Automated Quantitative Measures of Terminal Duct Lobular Unit Involution and Breast Cancer Risk

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Background

- Terminal duct lobular units (TDLUs) are the functional milk-producing glands in the breast. TDLUs involute with age whereby where lobules of Types 2 and 3 regress back to Type 1 (**Figure 1**).
- Prior studies using qualitative or semi-quantitative measures of TDLU involution found inverse associations with breast cancer risk. [1]
- We developed and validated a deep-learning computational method to automatically capture quantitative measures of TDLU involution in normal breast tissue. [2,3]

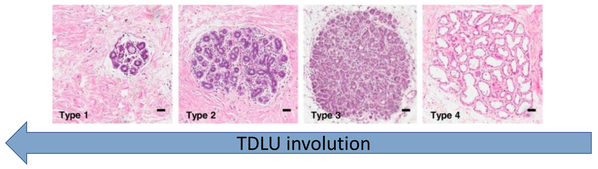

Figure 1. TDLUs are historically classified into four lobule types: Type 1 (least developed; <12 acini), Type 2 (intermediate in their degree of differentiation; ~50 acini), Type 3 (fully developed structures; ~80 acini), and Type 4 (occurs during pregnancy and lactation).

Aim

- To assess the association of quantitative TDLU involution measures obtained using our computational method with (1) established breast cancer risk factors and (2) breast cancer risk in the Nurses' Health Study (NHS) and NHSII.

Materials & Methods

- We applied our computational method to whole slide images (WSIs) from a nested case-control study within the NHS and NHSII cohorts. [4]
- Cases were women who reported a diagnosis of breast cancer subsequent to a diagnosis of benign breast disease (BBD). Controls were diagnosed with BBD but did not subsequently develop breast cancer. Cases and controls were matched on age at cancer diagnosis, year of BBD diagnosis, and years between BBD diagnosis and cancer diagnosis. WSIs of normal tissue from BBD biopsies were available for 287 cases and 1083 controls.
- Our computational method captured three standardized TDLU involution measures [5] and five novel measures (**Figure 2**).

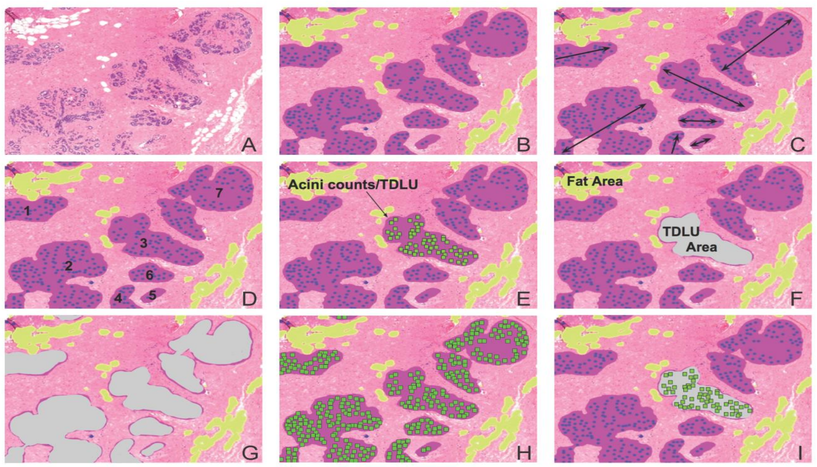

Figure 2. (A) A region of a whole slide image. **(B)** Our computational pathology method segments terminal duct lobular units (TDLUs; purple areas), detects acini (blue dots), and segments adipose tissue (yellow areas). Quantitative TDLU involution measures investigated in this study consisted of the three standardized measures (median TDLU span **(C)**, TDLU counts per non-adipose tissue area **(D)**, and median acini counts per TDLU **(E)**), and five novel measures (median TDLU area **(F)**, total TDLU area as a percentage of tissue area and non-adipose tissue area **(G)**, total number of acini (detected in TDLUs) per non-adipose tissue area **(H)**, and median acini density **(I)**).

Materials & Methods

- Qualitative assessments of TDLU involution by pathologists were conducted in a subset of 177 cases and 857 controls: no type 1 lobules, mixed type; and predominant lobule type 1 no type 3 lobules.
- Breast cancer risk factors included age at BBD biopsy, BBD histological subtypes, body size (body size at ages 5-10 and BMI at BBD diagnosis), and reproductive factors (age of menarche, parity, age at first birth, birth index, breast feeding, and menopausal status). Birth index incorporates age at first birth, number of children, and spacing of births.
- The associations between breast cancer risk factors and quantitative involution measures among controls were assessed using analysis of covariance (ANCOVA) adjusting for age at BBD biopsy.
- Each quantitative TDLU measure was categorized into quartiles as defined by the distribution among the controls; the relationship between each quantitative TDLU involution measure and breast cancer risk was evaluated using unconditional logistic regression, adjusting for the matching factors and BBD histological subtypes. Analyses were also stratified by parity and menopausal status.

Results

- Menopausal status and parity were significantly associated with all eight quantitative measures after adjusting for age (p<0.05).
- Select TDLU measures were associated with BBD histological subtype, BMI, and birth index (p<0.05).
- No quantitative measure was significantly correlated with body size at ages 5-10 years, age of menarche, age at first birth, or breastfeeding (p>0.05; **Figure 3**).

