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Characterizing Novel Radiologic And Pathologic Tissue-Based Risk Factors For Breast Cancer In African American Women With Benign Breast Disease

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CHARACTERIZING NOVEL RADIOLOGIC AND PATHOLOGIC TISSUE-BASED RISK FACTORS FOR BREAST CANCER IN AFRICAN AMERICAN WOMEN WITH BENIGN BREAST DISEASE

by

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DISSERTATION

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DEDICATION

To my family, for their love, support, and teaching me the value of education and service.

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CHAPTER 1: INTRODUCTION

I. Breast cancer disparities: mortality and tumor biology

Breast cancer is the most common cancer and second leading cause of death for women in the United States[1]. Although breast cancer incidence is equal among European American and African American women, African American women are especially burdened with a 42% higher breast cancer mortality rate[2]. The underlying causes of this survival disparity are still debated and have been partially attributed to social determinants including differential environmental exposure, access to care and quality of care issues[3–6]. Breast cancer stage of diagnosis further contributes to this disparity as African American women are not only more likely to be diagnosed with breast cancer at later stage disease, but their 5-year survival is lower than that of other ethnic groups at every stage[7].

Consistent reports that African American women suffer higher rates of young-onset breast cancer – cancer diagnosed before the age of 35 that is additionally associated with more aggressive disease – than women of all other ethnic groups point to differences in tumor biology[8–10]. Molecular grading and subtyping furthered this understanding. Even after adjusting for age and stage of disease, African American women are more likely to have high-grade tumors[11,12]. They are also more likely to be diagnosed with aggressive subtypes characterized by the lack of hormone receptors which are resistant to hormone therapy and marked by poorer survival[9,11,12]. Genetic profiles of stage I-III breast tumors in The Cancer Genome Atlas (TCGA) indicate more aggressive and recurrent tumors in African American women: these show higher intratumoral genetic heterogeneity, more basal-like (PAM50) signatures, and more TP53 mutations than tumors from European American women[13]. Transcriptional profiles of stage I-III breast tumors in TCGA also suggest differences by race. Resistin, associated with insulin resistance and obesity, is upregulated while LOC90784, a long non-coding RNA inversely associated with breast cancer stage and the triple negative subtype, is downregulated in tumors from African American women compared to tumors from European American women[14]. As our understanding of cancer biology

progresses, it is likely that additional mechanisms will be identified that contribute to racial differences and disparities in breast cancer.

II. Breast cancer risk factors

Breast cancer risk factors and risk models

Our understanding of breast cancer risk factors can be synthesized with breast cancer risk models such as the Gail model, more commonly known as the National Cancer Institute's Breast Cancer Risk Assessment Tool (BRCAT)[15]. This tool incorporates information from several risk factors including estrogen exposure, family history and prior biopsy history. Risk models provide patients, physicians and scientists estimates of absolute risk of developing breast cancer over a specified period, typically over the next five years or total lifetime. BRCAT can be used clinically to identify women at high risk who may benefit from increased screening for early detection of breast cancer or chemoprevention to reduce breast cancer risk.

Although useful, breast cancer risk models do not discriminate between women destined to develop or not to develop breast cancer with a high degree of accuracy. The ability to separate patients who are destined to become cases from controls is typically measured by the area under the receiver operator characteristic curve (AUC). The BRCAT has an AUC of 0.6 – a low to moderate level of discriminatory accuracy[16]. Risk model discriminatory accuracy can be improved by adding risk factors that capture new biologic information that reflect breast cancer risk or carcinogenesis. Models that have incorporate additional biological information not already captured by the BRCAT such as mammographic density and genetic SNP scores perform better with an AUC around 0.68[16]; however, the moderate level highlights the clear need to identify new biological risk factors to improve breast cancer risk assessment.

The BRCAT model was created using risk factors estimated from primarily European American women populations, and the use of this tool in African American women is limited as the model underestimates breast cancer risk in this population[17]. The Gail model was modified for use in African American populations using the Women's Contraceptive and Reproductive

Experiences (CARE) Study. Unfortunately, this model still underestimates breast cancer risk for African American women with a prior breast biopsy[17–19]. Risk models for this population can improve by providing robust, race-specific risk factor estimates from cohort studies.

