based on the extent and type of density: N1—nondense, no ducts visible; P1—ductal prominence occupying less than a fourth of the breast; P2—prominent ductal pattern occupying more than a fourth of the breast; DY—homogenous plaque-like areas of extreme density (15). This measure of MBD has high inter-reader agreement (16,17) and has been used in multiple previous studies and consistently shown to be associated with breast cancer risk (18–28). This clinically obtained measure was documented by the radiologists and retained at the Mayo Clinic, starting in 1985. The data extracted from these reports were analyzed for the current study.

### Ascertainment of Risk Factor Information

Information on the demographic and clinical variables including age, parity, family history of breast cancer, BMI, height, menopausal

status, and PMH therapy use was obtained from the Mayo medical record and questionnaires mailed to study participants or proxy, that is, next of kin of deceased patients (1). Family history of breast cancer was categorized as follows: strong—at least one first-degree relative with breast cancer before age 50 years, or two or more relatives with breast cancer with at least one being a first-degree relative; weak—any other family history of breast cancer; or none—no family history of breast cancer (1). Additional information regarding menopausal status was obtained from a clinical mammography database maintained at Mayo Clinic, a database of self-reported information gathered from patients at each mammogram visit.

### Statistical Analysis

***Descriptive Statistics and Associations With Demographic and Clinical Variables.*** For a quantitative summary of the data, we used frequencies and percentages for categorical variables, and means and standard deviation for continuous variables. To compare distributions of demographic and clinical variables across different categories of lobular involution and parenchymal pattern of MBD, we initially used $\chi^2$ tests of statistical significance. All variables that were univariately statistically significant were then included in a multivariable logistic regression model to assess the independent effects of these demographic and clinical variables. Separate logistic models were fit for lobular involution and for MBD parenchymal pattern.

***Associations of Lobular Involution and MBD With Breast Cancer Risk.*** The duration of follow-up was calculated as the number of days from benign biopsy to the date of the diagnosis of breast cancer, death, or last contact. In addition, for women who had undergone a prophylactic mastectomy or who had a diagnosis of lobular carcinoma in situ, follow-up was censored at the corresponding surgical or diagnosis date. The primary variables of interest included the two exposure variables— lobular involution (none, partial, and complete) and MBD parenchymal pattern (N1, P1, P2, and DY). The following established breast cancer risk factors that are measured to correspond to each woman's status at the time of the benign biopsy were examined as potential confounders: age (categorized as <45, 45–54, and ≥ 55 years) that was modeled as a categorical variable when directly assessing its association with breast cancer risk and as a continuous variable based on the original noninteger values when adjusting for its effect in other models; histological impression (categorized as nonproliferative disease, proliferative disease without atypia, and atypical hyperplasia); parity (categorized as nulliparous and parous); menopausal status (categorized as premenopausal and postmenopausal); PMH use (categorized as never use and ever use); family history of breast cancer (categorized as none, weak, and strong); and BMI (categorized into approximate quartiles). Crude incidence rates were calculated by dividing the number of observed events by the total number of person-years of observation, and 95% confidence intervals (CIs) for these rates were calculated based on the normal approximation to the Poisson distribution.

To assess associations between lobular involution, MBD, and potential confounding variables with breast cancer risk, we calculated hazard ratios (HRs) and corresponding 95% confidence intervals using Cox proportional hazards regression models, with time from the benign biopsy to breast cancer or last follow-up serving as the time scale. We formally evaluated the Cox regression proportional hazards assumptions for lobular involution and MBD hazard ratios by fitting and testing the corresponding exposure × time interaction terms and found no evidence of model violations ($P > .40$ for each exposure variable). We used Wald $\chi^2$ tests to examine the dose–response effects of lobular involution and MBD with breast cancer risk by ordering the categories from lowest (complete involution and N1) to highest (no involution and DY) hypothesized risk and including this ordered variable as a 1 *df* linear term in the Cox regression model. We first assessed associations with breast cancer risk after adjustment only for age because age is the strongest breast cancer risk factor for women in our cohort. Then, we simultaneously included all confounding variables in a multivariable Cox regression model. Finally, to examine the independent association of these two variables with breast cancer risk, in addition to adjusting for the risk factors listed above, we adjusted for MBD when assessing association for lobular involution and adjusted for lobular involution when assessing association for MBD.

