Research Article

Cancer Prevention Research

Relationship of Terminal Duct Lobular Unit Involution of the Breast with Area and Volume Mammographic Densities

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Abstract

Elevated mammographic density (MD) is an established breast cancer risk factor. Reduced involution of terminal duct lobular units (TDLU), the histologic source of most breast cancers, has been associated with higher MD and breast cancer risk. We investigated relationships of TDLU involution with area and volumetric MD, measured throughout the breast and surrounding biopsy targets (perilesional). Three measures inversely related to TDLU involution (TDLU count/mm^2, median TDLU span, median acini count/TDLU) assessed in benign diagnostic biopsies from 348 women, ages 40–65, were related to MD area (quantified with thresholding software) and volume (assessed with a density phantom) by analysis of covariance, stratified by menopausal status and adjusted for confounders. Among premenopausal women, TDLU count was directly associated with percent perilesional MD (P trend = 0.03), but not with absolute dense area/volume. Greater TDLU span was associated with elevated percent dense area/volume (P trend<0.05) and absolute perilesional MD ($P = 0.003$). Acini count was directly associated with absolute perilesional MD ($P = 0.02$). Greater TDLU involution (all metrics) was associated with increased nondense area/volume (P trend ≤ 0.04). Among postmenopausal women, TDLU measures were not significantly associated with MD. Among premenopausal women, reduced TDLU involution was associated with higher area and volumetric MD, particularly in perilesional parenchyma. Data indicating that TDLU involution and MD are correlated markers of breast cancer risk suggest that associations of MD with breast cancer may partly reflect amounts of at-risk epithelium. If confirmed, these results could suggest a prevention paradigm based on enhancing TDLU involution and monitoring efficacy by assessing MD reduction. *Cancer Prev Res; 9(2); 149–58. ©2015 AACR.*

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Introduction

Mammographic density (MD) is a representation of stromal and epithelial (fibroglandular) breast tissue content (1). Women with high (≥75%) MD have an approximately 4-fold increased risk of breast cancer compared with women with low density (<5%; ref. 2). Nonetheless, most women with high MD do not develop breast cancer, and many breast cancers occur among women with low MD. Therefore, understanding the characteristics of dense tissue that account for increased risk may improve the value of MD as a breast cancer risk marker. Recent studies linking involution of terminal duct lobular units (TDLU), the structures from which most breast cancers arise (3), and MD have provided important clues (4, 5).

TDLU involution, a normal process of aging, is characterized by a reduction in the number and size of TDLUs and their secretory substructures called acini (3). Likewise, MD decreases as both age and the amount of breast adipose tissue increase (6). Two large cohorts of women with benign breast disease (BBD) have found that women with reduced TDLU involution are at increased breast cancer risk (7, 8). Limited data also suggest that both elevated MD and benign breast tissue demonstrating reduced TDLU involution are independent risk factors for the development of breast cancer among women who have received a BBD biopsy diagnosis (5). To date, findings relating

TDLU involution to MD have largely been based on nonquantitative measures of involution, in which the extent of involution was classified visually (4, 5, 9). In these studies, MD was rated visually in categories (10) or quantified as a percentage of total breast area by computer-assisted software (11). These methods are reproducible in trained hands but are also subjective in nature.

Improved characterization of breast tissue composition on microscopic and macroscopic levels may now be possible using objective measures of both TDLU involution and MD. We have previously found quantitative measures that are inversely associated with TDLU involution (i.e., TDLU count, median TDLU span, and median acini count/TDLU) to be significantly related to breast cancer risk factors among women who donated normal breast tissues (12). In a separate study of women undergoing diagnostic breast biopsy, we determined that risk factor associations with quantitative area and volumetric MD measures exhibited some overlap but divergence as well, particularly for body mass index (BMI; ref. 13). Use of objective and reproducible methods of TDLU and MD assessment may improve our understanding of inter-relationships between these two factors influencing breast cancer risk and enhancing their utility for risk prediction (14, 15).

We investigated relationships of standardized measures of TDLU involution with quantitative area and novel volumetric measures of MD, measured throughout the breast and immediately surrounding lesions targeted for biopsy, among women who were referred for image-guided breast biopsy yielding benign diagnoses.

Materials and Methods

Study population

The NCI Breast Radiology Evaluation and Study of Tissues (BREAST) Stamp Project is a molecular epidemiologic study of MD undertaken at the University of Vermont College of Medicine and the University of Vermont Medical Center, as described previously (13). Briefly, 465 women who were referred for diagnostic image-guided breast biopsy were enrolled from 2007 to 2010. Eligible women were aged 40 to 65 years, did not have breast implants, were not taking breast cancer chemoprevention, and had no history of breast cancer, and no breast surgery within the past year.

Study participants completed a standard health history questionnaire (16). A research coordinator administered an interview to collect additional health information and measured participants' height and weight. A woman was considered postmenopausal if menstrual periods had stopped more than 12 months before interview, she had undergone bilateral oophorectomy, or she had undergone a hysterectomy and was 55 years of age or older; otherwise, a woman was considered premenopausal. Participants provided written informed consent (in accordance with Institutional Review Boards at the University of Vermont and NCI).

Pathology

Ultrasound-guided core needle (14-gauge) or stereotactic-guided vacuum-assisted (9-gauge) breast biopsies were routinely processed as formalin-fixed paraffin-embedded blocks, which were sectioned and stained with hematoxylin and eosin (H&E) for diagnosis. For study purposes, final diagnoses were categorized as nonproliferative BBD, proliferative (ductal hyperplasia; sclerosing adenosis), proliferative with atypia (atypical ductal or lobular hyperplasia), *in situ*, or invasive breast carcinoma based on review of pathology reports. For women who had $\geq$two unilateral biopsy targets, the two most invasive targets were selected. If there were $\geq$two bilateral targets, then one target from each breast was selected, sampling the tissue with the most severe diagnosis.