Current breast cancer risk models primarily estimate the risk of developing ER positive breast cancer over a specified period. Model accuracy could improve with the addition of ER negative or subtype-specific models. Several studies indicate that risk factors can vary in strength by breast cancer subtype[20–23]. As ER-negative and triple-negative breast cancers are more prevalent in African American women[1,9,12], African American women may be especially poised to benefit from subtype-specific models. Further incorporation of other biological variables that are more prevalent in African American women, such as obesity, may also improve risk models in this population.

Obesity and breast cancer risk

Obesity, or excess body fat, has long been associated with breast cancer and is more prevalent in the African American population, but this association is altered by menopausal status. Obesity is associated with a reduced breast cancer risk in premenopausal women but an increased breast cancer risk in postmenopausal women[24,25]. This conflicting risk is thought to be due to the influence of obesity on the hormonal milieu of patients. Estrogen exposure is highly associated with hormone receptor positive breast tumors[26]. In premenopausal women, excess body fat is associated with anovulatory or irregular menstrual cycles and lower circulating estrogen levels[27], subsequently leading to a decrease in breast cancer risk. Postmenopausal women not receiving hormone replacement therapy have low circulating levels of estrogen, so the aromatization of androgens to estrogens in excess adipose tissue can significantly increase estrogen levels that lead to increased risk of hormone receptor positive tumors[26,28]. As most breast cancers develop in post-menopausal women and are hormone-receptor positive, obesity is an important risk factor for breast cancer.

Estrogen receptor (ER) negative and triple negative tumors occur more frequently in premenopausal obese women compared to premenopausal normal weight women[29,30]. These tumors are generally more aggressive and have a poorer prognosis[31]. Though these tumors lack estrogen receptors, estrogen may play a role in pathogenesis. Patients with BRCA1- or BRCA2 mutations undergoing prophylactic oophorectomy, which effectively lowers total estrogen exposure, results in significant reduction of estrogen receptor negative breast cancer risk in both pre- and post-menopausal women[32]. Other mechanisms that may contribute to the risk increase associated with obesity are increased insulin on the Akt/mTOR signaling pathway, the release of inflammatory cytokines, and changes to the breast tissue microenvironment[33].

African American women experience an obesity prevalence about 50% higher than European American women, and 70% higher for women under the age of 40[34]. This increase in obesity may contribute to the increased incidence of young-onset and estrogen receptor negative breast cancers in African American women. In African Americans, an increased waist-to-hip ratio is also associated with triple negative tumors in both pre- and post-menopausal women[35]. Increased waist-to-hip ratio is associated with a visceral fat distribution where fat surrounds the abdominal organs. Visceral fat is associated with higher rates of metabolic syndrome, inflammation, and postmenopausal breast cancer[36].

Benign breast disease and the subsequent risk of breast cancer

Approximately 1.6 million breast biopsies are performed each year in the United States[37], and most result in non-malignant findings, or benign breast disease (BBD). Though these pathologies are not malignant, the presence of BBD on biopsy is associated with increased risk of developing in situ or invasive breast cancer[38]. BBD lesions can be categorized by Dupont and Page criteria for the presence of epithelial proliferation and atypical cells as these two characteristics confer higher risk of subsequent breast cancer in several cohorts of European American women[38,39]. BBD features are heterogeneous for breast cancer risk: columnar alterations, or morphological changes in the breast epithelium, are associated with increased risk

while lobular involution, or atrophy of fibroglandular breast tissue, is associated with decreased risk[38,40].

BBD has not been well studied in African American women as the majority of studies were conducted in European American populations. Worsham et al[41,42] included African American women in their analysis of BBD and breast cancer, but the sample size was limited and studied retrospectively. There is evidence that BBD presents differently in African American women - fibroadenomas, benign tumors of stromal and epithelial tissue, occur and recur more frequently in African American women than European American women[43]. As BBD may present differently in African American women, further study of BBD is imperative in this population to identify women who are at high risk of developing in situ or invasive breast cancer.