To evaluate the potential modifying effects that lobular involution and MBD may have on each other's association with breast cancer risk, we fitted and tested a lobular involution × MBD interaction term using the Cox regression model. We also examined the differences in breast cancer risk associated with combinations of specific categories of lobular involution and MBD. For these analyses, we recategorized MBD as a two-level variable: low density (N1 and P1), and high density (P2 and DY). When combined with the three-level measure of lobular involution, this resulted in six possible combinations of lobular involution and MBD. Because no inherent ordering of these joint lobular involution and MBD categories from lowest to highest breast cancer risk could be assigned to the six combination values, statistical comparisons were made for five of these paired categories (complete involution and P2 or DY; partial involution and N1 or P1; partial involution and P2 or DY; no involution and N1 or P1 and; no involution and P2 or DY); the combination of complete lobular involution with N1 or P1 parenchymal pattern of MBD serving as the reference. Statistical significance was assessed for all five of these comparisons simultaneously using a single *P* value that did not impose any ordering among the estimated hazard ratios.

All statistical tests were two-sided. *P* values less than .05 were considered statistically significant, and all analyses were carried out using the SAS (SAS Institute, Inc, Cary, NC) software system.

## Results

### Patient Characteristics of the Nested Cohort Study

Between January 1, 1985, and December 31, 1991, 3271 women in the Mayo BBD cohort were diagnosed with BBD. A measure of MBD was available within 6 months of BBD diagnosis for 2666 women (82%) who formed the study cohort. There were no statistically significant differences between the study participants (n = 2666) and those not included because of lack of mammogram availability (n = 605), based on age, breast cancer case status, parity, BMI, or family history (data not shown). After a mean duration of 13.3 years (standard deviation = 4.7) of follow-up, 172 of 2666 women

(6.5%) subsequently developed breast cancer (case subjects). Table 1 shows characteristics of the study cohort. The age distribution of the cohort showed that the groups of women aged less than 45 years or 45–54 years, each composed approximately one-fourth of the cohort, whereas women 55 years or older composed 48.9% of the cohort. The mean age at biopsy was 54.7 years (95% CI = 54.2 to 55.2 years). A statistically significantly increased risk of breast cancer was noted in women who were 55 years or older (HR = 2.07, 95% CI = 1.37 to 3.14, $P < .001$), who had atypical hyperplasia on biopsy (HR = 3.69, 95% CI = 2.30 to 5.91, $P < .001$), and who had strong family history for breast cancer (HR = 1.80, 95% CI = 1.24 to 2.61, $P = .004$). There were no statistically significant differences across case status with regard to parity ($P = .23$), BMI ($P = .91$), PMH use ($P = .44$), and menopause status ($P = .61$).

The distribution of patients by level of lobular involution and parenchymal patterns of MBD is shown in Table 2. Overall, 641 of 2666 women (24%) showed complete involution, 359 of 2666 women (13.5%) showed no involution, and 1666 of 2666 women (62.5%) showed partial involution. There was a large proportion (65%) of women with dense breasts (P2 and DY) in the cohort. For women with no involution, the majority (60.7%) had extremely dense (DY) pattern of mammographic density. However, among women with complete involution, the parenchymal patterns were almost equally represented in all four MBD categories (27.1%, 19.7%, 27.5%, and 25.7%, for N1, P1, P2, and DY, respectively).

We also compared the distributions of demographic and clinical variables across levels of lobular involution and parenchymal pattern of MBD. In univariate analyses, the following factors were associated with greater extent of lobular involution—older