Histologic assessment of TDLU involution

H&E-stained tissue sections were digitized at $\times$20 magnification (Aperio ScanScope CS) and were prepared for Web-based viewing and annotation with Digital Image Hub software (SlidePath/Leica; ref. 12). The lasso tool in Digital Image Hub was used to manually outline and measure total tissue area (mm^2) per section. Normal TDLUs per section were enumerated by a pathologist (M.E. Sherman); for women with TDLUs observed, menopausal-specific quartiles of the number of TDLUs per unit of tissue area (TDLU count/mm^2) were calculated. Up to 10 TDLUs were evaluated for maximum diameter as "TDLU span" (measured with an electronic ruler in microns) to provide reliable estimates (17). A semiautomated image analysis tool was used to estimate the number of acini per TDLU as previously described (18, 19). Median values for each woman were used as summary measures of TDLU span and acini count. Menopausal status-specific quintiles of median TDLU span and tertiles of median acini count/TDLU were calculated and used in subsequent analyses. A previous study (12) demonstrated high intraobserver agreement (Spearman $r >$ 0.90) for the study pathologist (M.E. Sherman) for the TDLU measures and found that TDLU measures were inversely correlated with the subjective impression of TDLU involution that had been previously linked to MD and breast cancer risk (5).

MD assessment

Digital raw mammographic images were transferred to the University of California at San Francisco for quantitative area and volumetric density assessment. This analysis was restricted to prebiopsy craniocaudal views of the ipsilateral breast. The median (range) number of days between the mammogram selected for analysis and subsequent breast biopsy was 13 (0–294) days; for the vast majority (95%) of women, the selected mammogram was acquired within 47 days prior to biopsy.

Area density. Area measures of density were estimated as described previously (20), using computer-assisted thresholding software comparable with other validated methods (11, 21). One trained experienced reader (20, 21) measured absolute dense area (cm^2) by setting a pixel threshold for dense tissue. The percentage of dense area was calculated by dividing absolute dense breast area by total breast area (i.e., absolute dense area + absolute nondense area) and multiplying by 100.

Volume density. Absolute fibroglandular tissue volume (cm^3) and percent fibroglandular volume were estimated from the same images using Single X-ray Absorptiometry (SXA) as described previously (22). An SXA breast density phantom was affixed to the compression paddle and included in the X-ray field. Mammographic grayscale values were compared with values of the SXA phantom with a known fibroglandular volume composition and thickness (22) using two reference compositions:

Crisco (J. M. Smucker Co.) as 0% fibroglandular tissue reference and proprietary material from Computerized Imaging Reference Systems, Inc. (CIRS, Inc.) equivalent to 100% fibroglandular tissue. In this way, volumetric measures were achieved using a planar image. Previous estimates of reproducibility for SXA test phantoms demonstrated a repeatability SD of 2%, with a ±2% accuracy for the entire thickness and density ranges (22).

Perilesional volume density. To compute localized density measures surrounding lesions targeted for biopsy (i.e., perilesional), a radiologist (J.M. Johnson) recorded the location and radius of the biopsy target on the prebiopsy standard digital mammogram (i.e., Digital Imaging and Communications in Medicine format). Absolute perilesional fibroglandular volume (cm^3) and percent perilesional fibroglandular volume were estimated using SXA within a volume twice the size of, but excluding, the biopsy target, centered at the biopsy site (Fig. 1; ref. 15). A repeat set of 25 images was assessed for reliability. The intraclass correlation coefficients for percent perilesional fibroglandular volume, absolute perilesional fibroglandular volume, and total perilesional volume were 0.99, 0.72, and 0.71, respectively, indicating good to excellent reproducibility.

Analytic population

Of the 465 women who consented to the telephone interview, 12 were not subsequently biopsied, and 81 were diagnosed with breast cancer and were excluded. We also excluded 6 women without biopsy tissue available for research, 14 missing prebiopsy SXA density data, 1 woman whose images were not suitable for TDLU assessment, and 3 women missing perilesional density measures, resulting in a final analytic population of 348 women. Of the 348 women, 47 had TDLU and perilesional MD data for two biopsy targets, resulting in a total of 395 biopsy targets for inclusion in the present analysis.

Statistical analyses

Descriptive statistics for TDLU and MD measures were calculated, and Spearman rank correlation coefficients were estimated for their associations with age and BMI (kg/m^2). A lowess function was used to estimate and plot the average of TDLU count and percent dense area/volume as a function of age. Associations of TDLU measures with MD were stratified by menopausal status, given that both measures have been shown to differ by menopausal status (12, 23). To compare mean quantitative MD measures across categories of TDLU measures, we used analysis of covariance (ANCOVA) models. Density measures were square-root transformed to better approximate normal distributions. For ease of interpretation, the least square means and standard errors from ANCOVA were back transformed, and corresponding 95% confidence intervals were calculated (see Appendix for details). We conducted analysis at the biopsy target level using SAS PROC GENMOD; correlations among biopsies from the same woman