Breast density and breast cancer risk

Breast density describes the appearance of breast tissue on mammogram – fibroglandular tissue appears dense or radiopaque and adipose tissue appears non-dense or radiolucent. Both qualitative and quantitative assessments of breast density are strongly associated with breast cancer risk[44–46]. Dense tissue areas on mammograms are associated with location[47] and hormone receptor status[48] of subsequently arising cancer. Dense areas on mammogram can also mask small tumors, delaying cancer diagnosis.

The majority of breast density studies were conducted in European American or European populations and has not been well studied in African American women. The multi-center Breast Cancer Screening Consortium[49] included about 2800 African American women, but as this constituted around only 6% of study participants, did not provide race-specific estimates for breast cancer risk. Other studies have suggested differences by race[50] and McCarthy et al[51] found that African American women were more likely to have dense breasts than European American women once adjusting for age and BMI. Additionally, very few studies[49,52] have been able to examine breast density in women with BBD. Assessing both density and BBD is important to understanding how these factors may modify or interact relationships with breast cancer risk.

III. Rationale

This dissertation examines radiologic and pathologic tissue-based risk factors for breast cancer in African American women, an underserved population experiencing increasing breast cancer incidence and high mortality burden. Ultimately, we hope to improve risk estimation in this population for better clinical management, to prevent or detect breast cancers earlier to improve breast cancer survival. The importance of breast cancer risk factors is not limited to absolute risk estimates: risk factors can also reveal information about breast carcinogenesis. Long-term goals of this dissertation are to further our understanding of breast carcinogenesis in African American women and find features that may predict breast cancer in this population. In this dissertation, we characterize several known and previously not described risk factors on mammograms and pathology tissue from the Detroit BBD cohort to provide race-specific risk factor estimates and new biological information to improve breast cancer risk models in African American women.

The Detroit BBD cohort[53] comprises African American women aged 18 to 84 diagnosed with BBD between 1997 and 2010 in the metropolitan Detroit area. Women with a history or diagnosis within six months of invasive or *in situ* breast carcinoma were excluded from this Institutional Review Board approved study. Breast biopsy tissue was examined by study pathologists for Dupont and Page criteria[39] and individual BBD lesions. In situ and invasive breast carcinomas were ascertained by linkage to the Metropolitan Detroit Cancer Surveillance System (MDCSS), a founding member of the National Cancer Institute's Surveillance Epidemiology and End Results (SEER) program. This dissertation utilizes the strengths of this existing BBD cohort and biospecimens to further characterize current and novel breast cancer risk factors in African American women. As tissue reflects both genetic and environmental determinants of disease, this is a valuable resource to understand breast cancer risk.

The specific aims of the dissertation were:

1. To determine whether risk of subsequent breast cancer associated with fibroadenomas on benign breast biopsy in African American women differs from European American women.
2. To characterize the association between qualitative density and parenchymal patterns on mammographic images and breast cancer.
3. To examine whether crown-like structures on benign breast biopsy were associated with breast cancer risk.

CHAPTER 2: BREAST FIBROADENOMAS ARE NOT ASSOCIATED WITH SUBSEQUENT BREAST CANCER RISK IN AN AFRICAN AMERICAN COHORT

I. Introduction

Over 1.5 million breast biopsies are pathologically assessed annually in the United States, indicated by abnormal mammography findings or patient complaints[37]. Most biopsies are not malignant, but instead exhibit a number of pathological lesions that constitute benign breast disease (BBD). Biopsies that exhibit proliferative disease or cellular atypia, as defined by Dupont and Page criteria, are consistently associated with increases in breast cancer risk[38,39,53]. These pathologic criteria have been included in risk assessment models to identify women at high risk of developing breast cancer. Several current risk assessment models, including the frequently used Breast Cancer Risk Assessment tool, incorporate information on the number of prior biopsies and the presence of atypia, but do not account for other BBD lesions that may independently increase breast cancer risk[15]. Reliable estimates of breast cancer risk associated with individual lesions can improve risk models, allowing physicians to better identify women at high risk of developing breast cancer who may benefit from additional screening or chemoprevention.