**Table 1.** Association of demographic and clinical variables with risk of breast cancer*

| Characteristic | No. of women (n = 2666), No. (%) | No. of women with breast cancer (n = 172)† | No. of person-years of follow-up | HR (95% CI)‡ | $P_{trend}$§ |
|---|---|---|---|---|---|
| Age at BBD diagnosis, y | | | | | <.001 |
| <45 | 696 (26.1) | 29 | 9667 | 1.00‖ | |
| 45–54 | 667 (25.0) | 45 | 9507 | 1.58 (0.99 to 2.52) | |
| ≥55 | 1303 (48.9) | 98 | 16198 | 2.07 (1.37 to 3.14) | |
| Histology | | | | | <.001 |
| Nonproliferative disease | 1556 (58.4) | 64 | 20859 | 1.00‖ | |
| Proliferative disease without atypia | 954 (35.8) | 84 | 12524 | 2.09 (1.51 to 2.89) | |
| Atypical Hyperplasia | 156 (5.9) | 24 | 1990 | 3.69 (2.30 to 5.91) | |
| Parity | | | | | .23 |
| Nulliparous | 358 (14.6) | 27 | 4579 | 1.00‖ | |
| Parous | 2087 (85.4) | 134 | 27899 | 0.75 (0.50 to 1.14) | |
| Missing¶ | 221 | 11 | | | |
| Body mass index, kg/m$^2$ | | | | | .91 |
| 15–22 | 498 (25.2) | 32 | 7396 | 1.00‖ | |
| 23–25 | 506 (25.6) | 32 | 7644 | 0.90 (0.55 to 1.47) | |
| 26–29 | 523 (26.5) | 38 | 7840 | 1.00 (0.62 to 1.61) | |
| 30–66 | 449 (22.7) | 29 | 6635 | 0.92 (0.55 to 1.52) | |
| Missing¶ | 690 | 41 | | | |
| Postmenopausal Hormone Use | | | | | .44 |
| Never | 947 (44.0) | 54 | 13214 | 1.00‖ | |
| Ever | 1205 (56.0) | 85 | 17740 | 1.15 (0.82 to 1.61) | |
| Missing¶ | 514 | 33 | | | |
| Menopause status | | | | | .61 |
| Premenopausal | 796 (30.7) | 37 | 11065 | 1.00‖ | |
| Postmenopausal | 1796 (69.3) | 128 | 23660 | 1.12 (0.69 to 1.81) | |
| Missing¶ | 74 | 7 | | | |
| Family history of breast cancer# | | | | | .004 |
| None | 1542 (64.3) | 93 | 20901 | 1.00‖ | |
| Weak | 509 (21.2) | 36 | 7349 | 1.15 (0.78 to 1.70) | |
| Strong | 349 (14.5) | 39 | 4912 | 1.80 (1.24 to 2.61) | |
| Missing¶ | 266 | 4 | | | |

* Nested cohort study of women with benign breast disease (BBD) diagnosed at the Mayo Clinic between January 1, 1985, and December 31, 1991. CI = confidence interval; HR = hazard ratio.

† After a mean follow-up of 13.3 years, 172 of 2666 women developed breast cancer.

‡ Analysis of breast cancer risk was done using the Cox proportional hazards model. Association of risk with age at BBD diagnosis was unadjusted; all other associations with risk are adjusted for age.

§ $P_{trend}$ values were calculated using two-sided Wald test for trend.

‖ Referent category in the Cox proportional hazards model.

¶ Subjects for whom information for that demographic or clinical variable was not available from questionnaires or medical records.

# Strong—at least one first-degree relative with breast cancer before age 50 years, or two or more relatives with breast cancer with at least one being a first-degree relative; weak—any other family of breast cancer; None—no family history of breast cancer.

**Table 2.** Overall distribution of lobular involution and parenchymal pattern of mammographic breast density*

| Wolfe parenchymal pattern† | Extent of lobular involution‡ | | | |
|---|---|---|---|---|
| | None,‡ No. (%) | Partial,‡ No. (%) | Complete,‡ No. (%) | Total, No. (%) |
| Overall | 359 (13.5) | 1666 (62.5) | 641 (24.0) | 2666 (100) |
| N1 | 56 (15.6) | 325 (19.5) | 174 (27.1) | 555 (20.8) |
| P1 | 31 (8.6) | 221 (13.3) | 126 (19.7) | 378 (14.2) |
| P2 | 54 (15.0) | 412 (24.7) | 176 (27.5) | 642 (24.1) |
| DY | 218 (60.7) | 708 (42.5) | 165 (25.7) | 1091 (40.9) |

* Nested cohort study of women with benign breast disease diagnosed at the Mayo Clinic between January 1, 1985, and December 31, 1991.

† Wolfe parenchymal pattern: N1—nondense, no ducts visible; P1—ductal prominence occupying less than a fourth of the breast; P2—prominent ductal pattern occupying more than a fourth of the breast; DY—homogenous plaque-like areas of extreme density.