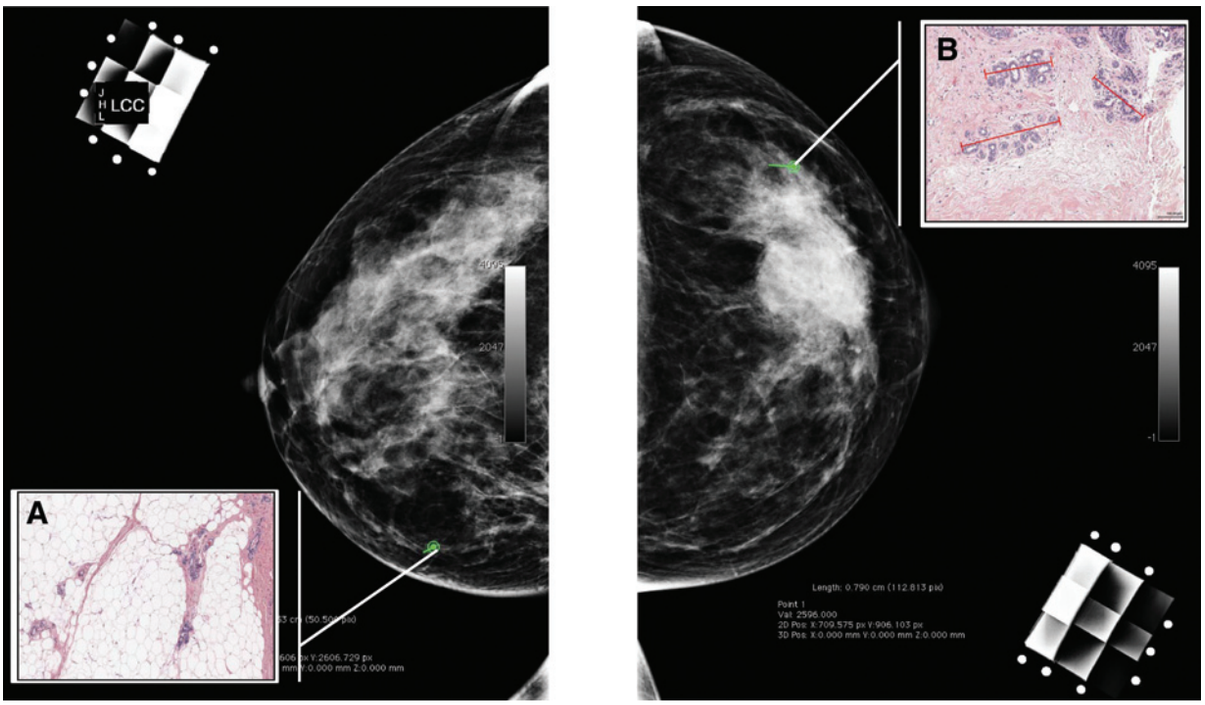

Figure 1.
Representative full-field digital mammograms from two premenopausal study participants. The digital mammogram is acquired with the density phantom in the corner of the image to allow for automated computation of volumetric MD. To compute perilesional MD, the radiologist recorded the biopsy location and radius of the biopsy target (noted in green) on a craniocaudal view of the prebiopsy digital mammogram. Percent perilesional fibroglandular volume was estimated at a volume twice the size of but excluding the biopsy target, centered at the biopsy site. H&E images from each participant's breast biopsy are also shown. In this example, panel A represents a breast biopsy specimen with marked TDLU involution and with comparable MD estimates of percent fibroglandular volume (43.9%) and percent perilesional fibroglandular volume (43.8%). In contrast, panel B depicts a breast biopsy specimen with limited TDLU involution, as reflected in the increased number of TDLUs (TDLU spans are annotated and measured in microns using a digital ruler) and number of acini within the TDLUs; the mammogram in B has lower percent fibroglandular volume (36.3%) as compared with percent perilesional fibroglandular volume (60.2%).

Table 1. Characteristics of women referred to an image-guided breast biopsy and diagnosed with benign breast disease, The BREAST Stamp Project, 2007–2010

Characteristic ($n = 348$ women)	Premenopausal *n* (%)	Postmenopausal *n* (%)
Age at mammogram (years)		
39–44	74 (32.7)	1 (0.8)
45–49	94 (41.6)	5 (4.1)
50–54	55 (24.3)	29 (23.8)
55–59	3 (1.3)	47 (38.5)
60–65	0 (0.0)	40 (32.8)
White, non-Hispanic race	213 (94.2)	110 (90.2)
College/graduate school degree	197 (87.2)	95 (77.9)
BMI, kg/m^2		
<25	112 (49.6)	48 (39.3)
25–<30	64 (28.3)	35 (28.7)
30+	50 (22.1)	39 (32.0)
Age at menarche (years)		
≤12	83 (36.7)	46 (37.7)
13	86 (38.1)	41 (33.6)
≥14	54 (23.9)	32 (26.2)
Parity		
Nulliparous	57 (25.2)	30 (24.6)
1	22 (9.7)	23 (18.9)
2	94 (41.6)	43 (35.2)
3+	53 (23.5)	26 (21.3)
Age at first birth (years)		
<25	56 (33.1)	54 (58.7)
25–<30	56 (33.1)	22 (23.9)
30+	57 (33.7)	15 (16.3)
Menopausal hormone therapy use		
Never	194 (85.8)	69 (56.6)
Former	22 (9.7)	33 (27.0)
Current	3 (1.3)	15 (12.3)
Cigarette smoking, 100+ cigarettes/lifetime		
Never	125 (55.3)	51 (41.8)
Former	72 (31.9)	47 (38.5)
Current	19 (8.4)	16 (13.1)
Breast biopsy prior to enrollment	69 (30.5)	49 (40.2)
Family history of breast cancer in a first-degree female relative	52 (23.0)	29 (23.8)
Biopsy type		
Ultrasound-guided	119 (52.7)	49 (40.2)
Stereotactic-guided	102 (45.1)	71 (58.2)
Both	5 (2.2)	2 (1.6)
Biopsy laterality and number		
Left		
One biopsy	101 (44.7)	44 (36.1)
Two biopsies	11 (4.9)	7 (5.7)
Right		
One biopsy	96 (42.5)	60 (49.2)
Two biopsies	11 (4.9)	8 (6.6)
Bilateral biopsies	7 (3.1)	3 (2.5)
Pathologic diagnosis[a]		
Benign	95 (42.0)	48 (39.3)
Proliferative	113 (50.0)	58 (47.5)
Proliferative with atypia[b]	18 (8.0)	16 (13.1)
Characteristic (per biopsy target, $n = 395$ biopsies)		
Biopsy type		
Ultrasound-guided	140 (54.9)	53 (37.9)
Stereotactic-guided	115 (45.1)	87 (62.1)
Pathologic diagnosis		
Benign	111 (43.5)	56 (40.0)
Proliferative	125 (49.0)	66 (47.1)
Proliferative with atypia[c]	19 (7.5)	18 (12.9)

NOTE: Missing values were excluded from percentage calculations.

[a]Among women with multiple biopsies, this was the worst pathologic diagnosis.

[b]Includes $n = 9$ atypical ductal, $n = 7$ atypical lobular hyperplasia, and $n = 2$ with both diagnoses among premenopausal women, and $n = 6$ atypical ductal hyperplasia and $n = 10$ atypical lobular hyperplasia diagnoses among postmenopausal women.