One type of BBD, fibroadenomas, are well-circumscribed benign tumors of epithelial and stromal tissue[54] (Figure 1). Breast fibroadenomas most frequently occur in women in their 20s[54] but can occur at any age; it is estimated that 10% of women have breast fibroadenomas[55]. A recent meta-analysis of 11 studies reported an increase in breast cancer risk by 41% (95% CI: 11-80%) for women diagnosed with a fibroadenoma compared to women without fibroadenoma on biopsy; however, this estimate exhibits significant statistical heterogeneity[56]. Furthermore, the studies in this meta-analysis were primarily from European ancestral populations, and several were conducted prior to the widespread use of screening mammography in the 1980s. Although African American women experience a higher incidence and recurrence of fibroadenomas at a

younger age[43,57], breast cancer risk associated with this lesion has not been independently assessed in this population of women.

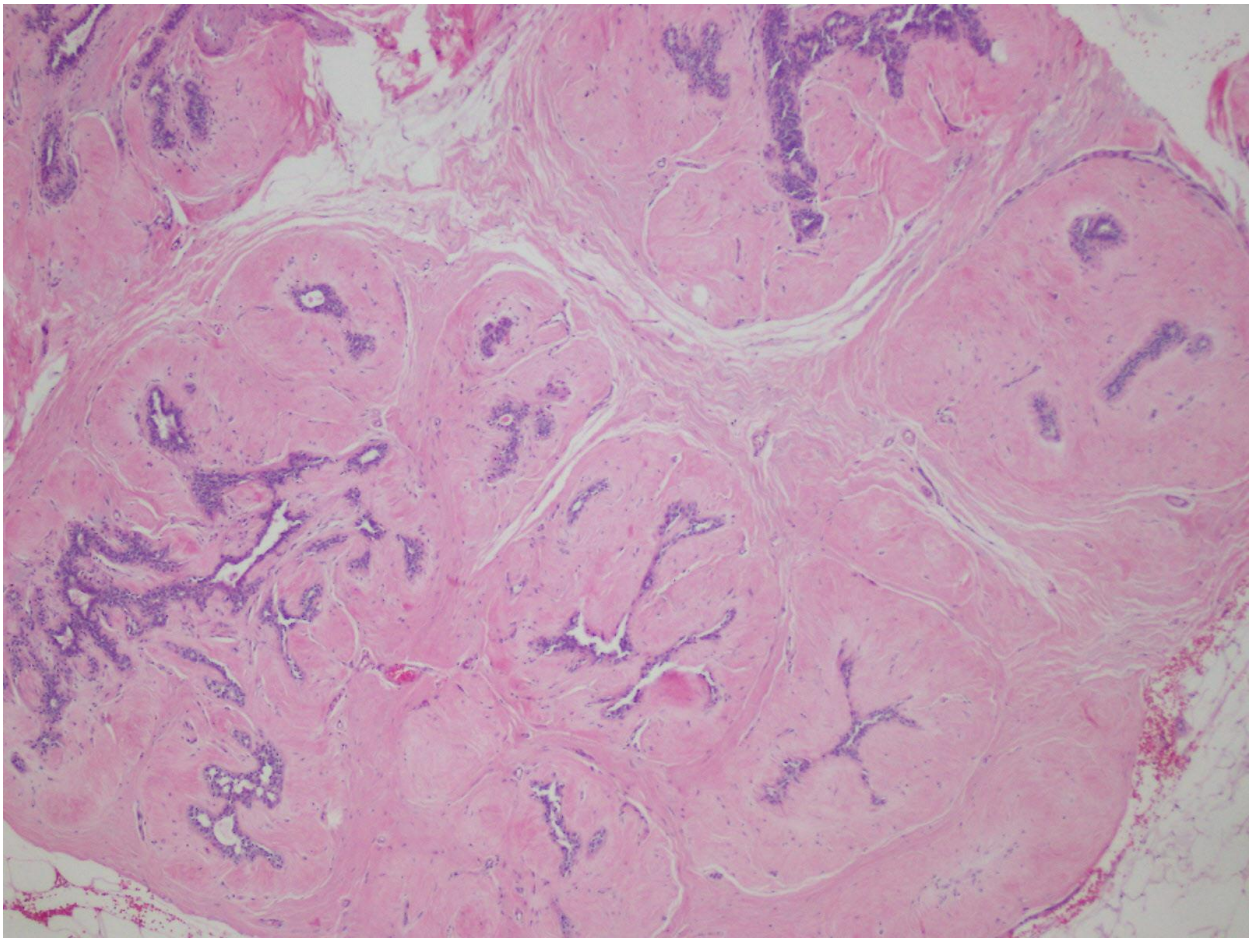


Fig. 1 Fibroadenoma. Fibroadenomas are benign tumors of stromal and epithelial tissue that are typically well-circumscribed and mobile within the tissue. The fibroadenoma shown here exhibits purple epithelial tissue surrounded by pink fibrotic stromal tissue (hematoxylin-eosin; original magnification 100x)