‡ Extent of lobular involution was classified as none (0% lobules involuted), partial (1%–74% lobules involuted), and complete (≥75% lobules involuted).

age (≥55 years), nonproliferative histology, nulliparity, increased BMI, no PMH use, postmenopausal status, and no family history. The following factors were associated with less dense breast tissue—older age (≥55 years), nonproliferative histology, parity, increased BMI, no PMH use, postmenopausal status, and no family history. After including these variables in multivariable logistic regression analyses, BMI and PMH use were no longer associated with lobular involution, and menopausal status and family history were no longer associated with parenchymal pattern. All other variables remained statistically significant ($P < .05$) (data not shown) and were included in the models examining lobular involution, MBD, and the risk of breast cancer.

### Lobular Involution, MBD, and Breast Cancer Risk

Next we examined the association of lobular involution and parenchymal pattern of MBD with breast cancer risk in women diagnosed with BBD (Table 3). Age-adjusted analyses showed statistically significant associations of lobular involution ($P < .001$) and MBD ($P = .002$) with breast cancer risk. Adjusting for confounders (age, BBD histology, parity, BMI, menopausal status, and family history) in multivariable analyses revealed slightly attenuated, though still statistically significant, associations of lobular involution ($P = .002$) and MBD ($P = .018$) with risk. Additional adjustment for MBD did not change the association between lobular involution and breast cancer risk ($P = .002$). Women with no or partial lobular involution showed statistically significantly increased risk of breast cancer compared with women with complete involution (none: HR = 2.62, 95% CI = 1.39 to 4.94; partial: HR = 1.61, 95% CI = 1.03 to 2.53; $P_{trend} = .002$), using the complete involution category as the referent. Similarly, additional adjustment for lobular involution did not change the association between MBD and breast cancer risk. Women with dense breasts showed

**Table 3.** Age-adjusted and multivariable-adjusted risk of breast cancer by levels of lobular involution and parenchymal pattern of mammographic breast density*

| Characteristic | No. of women (%) | No. of women with breast cancer | No. of person-years of follow-up | Age adjusted† | | Multivariable adjusted§ | | Multivariable adjusted‖ | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | HR (95% CI) | $P_{trend}$‡ | HR (95% CI) | $P_{trend}$‡ | HR (95% CI) | $P_{trend}$‡ |
| Involution¶ | | | | | <.001 | | .002 | | .002 |
| Complete | 641 (24.0) | 28 | 7975 | 1.00# | | 1.00# | | 1.00# | |
| Partial | 1666 (62.5) | 120 | 22503 | 2.10 (1.37 to 3.23) | | 1.62 (1.03 to 2.53) | | 1.61 (1.03 to 2.53) | |
| None | 359 (13.5) | 24 | 4895 | 2.96 (1.59 to 5.51) | | 2.62 (1.40 to 4.92) | | 2.62 (1.39 to 4.94) | |
| Parenchymal pattern** | | | | | .002 | | .018 | | .022 |
| N1 | 555 (20.8) | 23 | 7255 | 1.00# | | 1.00# | | 1.00# | |
| P1 | 378 (14.2) | 19 | 4779 | 1.19 (0.65 to 2.18) | | 1.22 (0.66 to 2.24) | | 1.23 (0.67 to 2.26) | |
| P2 | 642 (24.1) | 56 | 8580 | 2.08 (1.28 to 3.37) | | 1.96 (1.20 to 3.21) | | 1.96 (1.20 to 3.21) | |
| DY | 1091 (40.9) | 74 | 14758 | 1.92 (1.19 to 3.10) | | 1.70 (1.05 to 2.77) | | 1.67 (1.03 to 2.73) | |

* Nested cohort study of women with benign breast disease (BBD) diagnosed between January 1, 1985, and December 31, 1991 at the Mayo Clinic. All analyses were done using the Cox proportional hazards model. CI = confidence interval; HR = hazard ratio.

† Age-adjusted univariate analysis.

‡ $P_{trend}$ values were calculated using two-sided Wald test for trend.

§ Multivariable analysis adjusting for age, BBD histology, body mass index (BMI), parity, menopause status, and family history.

‖ Multivariable analysis adjusting for age, BBD histology, BMI, parity, menopause status and family history, and parenchymal pattern (for lobular involution) or lobular involution (for parenchymal pattern).