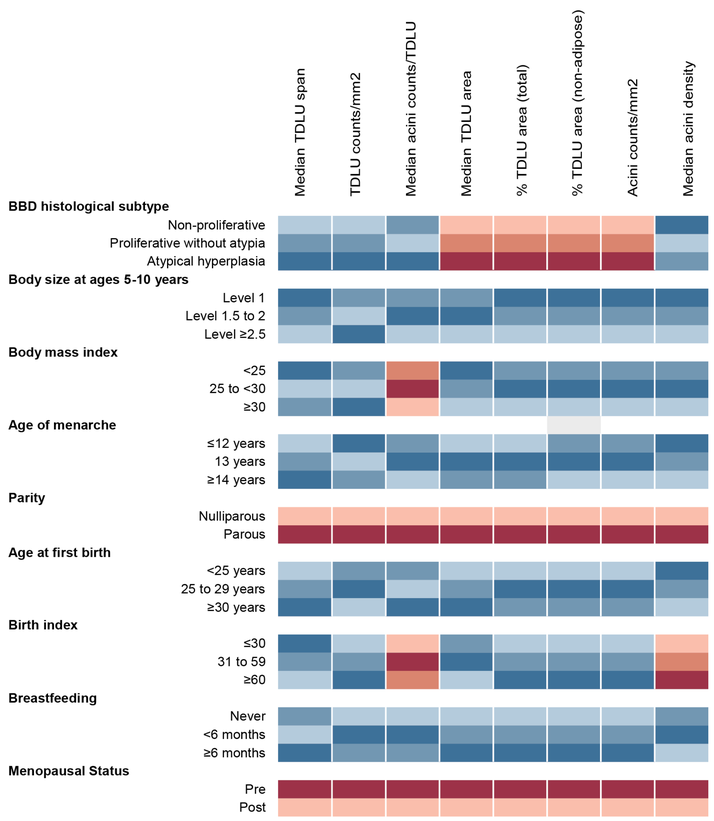

Figure 3. Summary of the associations between breast cancer risk factors and quantitative terminal duct lobular unit (TDLU) measures among 1083 controls. The numbers are p-values derived from ANCOVA analyses using age-adjusted means. Pink cells indicate significant associations achieving p<0.05. Color intensity of the cells reflect the ranking of their age-adjusted means.

Results

- No quantitative TDLU involution measure in normal breast tissue was associated with subsequent breast cancer risk (p-trends ranged from 0.409 to 0.984; **Table 1**).
- These null findings were consistent within strata of parity and menopausal status.
- In the subset of women with qualitative assessment, categories of TDLU involution were also not associated with breast cancer risk (mixed type versus no type 1, adjusted OR=1.15, 95% CI 0.69-1.98; predominant lobule type 1 no type 3 versus no type 1, adjusted OR=0.95, 95%CI 0.54-1.71).

Table 1. The association between automated terminal duct lobular unit (TDLU) measures and breast cancer risk was evaluated using unconditional logistic regression models adjusting for the matching factors and BBD histological subtypes to estimate odd ratios (ORs) and 95% confidence intervals (CI). Each quantitative TDLU measure was categorized into quartiles as defined by the distribution among the controls. The median value for each quartile was included as a continuous variable in the unconditional logistic regression together with matching factors and BBD histological subtypes to obtain the p-trend value (Wald test).

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*p*-trend
Median TDLU span					
Cases/Controls, *n*	65/271	72/270	73/271	77/271	
Model	Ref	0.94 (0.64,1.39)	0.92 (0.62,1.37)	0.89 (0.59,1.33)	0.562
TDLU counts/mm2					
Cases/Controls, *n*	67/271	73/270	71/271	76/271	
Model	Ref	1.04 (0.71,1.53)	0.96 (0.65,1.41)	1.15 (0.79,1.69)	0.492
Median acini counts/TDLU					
Cases/Controls, *n*	26/121	79/348	89/311	93/303	
Model	Ref	0.94 (0.57,1.57)	1.00 (0.61,1.69)	1.05 (0.64,1.77)	0.585
Median TDLU area					
Cases/Controls, *n*	58/271	78/270	66/271	85/271	
Model	Ref	1.15 (0.78,1.71)	0.87 (0.57,1.31)	1.1 (0.73,1.66)	0.895
% TDLU area (total)					
Cases/Controls, *n*	58/271	82/270	63/271	84/271	
Model	Ref	1.15 (0.78,1.71)	0.86 (0.57,1.30)	1.04 (0.69,1.58)	0.897
% TDLU area (non-adipose)					
Cases/Controls, *n*	58/271	87/270	57/271	85/271	
Model	Ref	1.23 (0.84,1.82)	0.80 (0.52,1.21)	1.1 (0.73,1.66)	0.984
Acini counts/mm2					
Cases/Controls, *n*	64/271	71/270	69/271	83/271	
Model	Ref	0.98 (0.67,1.45)	0.86 (0.58,1.29)	1.03 (0.69,1.53)	0.830
Median acini density					
Cases/Controls, *n*	57/271	89/270	61/271	80/271	
Model	Ref	1.54 (1.05,2.27)	1.05 (0.70,1.58)	1.36 (0.92,2.01)	0.409

Conclusion

- In this large dataset, automated quantitative metrics of TDLU involution were associated with aging and reproductive breast cancer risk factors.
- However, our automated estimates of TDLU involution in normal tissue from BBD biopsies were not associated with breast cancer risk.
- Further work will include applying our method to assess TDLU involution and breast cancer risk in other large epidemiological cohorts.

Acknowledgments

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