African American women suffer a 42% higher breast cancer mortality rate than European American women[2], a burden that partly stems from differences in tumor biology. African American women are more likely to develop breast cancer at a younger age[8–10] and more likely to be diagnosed with aggressive tumors characterized by high molecular grade[11,12] and lack of hormone receptors[9,11,12]. Despite this survival disparity, prior investigations on BBD and breast cancer risk

focused on primarily European American cohorts. A study cohort from Henry Ford Hospital in Detroit was the first to include a considerable number (1200+) of African American women[41,42,58], but studies in African American women are largely lacking. The goal of this study is to examine in a contemporary cohort whether breast cancer risk associated with fibroadenoma differs for African American women, a population who are more likely to present with fibroadenomas and more likely to develop aggressive breast cancers that respond poorly to treatment.

II. Materials and Methods

Study design

We conducted a retrospective cohort study to investigate the subsequent breast cancer risk associated with a fibroadenoma on biopsy. This cohort consists of African American women diagnosed with BBD between 1997 and 2010 in metropolitan Detroit who were passively followed for breast cancer current to December 2015. Our main exposure of interest was the presence or absence of fibroadenoma on biopsy; our main outcome of interest was the diagnosis of *in situ* or invasive breast carcinoma.

We first identified which features were more common by presence or absence of fibroadenoma on biopsy. In our analyses of associated breast cancer risk in this cohort, we adjusted for likely confounders including age and previously identified categorizations of BBD: epithelial proliferation and cellular atypia. To understand this breast cancer risk in the context of population level risk, we finally compared *in situ* and invasive breast carcinoma incidence in this cohort to that of the larger metropolitan Detroit population.

Study population

African American women with their first benign breast biopsies conducted between 1997 and 2010 were identified using University Pathology Group (UPG; Detroit, MI) records. UPG provides pathology services to hospitals in metropolitan Detroit including Sinai Grace, Harper Hospital, and Karmanos Cancer Institute. Women aged 18 to 84 at time of benign breast biopsy were eligible for this Institutional Review Board approved study. Exclusionary criteria included: a

diagnosis of invasive or *in situ* breast carcinoma before or within six months of the breast biopsy, a history of mastectomy or reduction mammoplasty, lipoma, fat necrosis, epidermal cysts, hematoma, accessory structure, phyllodes tumor, or a lymph node biopsy without breast tissue. For this type of study, the Wayne State University Institutional Review Board determined that written informed consent was not required. Data on age at biopsy, date of birth and date of biopsy for all women in this cohort were collected for this study.

Histology review

Core needle and excisional benign biopsies were microscopically reviewed by blinded study pathologists using original hematoxylin and eosin (H&E) slides. Slides from the first biopsy were assessed for the presence of BBD lesions and lobular involution similar to the Mayo Clinic's BBD study[38]. In total, 12 pathologic lesions including apocrine metaplasia, calcifications, columnar alterations, cysts, duct ectasia, ductal hyperplasia, fibroadenoma, fibrosis, intraductal papilloma, lobular hyperplasia, radial scars, and sclerosing adenosis were assessed. A biopsy could indicate the presence of one or multiple lesions.

The biopsies were additionally categorized into three groups using criteria described by Dupont and Page[39] to control for the presence of proliferative disease and cellular atypia, previously shown to be strongly associated with breast cancer risk. Biopsies were categorized as non-proliferative disease if these included only fibroadenoma, cysts, fibrosis, ductal ectasia, mild ductal hyperplasia, mild lobular hyperplasia, apocrine metaplasia, radial scars, calcifications, and/or columnar alterations. Biopsies that also included intra-ductal papilloma, sclerosing adenosis, moderate to florid ductal hyperplasia, moderate to florid lobular hyperplasia and/or columnar alterations with hyperplasia were categorized as proliferative disease. Biopsies that included atypia (in addition to ductal hyperplasia, lobular hyperplasia, fibroadenoma and/or columnar alterations) were categorized as proliferative disease with atypia.