¶ Extent of lobular involution was classified as none (0% lobules involuted), partial (1%–74% lobules involuted), and complete (≥75% lobules involuted).

# Referent category in the Cox proportional hazards model.

** Wolfe parenchymal pattern: N1—nondense, no ducts visible; P1—ductal prominence occupying less than a fourth of the breast; P2—prominent ductal pattern occupying more than a fourth of the breast; DY—homogenous, plaque-like areas of extreme density.

statistically significantly increased risk of breast cancer compared with women with nondense breasts (for DY: HR = 1.67, 95% CI = 1.03 to 2.73; for P2: HR = 1.96, 95% CI = 1.20 to 3.21; for P1: HR = 1.23, 95% CI = 0.67 to 2.26; $P_{trend}$ = .02), using the N1 category as the referent.

Furthermore, we examined the effect of combination of lobular involution and MBD categories on breast cancer risk (Table 4). Age-adjusted analysis showed statistically significantly increased risk of breast cancer in women with no lobular involution and dense breasts (P2, DY) compared with women with complete involution and nondense breasts as the referent (HR = 5.14, 95% CI = 2.18 to 12.1). Adjusting for confounders (age, BBD histology, parity, BMI, menopause status, and family history) slightly attenuated the risk association (HR = 4.08, 95% CI = 1.72 to 9.68). We also found that when looking within each category of MBD, women with no involution were at higher risk compared with those with complete involution (Table 4). Similarly, when we look within each category of involution, women with dense breasts were at higher risk compared with women with nondense breasts (Table 4). We found no evidence of effect modification between lobular involution and BBD (test for interaction, $P$ = .60), consistent with these risk factors operating independently on risk of breast cancer.

## Discussion

In this study, we investigated the independent contributions of lobular involution and MBD to breast cancer risk in a cohort of 2666 women with BBD, followed for a mean of 13.3 years. This study, to our knowledge, is the first to show that lobular involution and MBD are independently associated with breast cancer risk. Our findings also reveal that having a combination of dense breasts and no lobular involution was associated with higher breast cancer risk than having nondense or fatty breasts and complete involution.

Previously, our research team showed that progressive lobular involution was statistically significantly associated with reduced breast cancer risk (4). The association was present even within populations of women at high risk such as those at older age, with atypical hyperplasia, or strong family history of breast cancer. The decrease in risk with complete involution may simply reflect fewer numbers of epithelial cells at risk for malignant transformation, or shorter exposure of epithelial cells to carcinogenic influences (4). Further studies are needed to clarify the biological mechanisms underlying lobular involution and its contribution to breast cancer risk.

MBD is a strong risk factor for breast cancer and women with dense tissue occupying more than 60%–75% of the breast have a four- to six-fold increased risk of breast cancer compared with those who have little or no density (5,6). The few histological studies of MBD reported so far have suggested that both breast epithelium and stroma may contribute to MBD (29,30). The current study found that the majority (approximately 76%) of women with no involution of breast tissue had dense breasts (P2, DY pattern). However, for women with complete lobular involution, there were fairly similar proportions of women in each of the MBD categories. One possible explanation is that as lobular involution occurs, the atrophic breast glandular epithelium is initially replaced by stroma and later by fatty tissue. Hence, complete involution with dense tissue on mammography may indicate that although the epithelium is atrophic, the dense tissue reflects the stromal contribution to MBD.

The current analysis shows that women with greatest risk for breast cancer were those with no lobular involution and mammographically dense breasts (incidence rate = 507 per 100 000 person-years, 95% CI = 279 to 736) compared with women with complete

**Table 4.** Risk of breast cancer for combinations of lobular involution and parenchymal pattern of mammographic breast density*