[c]Includes $n = 9$ atypical ductal, $n = 8$ atypical lobular hyperplasia, and $n = 2$ with both diagnoses among premenopausal women, and $n = 7$ atypical ductal and $n = 11$ atypical lobular hyperplasia diagnoses among postmenopausal women.

were accounted for in the variance calculation (24). All models were adjusted for age and BMI, which are known to be strongly associated with MD and TDLU measures. Potential confounders were identified separately for pre- and postmenopausal women using stepwise selection for each density measure with an inclusion/exclusion criteria of $P < 0.05$. Analyses among premenopausal women were additionally adjusted for history of breast biopsy and pathologic diagnosis; models evaluating associations for TDLU count were also adjusted for smoking status (percent and nondense area/volume measures) and biopsy type (nondense area/volume measures). Among postmenopausal women, models were additionally adjusted for pathologic diagnosis; models relating TDLU count to nondense area/volume measures were also adjusted for biopsy type. Probability values of <0.05 were considered statistically significant. All tests of statistical significance were two-tailed. Analyses were performed using SAS software (SAS Institute Inc.).

Results

Participant characteristics

The mean (SD) age of premenopausal ($n = 226$) and postmenopausal ($n = 122$) participants was 46 (4) and 57 (4) years, respectively. Most participants were non-Hispanic white, college graduates, and parous (Table 1). Compared with premenopausal women, postmenopausal women were more likely to be obese, to ever smoke cigarettes, and to have a breast biopsy prior to study enrollment. Premenopausal women were more likely to have had an ultrasound-guided breast biopsy (52.7%), whereas postmenopausal women were more likely to have had a stereotactic-guided biopsy (58.2%). Proliferative disease with atypia was diagnosed more frequently among postmenopausal women.

Distributions of TDLU and MD measures

Among all women, TDLU count (Fig. 2) and percent area and volume density measures were inversely associated with age (Supplementary Figs. S1 and S2) and BMI ($P < 0.001$; Table 2). TDLU count was weakly correlated with TDLU span and acini count/TDLU, whereas TDLU span and acini count/TDLU were more strongly correlated (Supplementary Table S1).

Median percent and absolute area and volume densities were higher among pre- versus postmenopausal women, whereas postmenopausal women tended to have higher measures of nondense area and volume (Table 2). Median percent perilesional fibroglandular volume tended to be higher than that of the entire breast. Significant positive correlations were observed between percent and absolute fibroglandular volumes of the entire breast and that surrounding the biopsy site (Supplementary Table S2). Similarly, nondense volume of the entire breast was positively correlated with perilesional nondense volume.

TDLU and MD measures among women with two biopsies

TDLU count was moderately correlated between biopsy sites among pre- ($r = 0.58$, $P = 0.001$) and postmenopausal ($r = 0.50$,

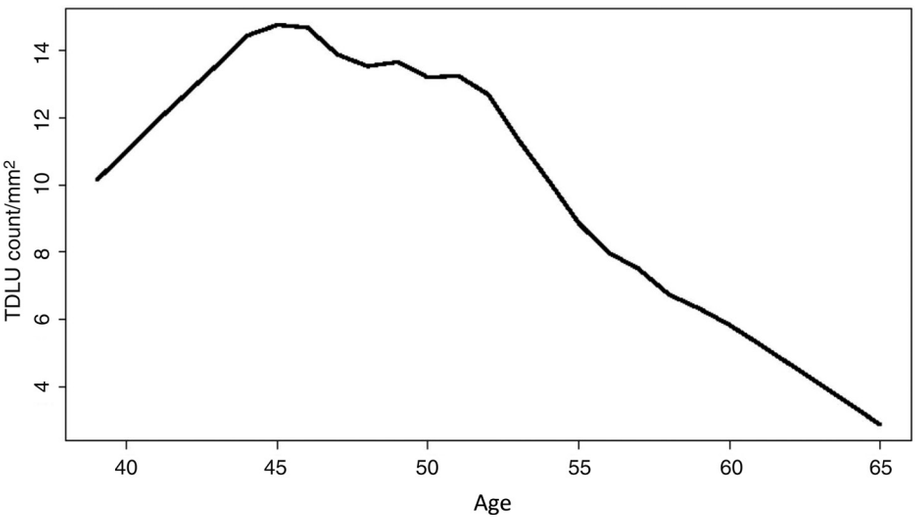

Figure 2.
Average TDLU count by age. A lowess function was used to estimate the average of TDLU counts as a function of age.

$P = 0.03$) women (data not shown). TDLU span and acini count/TDLU were not correlated between biopsy sites in premenopausal women ($n = 21$ with two evaluable biopsies: $r = 0.04$, $P = 0.86$ and $r = 0.33$, $P = 0.15$, respectively), but were highly correlated among postmenopausal women ($n = 10$ with two evaluable biopsies: $r = 0.99$, $P < 0.001$ and $r = 0.73$, $P = 0.02$, respectively).

Among women with two biopsies, percent perilesional fibroglandular volumes were strongly and positively correlated among both pre- ($r = 0.83$, $P < 0.001$) and postmenopausal ($r = 0.82$, $P < 0.001$) women. Among premenopausal women, positive correlations were also observed for absolute perilesional fibroglandular volumes ($r = 0.44$, $P = 0.01$) as well as nondense perilesional volumes ($r = 0.84$, $P < 0.001$) for both biopsy sites. In contrast, among postmenopausal women, we did not observe significant correlations for absolute perilesional fibroglandular volumes ($r = 0.06$, $P = 0.82$) or nondense perilesional volumes ($r = 0.33$, $P = 0.18$) for the two biopsy sites.