Biopsies classified as showing atypia and a random sample of all other biopsies were re-assessed by a blinded study pathologist at the Mayo Clinic. Breast biopsies that could not be

assessed for fibroadenoma presence due to limited tissue were excluded from analysis (N=23). Data on the presence or absence of the pathologic lesions, proliferative disease and cellular atypia were collected for this study.

In situ and invasive carcinoma ascertainment

Women who developed *in situ* or invasive breast carcinoma were identified through hospital medical records and also through the use of the Metropolitan Detroit Cancer Surveillance System (MDCSS), a founding member of the National Cancer Institute's Surveillance Epidemiology and End Results (SEER) program for more complete follow-up. MDCSS collects cancer incidence, treatment and survival data in the tri-county metropolitan Detroit area. Utilization of both data sources allowed the identification of cancers in women residing in the entire tri-county metropolitan Detroit area. Women were matched between UPG records and MDCSS using name, date of birth, and/or social security number; follow-up information was complete to December 31, 2015. Data on the diagnosis of *in situ* or invasive breast cancer, date of diagnosis and vital status were collected for this study.

Statistical Analysis

Our first objective was to examine whether fibroadenomas were associated with other benign lesions on biopsy; we evaluated these associations using chi-square tests. Our second objective was to examine the risk of breast cancer associated with fibroadenomas relative to other non-fibroadenoma BBD. This objective was evaluated within the Detroit cohort using relative risk ratios and 95% confidence intervals calculated using multivariable log-binomial regression and adjusting for age at biopsy. Regression models were further adjusted with the Dupont and Page criteria (epithelial proliferation and cellular atypia on biopsy), likely confounders consistently identified in prior studies of BBD, and backwards selection based on Bayesian information criterion (BIC) to fully adjust analyses for other potential confounders. Our third objective was to examine whether breast cancer risk associated with fibroadenoma presence on biopsy differed by likely menopausal status; we evaluated this risk difference by stratifying the regression models

by age (below or above 50 years). Our fourth objective was to examine whether the time to breast cancer diagnosis differed by fibroadenoma presence on biopsy. We evaluated this time to diagnosis using competing risk analysis with death due to any cause other than breast cancer considered as a competing risk.

Our last objective was to examine the excess risk of breast cancer associated with having a biopsy, with or without fibroadenoma, compared to that of the general population. We estimated this excess risk using age-adjusted standardized incidence ratios (SIRs) calculated from SEER estimates of *in situ* and invasive breast cancer incidence in African American women in MDCSS from 1999 to 2015.

III. Results

Distribution of BBD features and characteristics by fibroadenoma status

3,845 benign breast biopsies were assessed in this African American cohort, 1,798 (47%) of which were diagnosed with fibroadenoma. Median length of follow-up was 13.0 years (range 0.5 – 19.0 years); median time to breast cancer diagnosis was 7.3 years (range 0.7 – 18.5 years). Fibroadenomas showed high concordance between study pathologists from KCI and the Mayo clinic (86.9%; Cohen's κ = 0.7022, Table 1) and the highest Cohen's κ of all BBD lesions described on biopsy.

Table 1. Inter-observer agreement on fibroadenoma presence between study pathologists in a subset of the Detroit Benign Breast Disease cohort, 1997-2010

		KCI [b] read		
	N (%)	Absent	Present	Total
Mayo [a] read	Absent	92 (63.89%)	6 (4.17%)	98
	Present	12 (8.33%)	34 (23.61%)	46
	Total	104	40	144[c]

[a]Mayo pathologist Daniel W. Visscher, MD
[b]KCI pathologists Rouba Ali-Fehmi, MD and Susdeshna Bandyopadhyay, MD
[c]145 blocks were reviewed in total. One case could not be assessed for fibroadenoma presence and contributed to the final concordance rate, discordance rate, and Cohen's κ