| Combination of lobular involution† and parenchymal pattern‡ | No. of women | No. of women with breast cancer | No. of person-years of follow-up | Incidence rate§ (95% CI) | Age-adjusted,‖ HR (95% CI) | P¶ | Multivariable adjusted,# HR (95% CI) | P¶ |
|---|---|---|---|---|---|---|---|---|
| Complete† and N1 or P1‡ | 300 | 9 | 3644.46 | 247 (86 to 408) | 1.00** | <.001 | 1.00** | .006 |
| Complete† and P2 or DY‡ | 341 | 19 | 4330.29 | 439 (242 to 636) | 1.85 (0.84 to 4.10) | | 1.66 (0.75 to 3.70) | |
| Partial† and N1 or P1‡ | 546 | 28 | 7241.11 | 387 (244 to 530) | 2.03 (0.95 to 4.31) | | 1.57 (0.73 to 3.36) | |
| Partial† and P2 or DY‡ | 1120 | 92 | 15261.88 | 603 (480 to 726) | 3.88 (1.92 to 7.83) | | 2.70 (1.32 to 5.53) | |
| None† and N1 or P1‡ | 87 | 5 | 1149.02 | 435 (54 to 817) | 3.77 (1.22 to 11.6) | | 3.24 (1.05 to 9.98) | |
| None† and P2 or DY‡ | 272 | 19 | 3745.81 | 507 (279 to 736) | 5.14 (2.18 to 12.1) | | 4.08 (1.72 to 9.68) | |

* Nested cohort study of women with benign breast disease (BBD) diagnosed at the Mayo Clinic between January 1, 1985, and December 31, 1991. HR = hazard ratio; CI = confidence interval.

† Extent of Lobular involution was classified as none (0% lobules involuted), partial (1 to 74% lobules involuted), or complete (≥75% lobules involuted).

‡ Wolfe parenchymal pattern: N1—nondense, no ducts visible; P1—ductal prominence occupying less than a fourth of the breast; P2—prominent ductal pattern occupying more than a fourth of the breast; DY—homogenous plaque-like areas of extreme density.

§ Crude incidence rates per 100 000 person-years. 95% confidence intervals calculated based on large sample normal approximation to the Poisson distribution.

‖ Age-adjusted univariate analysis; analysis was done using the Cox proportional hazards model.

¶ *P* values were calculated using two-sided Wald test with 5 df.

# Multivariable analysis adjusting for age, BBD histology, body mass index, parity, menopause status, and family history.

** Referent category in the Cox proportional hazards model.

involution and mammographically nondense breasts (incidence rate = 247 per 100 000 person-years, 95% CI = 86 to 408). Lack of lobular involution suggests an epithelial-rich environment (4). On the other hand, mammographically dense tissue, as noted earlier, has been thought to represent both epithelial and stromal components (29,30). It could be hypothesized that the stromal-rich environment in mammographically dense breasts results in a preponderance of growth factors that may stimulate the epithelium in a non-involuted breast, setting the stage for malignant transformation (31). In fact, several studies have shown correlation between tissue-based or circulating growth factors and MBD (32–34). Further tissue-based studies with inclusion of stromal markers are needed to clarify the risk mechanisms.

Our study findings have the potential to translate to improvements in breast cancer risk prediction. Knowledge of a woman's breast cancer risk is an important component of her decision making regarding appropriate screening and use of risk reduction strategies such as chemoprevention (tamoxifen or raloxifene). The urgent need to accurately identify women at elevated risk of breast cancer has been emphasized in multiple reports (35,36). Currently, the commonly used breast cancer risk prediction tool, the Gail model, has been shown to provide useful risk stratification when applied at the population level (37). However, in a study applying the Gail model to estimate risk for women with atypical hyperplasia, a high-risk subgroup among women with BBD, the concordance between predicted and observed outcomes was low (concordance statistic = 0.50, 95% CI = 0.44 to 0.55) (38). In order to accurately classify women into high-risk or low-risk groups, predictive risk markers must be identified and incorporated into risk models. Recent efforts to incorporate MBD into risk prediction models have shown slight improvement in discriminatory accuracy (5,39,40). Our findings that lobular involution, a tissue-based marker, and MBD, a radiological marker, are independent risk factors for breast cancer, and that the combination of these factors can stratify risk, supports the potential for incorporation of both these markers in future breast cancer risk prediction models.

The strength of this study is that it was conducted in a large, well-characterized cohort of women with BBD, with information on both lobular involution and MBD, and long-term follow-up for breast cancer events. However, we acknowledge that our study has a few limitations. Both the parenchymal pattern measure of MBD and three-category measure of lobular involution were subjective measures. However, the parenchymal pattern measure has been used in multiple prior studies and shown to be associated with breast cancer risk (15,19–21,28). Moreover, we are currently studying ways to quantify lobular involution (41), which will likely strengthen any associations seen with risk. Although the study population was predominantly white, it was representative of the upper Midwest population of the United States, and we acknowledge the need for continued research in diverse populations.