Associations between measures of TDLU involution and MD

Premenopausal women. Among premenopausal women, TDLU count was positively associated with all percent density measures (Table 3); although adjustments attenuated associations, associations of TDLU count and percent perilesional fibroglandular volume remained significant (adjusted mean percent density for the highest vs. lowest quintiles of TDLU count: 49.9% vs. 41.0%, respectively; P trend = 0.03). Figure 1 illustrates how differential TDLU associations with global versus localized density measures might occur by showing H&E images and corresponding mammograms from two premenopausal participants with low and high TDLU count: Panel A represents a breast biopsy specimen with marked TDLU involution and with comparable MD estimates of percent global fibroglandular volume (43.9%) and percent perilesional fibroglandular volume (43.8%). In contrast, panel B depicts a breast biopsy specimen with limited TDLU involution, and the corresponding mammogram has lower percent global fibroglandular volume (36.3%) as compared with percent perilesional fibroglandular volume (60.2%).

Table 2. Distribution of TDLU and MD measures among women with benign breast disease, stratified by menopausal status

	Overall				Premenopausal				Postmenopausal			
	Median	Range	Correlation with age, *r*	Correlation with BMI, *r*	Median	Range	Correlation with age, *r*	Correlation with BMI, *r*	Median	Range	Correlation with age, *r*	Correlation with BMI, *r*
TDLU measures												
TDLU count/100 mm^2	10.0	0–199.2	−0.25[a]	−0.29[a]	16.0	0–199.2	−0.14[b]	−0.33[a]	5.5	0–172.4	−0.30[a]	−0.20[b]
Median TDLU span, μ	258	78–809	−0.34[a]	−0.07	284	78–809	−0.16[b]	−0.05	224	83–568	0.02	−0.02
Median acini count per TDLU	11.0	1–68	−0.36[a]	−0.10	13.0	1–68	−0.13	−0.03	8.0	2–33.5	−0.03	−0.11
MD measures												
Area measures												
Percent dense area (%)	25.9	0–88.9	−0.27[a]	−0.53[a]	32.8	0–88.9	−0.01	−0.47[a]	16.3	0–82.2	−0.14	−0.59[a]
Absolute dense area (cm^2)	29.9	0–139.1	−0.14[b]	−0.15[b]	35.9	0–139.1	0.05	−0.02	24.7	0–130.1	−0.11	−0.32[a]
Nondense area (cm^2)	95.7	11.6–441.2	0.28[a]	0.71[a]	79.5	11.6–441.2	0.07	0.71[a]	132.0	14.9–385.5	0.09	0.71[a]
Volume measures												
Percent FGV (%)	35.1	0.6–99.3	−0.30[a]	−0.61[a]	40.9	0.6–98.8	−0.11	−0.60[a]	29.4	1.5–99.3	−0.14	−0.62[a]
Absolute FGV (cm^3)	186.2	6.7–683.5	−0.07	0.29[a]	193.6	6.7–683.5	−0.04	0.36[a]	173.6	31–637.8	−0.02	0.21[b]
Nondense volume (cm^3)	345.6	1.6–2126	0.24[a]	0.73[a]	282.1	3.5–2126	0.08	0.74[a]	458.8	1.6–1977	0.08	0.71[a]
Perilesional volume measures												
Percent perilesional FGV (%)	40.2	0–100	−0.31[a]	−0.51[a]	48.2	0–100	−0.14[b]	−0.51[a]	31.7	0–100	−0.06	−0.47[a]
Absolute perilesional FGV (cm^3)	6.0	0–91.5	−0.26[a]	−0.11[b]	7.5	0–86.6	−0.23[a]	0.01	4.3	0–91.5	0.004	−0.24[b]
Nondense perilesional volume (cm^3)	6.8	0–137.3	0.05	0.36[a]	6.3	0–126.1	−0.07	0.43[a]	7.8	0–137.3	0.13	0.21[b]

NOTE: Correlations between MD and TDLU measures (continuous) with age and BMI were assessed by Spearman partial rank-order correlation (*r*); [a], $P < 0.001$; [b], $P < 0.05$.
We computed the median acini count and median TDLU span for each biopsy with TDLUs observed ($n = 194$ and $n = 101$ biopsy targets among pre- and postmenopausal women, respectively).
Abbreviations: BMI, body mass index; FGV, fibroglandular volume; MD, mammographic density; TDLU, terminal duct lobular unit.

Table 3. Association between TDLU and percent dense area/volume MD measures among premenopausal women with benign breast disease, The BREAST Stamp Project

		Percent dense area/volume measures											
		Percent dense area (%)				Percent fibroglandular volume (%)				Percent perilesional fibroglandular volume (%)			
		Unadjusted		Adjusted[a]		Unadjusted		Adjusted[a]		Unadjusted		Adjusted[a]	
TDLU measure	N[b]	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
TDLU count/100 mm^2													
0	61	21.3	17.6–25.0	24.6	21.4–27.7	33.1	29.8–36.4	38.3	35.1–41.5	36.8	32.7–40.9	41.0	36.3–45.7
1.0–<11.3	48	26.9	22.6–31.1	27.5	23.0–32.0	38.7	33.9–43.4	40.1	35.9–44.4	41.2	36.0–46.5	42.0	36.8–47.2
11.3–<22.7	49	36.8	32.7–40.9	34.3	29.7–38.9	45.9	41.3–50.6	43.7	39.6–47.9	51.5	46.3–56.7	47.5	42.2–52.8
22.7–<48.3	49	29.3	25.5–33.0	27.0	23.4–30.6	43.1	38.5–47.7	40.8	36.9–44.7	52.0	46.5–57.5	47.6	42.6–52.6
48.3–199.2	48	36.7	32.9–40.6	31.9	27.2–36.5	52.0	48.1–55.9	44.8	40.7–49.0	59.5	54.9–64.1	49.9	44.8–55.0
P value for trend		**0.0003**		0.07		<**0.0001**		0.11		<**0.0001**		**0.03**	
Median TDLU span, μ													
78–<211	38	27.7	24.1–31.3	25.0	21.7–28.4	37.9	33.3–42.4	35.8	32.1–39.5	42.7	37.0–48.3	40.6	35.4–45.8
211–<260	39	30.8	25.5–36.2	29.2	23.5–35.0	43.4	38.1–48.7	41.9	37.0–46.8	50.8	44.9–56.7	48.7	43.0–54.4
260–<302	39	29.5	25.3–33.7	26.3	22.3–30.2	43.6	38.4–48.8	40.5	36.0–45.1	50.0	44.0–56.0	45.7	40.4–51.1
302–<385	39	39.3	34.7–43.9	37.0	32.0–41.9	50.9	45.8–55.9	47.5	43.0–52.1	59.0	52.7–65.2	54.4	48.7–60.1
385–809	39	34.9	30.3–39.5	32.7	27.7–37.7	48.6	43.4–53.7	47.1	42.3–51.9	52.4	46.6–58.3	50.0	44.2–55.8
P value for trend		**0.02**		**0.01**		**0.02**		**0.003**		**0.04**		**0.04**	
Median acini count per TDLU													
1–10	62	30.8	27.0–34.7	27.9	24.2–31.5	42.9	38.6–47.3	39.7	36.0–43.4	50.9	45.9–55.9	47.2	42.5–51.9
10.5–16.5	62	30.3	27.1–33.6	30.0	25.6–34.4	42.3	39.0–45.6	42.2	38.7–45.7	48.1	44.4–51.9	47.3	42.9–51.6
17–68	67	36.0	32.2–39.8	33.8	29.1–38.5	49.5	45.1–53.8	47.0	42.6–51.5	54.2	49.0–59.4	50.6	45.3–55.9
P value for trend		0.18		0.14		0.14		0.06		0.50		0.57	

NOTE: *P* values <0.05 are presented in bold font.

Abbreviations: CI, confidence interval; MD, mammographic density; TDLU, terminal duct lobular unit.

[a]Models were adjusted for age, BMI, history of breast biopsy, and pathologic diagnosis. TDLU counts were additionally adjusted for smoking status.

[b]*N* represents the number of biopsy targets.

Among women with TDLUs observed, TDLU span was positively associated with all percent density measures both before and after covariate adjustments (P trend < 0.05; Table 3). No statistically significant trends were observed between acini count/TDLU and any percent density measure.

In multivariate models, TDLU measures were not significantly associated with global absolute dense area and volume measures for the entire breast (Table 4). However, both TDLU span and acini count/TDLU were positively associated with absolute perilesional fibroglandular volume (adjusted mean absolute perilesional fibroglandular volume for the highest vs. lowest quintiles of TDLU span = 12.2 cm^3 vs. 6.7 cm^3, respectively; P trend = 0.003; and for the highest vs. lowest tertiles of acini count = 10.9 cm^3 vs. 6.8 cm^3, respectively; P trend = 0.02).

Table 4. Association between TDLU and absolute dense area/volume MD measures among premenopausal women with benign breast disease, The BREAST Stamp Project

		Absolute dense area/volume measures											
		Absolute dense area (cm^2)				Absolute fibroglandular volume (cm^3)				Absolute perilesional fibroglandular volume (cm^3)			
		Unadjusted		Adjusted[a]		Unadjusted		Adjusted[a]		Unadjusted		Adjusted[a]	
TDLU measure	N[b]	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
TDLU count/100 mm^2													
0	61	29.8	25.2–34.4	32.9	27.8–38.1	213.0	194.6–231.4	219.4	198.9–239.9	7.7	6.4–9.0	7.5	6.0–9.0
1.0–<11.3	48	38.8	32.3–45.4	41.2	33.1–49.2	219.1	190.4–247.8	233.5	202.6–264.4	7.6	5.6–9.7	8.2	6.0–10.5
11.3–<22.7	49	44.1	38.8–49.4	46.9	39.8–53.9	209.8	190.9–228.6	233.0	210.3–255.8	11.5	9.0–14.0	10.9	8.4–13.4
22.7–<48.3	49	32.0	27.5–36.4	34.6	28.8–40.4	180.2	162.7–197.7	203.3	180.2–226.4	7.0	5.6–8.5	7.3	5.6–9.0
48.3–199.2	48	34.3	30.0–38.5	36.9	30.1–43.7	170.4	151.3–189.5	199.3	174.4–224.3	10.2	7.8–12.6	9.6	7.1–12.1
P value for trend		0.67		0.75		**0.009**		0.17		0.32		0.50	
Median TDLU span, μ													
78–<211	38	35.0	30.8–39.1	34.8	29.5–40.2	184.7	163.0–206.5	200.2	174.6–225.8	5.8	4.3–7.3	6.7	5.0–8.4
211–<260	39	36.7	29.2–44.3	38.9	30.0–47.9	192.5	167.8–217.1	210.5	185.8–235.3	8.2	6.5–9.9	8.5	6.3–10.8
260–<302	39	31.4	27.3–35.6	33.1	27.3–39.0	176.3	156.9–195.7	200.2	173.3–227.0	8.7	6.0–11.3	9.0	5.7–12.3
302–<385	39	46.5	40.4–52.7	49.5	41.0–57.9	219.3	195.3–243.3	242.4	212.1–272.8	11.3	8.9–13.8	11.1	8.7–13.5
385–809	39	37.4	32.2–42.7	39.5	31.9–47.2	200.2	178.3–222.2	221.5	192.0–251.0	11.7	8.8–14.5	12.2	9.4–15.0
P value for trend		0.25		0.14		0.27		0.17		**0.003**		**0.003**	
Median acini count per TDLU													
1–10	62	37.8	33.1–42.6	39.6	33.4–45.8	194.7	176.3–213.1	216.9	193.2–240.7	6.3	5.1–7.4	6.8	5.3–8.3
10.5–16.5	62	37.0	32.3–41.7	39.9	32.5–47.2	200.0	181.6–218.4	220.6	197.4–243.9	10.6	8.5–12.7	11.1	8.6–13.6
17–68	67	37.2	33.1–41.2	40.0	32.7–47.2	191.3	174.5–208.0	216.5	189.6–243.4	10.4	8.3–12.4	10.9	8.5–13.2
P value for trend		0.89		0.98		0.84		0.94		**0.01**		**0.02**	

NOTE: *P* values <0.05 are presented in bold font.