In conclusion, we report that lobular involution and MBD are both risk factors for breast cancer, and that each provides unique information about breast cancer risk. These findings emphasize the potential for inclusion of these factors in future breast cancer risk prediction models.

## References


1. Hartmann LC, Sellers TA, Frost MH, et al.. Benign breast disease and the risk of breast cancer. *N Engl J Med.* 2005;353(3):229–237.
2. Bassett LW, Liu TH, Giuliano AE, Gold RH. The prevalence of carcinoma in palpable vs. impalpable, mammographically detected lesions. *AJR Am J Roentgenol.* 1991;157(1):21–24.
3. Dupont WD, Page DL. Risk factors for breast cancer in women with proliferative breast disease. *N Engl J Med.* 1985;312(3):146–151.
4. Milanese TR, Hartmann LC, Sellers TA, et al. Age-related lobular involution and reduced risk of breast cancer. *J Natl Cancer Inst.* 2006;98(22): 1600–1607.
5. Vachon CM, van Gils CH, Sellers TA, et al. Mammographic density, breast cancer risk and risk prediction. *Breast Cancer Res.* 2007; 9(6):217.
6. Boyd NF, Guo H, Martin LJ, et al. Mammographic density and the risk and detection of breast cancer. *N Engl J Med.* 2007;56(3):227–236.
7. Cummings SR, Tice JA, Bauer S, et al. Prevention of breast cancer in postmenopausal women: approaches to estimating and reducing risk. *J Natl Cancer Inst.* 2009;101(6):384–398.
8. Ghosh K, Hartmann LC, Reynolds C, et al. Association between mammographic density and age-related lobular involution of the breast. *J Clin Oncol.* 2010;28(13):2207–2212. Epub 2010 Mar 29. doi:10.1200/JCO.2009.23.4120.
9. Hughes LE, Mansel RE. Breast anatomy and physiology, Chapter 2. In: Hughes LE, Mansel RE, Webster DJT, eds. *Benign Disorders and Diseases of the Breast: Concepts and Clinical Management.* 2nd ed. London, UK: W.B.Saunders 2000;7–20.
10. Vorrherr H. *The Breast: Morphology, Physiology, and Lactation.*New York, NY: Academic Press; 1974.
11. Kelemen LE, Pankratz VS, Sellers TA, et al. Age-specific trends in mammographic density: the Minnesota Breast Cancer Family Study. *Am J Epidemiol.* 2008;167(9):1027–1036.
12. Henson DE, Tarone RE. Involution and the etiology of breast cancer. *Cancer.* 1994;74(S1):424–429.
13. Ginsburg OM, Martin LJ, Boyd NF. Mammographic density, lobular involution, and risk of breast cancer. *Br J Cancer.* 2008;99(9):1369–1374.
14. Boyd NF, Rommens JM, Vogt K, et al. Mammographic breast density as an intermediate phenotype for breast cancer. *The Lancet Oncol.* 2005;6(10):798–808.
15. Wolfe JN. Breast patterns as an index for developing breast cancer. *AJR Am J Roentgenol.* 1976;126(6):1130–1137.
16. Gao J, Warren R, Warren-Forward H, Forbes JF. Reproducibility of visual assessment on mammographic density. *Breast Cancer Res Treat.* 2008;108(1):121–127.
17. Boyd NF, Wolfson C, Moskowitz M, et al. Observer variation in the classification of mammographic parenchymal patterns. *J Chronic Dis.* 1986;39(6):465–472.
18. Moskowitz M, Pemmaraju S, Russell P, Gardella L, Gartside P, DeGroot I. Observations on the natural history of carcinoma of the breast, its precursors, and mammographic counterparts. Part 2: mammographic patterns. *Breast Dis.* 1977;3:37–41.
19. Wilkinson E, Clopton C, Gordonson J, Green R, Hill A, Pike MC. Mammographic parenchymal pattern and the risk of breast cancer. *J Natl Cancer Inst.* 1977;59(5):1397–1400.
20. Boyd NF, O'Sullivan B, Campbell JE, et al. Mammographic signs as risk factors for breast cancer. *Br J Cancer.* 1982;45(2):185–193.
21. Brisson J, Merletti F, Sadowsky NL, Twaddle JA, Morrison AS, Cole P. Mammographic features of the breast and breast cancer risk. *Am J Epidemiol.* 1982;115(3):428–437.
22. Brisson J, Morrison AS, Kopans DB, et al. Height and weight, mammographic features of breast tissue, and breast cancer risk. *Am J Epidemiol.* 1984;119(3):371–381.
23. Carlile T, Kopecky KJ, Thompson DJ, et al. Breast cancer prediction and the Wolfe classification of mammograms. *JAMA.* 1985;254(8): 1050–1053.
24. Wolfe JN, Saftlas AF, Salane M. Mammographic parenchymal patterns and quantitative evaluation of mammographic densities: a case-control study. *AJR Am J Roentgenol.* 1987;148(6):1087–1092.