Abbreviations: CI, confidence interval; MD, mammographic density; TDLU, terminal duct lobular unit.

[a]Models were adjusted for age, BMI, history of breast biopsy, and pathologic diagnosis.

[b]*N* represents the number of biopsy targets.

Table 5. Association between TDLU and nondense area/volume MD measures among premenopausal women with benign breast disease, The BREAST Stamp Project

		Nondense area/volume measures											
		Nondense area (cm^2)				Nondense volume (cm^3)				Nondense perilesional volume (cm^3)			
		Unadjusted		Adjusted[a]		Unadjusted		Adjusted[a]		Unadjusted		Adjusted[a]	
TDLU measure	N[b]	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
TDLU count/100 mm^2													
0	61	122.3	107.0–137.6	110.5	100.7–120.4	477.6	407.4–547.7	400.7	350.5–451.0	13.8	10.4–17.3	10.7	7.6–13.7
1.0–<11.3	48	108.0	93.8–122.3	110.5	99.6–121.4	380.9	309.0–452.8	389.1	334.6–443.5	9.6	7.0–12.2	11.3	8.5–14.1
11.3–<22.7	49	75.8	65.1–86.4	90.6	81.1–100.1	274.4	217.4–331.5	341.0	296.1–385.9	10.7	7.4–13.9	12.4	9.2–15.6
22.7–<48.3	49	79.6	68.5–90.8	95.7	85.1–106.2	280.7	222.7–338.6	352.6	300.5–404.8	6.7	4.6–8.8	9.3	6.9–11.8
48.3–199.2	48	60.2	52.3–68.1	84.5	75.0–93.9	168.2	134.0–202.4	286.0	242.1–329.9	7.7	5.0–10.5	11.1	7.7–14.4
P value for trend		**<0.0001**		**0.004**		**<0.0001**		**0.02**		**0.03**		0.85	
Median TDLU span, μ													
78–<211	38	98.2	83.7–112.7	112.8	101.3–124.2	355.9	283.2–428.5	421.7	368.3–475.1	7.8	5.2–10.4	9.8	7.0–12.5
211–<260	39	79.7	66.8–92.7	92.1	79.2–104.9	275.3	210.3–340.4	325.6	268.9–382.4	7.7	5.5–9.9	8.8	6.2–11.4
260–<302	39	80.5	66.9–94.1	99.7	87.6–111.8	267.8	199.6–336.1	350.2	286.8–413.7	8.7	5.8–11.5	10.6	7.1–14.0
302–<385	39	71.5	60.0–82.9	85.6	74.9–96.4	226.1	170.9–281.3	294.1	243.9–344.3	8.3	5.1–11.4	9.4	6.4–12.5
385–809	39	72.3	61.3–83.3	86.2	76.8–95.7	241.6	186.7–296.5	292.2	246.3–338.1	11.0	7.1–14.8	12.2	8.4–16.1
P value for trend		**0.04**		**0.01**		0.06		**0.009**		0.34		0.40	
Median acini count per TDLU													
1–10	62	87.7	76.0–99.3	105.7	95.9–115.6	295.7	238.7–352.8	377.6	330.3–425.0	5.6	4.1–7.1	7.5	5.8–9.1
10.5–16.5	62	84.2	75.4–93.0	92.3	83.6–101.1	296.9	250.6–343.2	329.6	288.8–370.3	11.3	8.7–13.9	12.2	9.2–15.3
17–68	67	67.5	58.3–76.7	81.8	73.2–90.5	216.9	172.8–261.0	279.9	238.4–321.3	9.1	6.4–11.8	10.8	7.6–14.0
P value for trend		0.05		**0.02**		0.12		**0.04**		0.12		0.12	

NOTE: *P* values <0.05 are presented in bold font.

Abbreviations: CI, confidence interval; TDLU, terminal duct lobular unit.

[a]Models were adjusted for age, BMI, history of breast biopsy, and pathologic diagnosis. TDLU counts were additionally adjusted for smoking status and biopsy type.

[b]*N* represents the number of biopsy targets.

We observed inverse associations of TDLU count with nondense area (*P* trend = 0.004) and volume (*P* trend = 0.02; Table 5). Likewise, TDLU span and acini count/TDLU were inversely associated with both nondense area (*P* trend $\leq$ 0.02) and volume (*P* trend $\leq$ 0.04). TDLU measures were not associated with nondense perilesional volume.

Postmenopausal women. Among postmenopausal women, TDLU count was positively associated with all percent density measures, but after adjustments trends were no longer statistically significant (Supplementary Table S3). Neither TDLU span nor acini count/TDLU was significantly associated with percent density measures. Significant associations between TDLU measures and absolute dense area or volume measures were not identified (Supplementary Table S4). TDLU count was inversely associated with nondense area and volume, but trends were attenuated with adjustments (*P* trend for nondense area = 0.05 and *P* trend for nondense volume = 0.10; Supplementary Table S5). TDLU span and acini count/TDLU were not significantly related to nondense area/volume.

Discussion

This study demonstrates that there are substantial associations between reduced levels of TDLU involution in benign breast biopsies and higher MD, particularly among premenopausal women. Our results affirm similar results reported previously and extend these findings by applying objective quantitative measurements of both TDLU involution and MD, assessed as both an area and a volume, with somewhat stronger associations observed for the latter. These findings support the hypothesis that MD and measures of TDLU involution are associated in benign breast biopsies and may have implications for understanding mechanisms that mediate the increased risk of developing breast cancer among women with dense breasts.

A prior analysis of 2,667 women included in the Mayo Clinic BBD Cohort (4) found that greater degrees of TDLU involution, as assessed subjectively (i.e., none, partial, or complete involution) in surgical specimens, were associated with lower risk MD patterns based on visual estimation (10). These associations were later affirmed in a subset of 317 women using quantitative MD area measures derived from digitized films. Specifically, the latter analysis, which was not stratified by menopausal status, demonstrated that TDLU involution was inversely associated with percent dense area, positively associated with nondense area, and not associated with absolute dense area (4). We observed similar associations with both area and volumetric density measures among premenopausal women, supporting the validity of our quantitative measures of TDLU involution.

Among postmenopausal women in our study with an upper age of 65 years, elevated TDLU count was linked to higher percent MD in univariate analyses; however, in multivariate models which included age-adjustment, our analysis did not show strong trends between measures of TDLU involution and MD. With aging and menopause, TDLUs normally regress, and epithelium is replaced with fibrous tissue and fat (3, 25). Histologic studies have consistently shown that both epithelium and stroma are responsible for radiological density (6, 26, 27), though our understanding of how proportions of breast epithelium, stroma, and adipose tissues vary with age is less clear (6, 28, 29). We observed a wide range of TDLU count, size, and mammographic densities among postmenopausal women; however, it may have been more challenging to detect relationships independent of age in this group by applying quantitative measurements to small image-guided biopsies. Future studies of both pre- and postmenopausal women will be important for gaining further insight into how age-related changes in breast tissue composition may

manifest mammographically and how these changes relate to breast cancer risk across the life course.

An inherent limitation of area MD measures is that they are two-dimensional measurements of a three-dimensional organ. We had therefore hoped to achieve additional insight into the relationship between TDLU involution and MD using an automated volumetric method for digital mammography (22). Percent fibroglandular volume as measured using SXA has been found to be positively correlated with equivalent measures acquired from breast MRI images (30), and significantly associated with breast cancer risk (21, 31). We found that associations between TDLU quantifiers and density of the entire breast were remarkably consistent irrespective of whether density was measured as an area or volume. Though volumetric in its conceptual design, SXA still represents a density measure that is derived from a two-dimensional mammography system. Additional information might be gained from true three-dimensional breast imaging modalities, such as MRI or ultrasound tomography (32).

It has been suggested that measures of both involution (7, 33) and MD (34) reflect a global process occurring throughout the breast and are general markers of breast cancer risk. We therefore explored associations between TDLU measures with both global and localized volumetric measures of MD. For the subset of pre- and postmenopausal women with two biopsy sites, we observed a moderate level of agreement between the numbers of TDLUs measured from the two biopsy specimens within a woman. These findings are consistent with a study demonstrating strong intrawoman concordance of subjectively assessed involution measured in four quadrants of both breasts of 15 women who had undergone prophylactic mastectomy (33). In contrast, we found that TDLU size measures (span and acini count/TDLU) were more variable across biopsy specimens within a woman, though estimates were based on small numbers. At the radiologic level, we observed strong positive correlations for percent perilesional fibroglandular volumes measured for two biopsy specimens within a woman.

Although global and local volumetric density measures were highly correlated, among all women, median percent perilesional fibroglandular volume was approximately 5% higher than its global counterpart, likely reflecting the tendency for biopsy targets to occur in denser breast regions. This difference between median percent global and perilesional fibroglandular volumes was smaller in postmenopausal (2.3%) than premenopausal women (7.3%), among whom several TDLU associations with perilesional density measures were stronger than with global density measures. For example, whereas positive associations between TDLU count and global percent density measures were attenuated after adjustments, a statistically significant trend between increasing TDLU count and increasing percent perilesional fibroglandular volume persisted. In addition, we observed positive associations of TDLU span and acini count/TDLU with absolute perilesional but not global fibroglandular volume. Some stronger associations with localized versus global measures may be expected as our TDLU measures are based on specimens obtained through image-guided breast biopsies, which target epithelial-rich tissues. Studies using different methodologies to evaluate MD in subregions of the breast have found that tumors tend to arise in localized regions of radiodense tissue (35, 36). TDLUs are sparse in adipose-rich parenchyma.

We did not identify significant associations between acini count/TDLU with percent MD measures. The lack of association could reflect insufficient statistical power, given that these analyses were restricted to women with observable TDLUs. Further, acini-rich versus -depleted TDLUs may leave a similar radiologic footprint, given that the contribution of epithelium and nonfatty stroma to MD is similar as a first approximation (6, 26, 27).

The strengths of our study include the use of objective, quantitative measures of both TDLU involution and MD in a contemporary population of women undergoing digital mammography and diagnostic image-guided breast biopsy. However, our study and others relating involution to density (4, 5) have limited generalizability as they have primarily consisted of white, highly educated women, who were referred for a breast biopsy. Identifying biomarkers among women diagnosed with BBD is still of interest, as they are at elevated breast cancer risk, warranting prospective studies in more diverse populations.

Our findings, in concert with data suggesting that involution and density are independently associated with elevated breast cancer risk (5), argue for further research to understand the tissue correlates of MD. This research may enable further specification of breast cancer risk among women with similar levels of MD, particularly among those who have undergone a biopsy showing BBD. Prior studies have suggested that combining measures of TDLU involution and MD may improve breast cancer risk prediction (5). Our findings suggest that greater amounts of at-risk epithelium may partially account for the increased risk associated with elevated MD, although other factors such as a procarcinogenic microenvironment or the impact of systemic factors may be important. Although measures of TDLU involution and MD are generally thought to be representative of the entire breast, regional differences clearly occur, especially in the context of pathology. As such, studies evaluating local variation in MD may provide clues as to why cancers develop in one particular region of the breast.

Many cancer chemoprevention studies follow a "window of opportunity" or "presurgical" trial design in which women undergo an image-guided breast biopsy followed by an intervention (for example, randomization to chemoprevention or placebo) and later surgical resection (37, 38). In addition to comparing biomarkers before and after intervention in lesions as surrogates of efficacy, such studies offer opportunities to compare changes in morphology and biomarkers in TDLUs surrounding the lesions in the paired pre- and postintervention tissue samples. Addition of research biopsies prior to initiation of drug may also be performed to sample areas remote from the biopsy target. Given that MD (39) and TDLU content (40) are potentially modifiable breast cancer risk factors, further research is needed to assess whether these features may serve as useful intermediate endpoints in breast cancer prevention studies.

Disclosure of Potential Conflicts of Interest

S.D. Herschorn has ownership interest in Hologic, IC. No potential conflicts of interest were disclosed by the other authors.

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