25. Brisson J, Verreault R, Morrison AS, Tennina S, Meyer F. Diet, mammographic features of breast tissue, and breast cancer risk. *Am J Epidemiol.* 1989;130(1):14–24.
26. Saftlas AF, Wolfe JN, Hoover RN, et al. Mammographic parenchymal patterns as indicators of breast cancer risk. *Am J Epidemiol.* 1989; 129(3):518–526.
27. Brisson J. Family history of breast cancer, mammographic features of breast tissue, and breast cancer risk. *Epidemiology.* 1991;2(6):440–444.
28. Byrne C, Schairer C, Wolfe J, et al. Mammographic features and breast cancer risk: effects with time, age, and menopause status. *J Natl Cancer Inst.* 1995;87(21):1622–1629.
29. Li T, Sun L, Miller N, et al. The association of measured breast tissue characteristics with mammographic density and other risk factors for breast cancer. *Cancer Epidemiol Biomarkers Prev.* 2005;14(2):343–349.
30. Ghosh K, Brandt KR, Reynolds CA, et al. Histologic markers of mammographic breast density: core-needle biopsy tissue from healthy volunteers. *Cancer Res.* 2009;69(2 suppl S):263S. Abstract 4037.
31. Wiseman BS, Werb Z. Stromal effects on mammary gland development and breast cancer. *Science.* 2002;296(5570):1046–1049.
32. Guo YP, Martin LJ, Hanna W, et al. Growth factors and stromal matrix proteins associated with mammographic densities. *Cancer Epdemiol Biomarkers Prev.* 2001;10(3):243–248.
33. Diorio C, Pollak M, Byrne C, et al. Insulin-like growth factor-I, IGF-binding protein-3, and mammographic breast density. *Cancer Epidemiol Biomarkers Prev.* 2005;14(5):1065–1073.
34. Byrne C, Colditz GA, Willett WC, Speizer FE, Pollak M, Hankinson SE. Plasma insulin-like growth factor (IGF) I, IGF-binding protein 3, and mammographic density. *Cancer Res.* 2000;60(14):3744–3748.
35. Freedman AN, Seminara D, Gail MH, et al. Cancer risk prediction models: a workshop on development, evaluation, and application. *J Natl Cancer Inst.* 2005;97(10):715–723.
36. Elmore JG, Fletcher SW. The risk of cancer risk prediction: what is my risk of getting breast cancer? *J Natl Cancer Inst.* 2006;98(23):1673–1675.
37. Gail MH, Brinton LA, Byar DP, et al. Projecting individualized probabilities of developing breast cancer for white females who are being examined annually. *J Natl Cancer Inst.* 1989;81(24):1879–1886.
38. Pankratz VS, Hartmann LC, Degnim AC, et al. Assessment of the accuracy of the Gail model in women with atypical hyperplasia. *J Clin Oncol.* 2008;26(33):5374–5379. Epub 2008 Oct 14.
39. Barlow WE, White E, Ballard-Barbash R, et al. Prospective breast cancer risk prediction model for women undergoing screening mammography. *J Natl Cancer Inst.* 2006;98(17):1204–1214.
40. Tice JA, Cummings SR, Ziv E, Kerlikowske K. Mammographic breast density and Gail model for breast cancer risk prediction in a screening population. *Breast Cancer Res Treat.* 2005;94(2):115–122.
41. McKian KP, Reynolds CA, Anderson S. A novel breast tissue feature strongly associated with risk of breast cancer. *J Clin Oncol.* 2009;27(35): 5893–5898.

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