Women with a fibroadenoma on biopsy were more likely to be younger than women without a fibroadenoma on biopsy ($p<0.001$) (Table 2). Women with fibroadenoma on biopsy had 1.6 times higher odds of having a core-needle biopsy than excisional biopsy compared to women without a fibroadenoma on biopsy ($p<0.001$). The presence of other benign breast lesions was less likely to be indicated on biopsies containing a fibroadenoma. Women with fibroadenoma on biopsy were 4 to 5.6 times lower odds of exhibiting cysts or intraductal papilloma on biopsy than women without fibroadenoma on biopsy (both $p<0.001$). Women with fibroadenoma on biopsy had a 3 to 4 times lower odds of exhibiting apocrine metaplasia, fibrosis or columnar alterations on biopsy than women without fibroadenoma on biopsy (all $p<0.001$). Women with fibroadenoma on biopsy had a 2 to 3 times lower odds of exhibiting ductal hyperplasia, lobular hyperplasia, ductal ectasia, sclerosing adenosis or radial scars on biopsy than women without fibroadenoma on biopsy (lobular hyperplasia $p=0.008$; all other $p<0.001$). Women with fibroadenoma on biopsy were 1.67 times lower odds of exhibiting calcifications on biopsy than women without fibroadenoma on biopsy ($p<0.001$). Additionally, biopsies with a fibroadenoma were less likely to be classified as proliferative disease (25.0%) or proliferative disease with atypia (1.3%) compared to biopsies without a fibroadenoma (51.5% and 6.1%, respectively).

Several associations between fibroadenomas and other benign breast lesions on biopsy remained after separating women by likely menopausal status by age (Tables 3-4). Notable exceptions were calcifications and proliferative disease with atypia on biopsy by fibroadenoma status. Under the age 50, women with fibroadenoma on biopsy were 3 times less likely to exhibit calcifications on biopsy compared to women without a fibroadenoma on biopsy; in women aged 50 or older, women with fibroadenoma on biopsy were 1.26 times more likely to exhibit calcifications on biopsy compared to women without a fibroadenoma on biopsy (95% confidence interval 2.4 – 3.8 and 1.03 – 1.54, respectively). Under the age 50, women with fibroadenoma

on biopsy were 20 times less likely to exhibit proliferative disease with atypia on biopsy compared to women without a fibroadenoma on biopsy; in women aged 50 or older, women with fibroadenoma on biopsy were 4.6 times less likely to exhibit proliferative disease with atypia on biopsy compared to women without fibroadenoma on biopsy (95% confidence interval 9.1 – 100 and 2.8 – 7.7, respectively).

Table 2. Distribution of benign breast features and other characteristics by fibroadenoma status in African American women in the Detroit BBD cohort, 1997-2010

Characteristic	Fibroadenoma status, N (%)[a]		Odds ratio (95% CI)	*P* value[b]
	Absent	Present		
	2047 (53.2)	1798 (46.8)		
Age at benign biopsy				<0.001
<40	387 (18.9)	573 (31.9)	Ref	
40-49	692 (33.8)	582 (32.4)	0.57 (0.48 – 0.67)	
50-59	577 (28.2)	374 (20.8)	0.44 (0.36 – 0.53)	
60-69	249 (12.2)	164 (9.1)	0.45 (0.35 – 0.56)	
70+	142 (6.9)	105 (5.8)	0.50 (0.38 – 0.66)	
Biopsy Type				<0.001
Excisional	826 (40.4)	536 (30.8)	Ref	
Core Needle	1221 (59.6)	1262 (70.2)	1.59 (1.39 – 1.82)	
Apocrine Metaplasia				<0.001
Absent	1202 (58.7)	1401 (82.3)	Ref	
Present	845 (41.3)	301 (17.7)	0.31 (0.26 – 0.36)	
Ductal Hyperplasia				<0.001
Absent	1272 (62.1)	1365 (80.6)	Ref	
Present	775 (37.9)	329 (19.4)	0.40 (0.34 – 0.46)	
Lobular Hyperplasia				0.008
Absent	2012 (98.3)	1662 (99.3)	Ref	
Present	34 (1.7)	11 (0.7)	0.40 (0.19 – 0.76)	
Calcifications				<0.001
Absent	1209 (59.1)	1229 (70.8)	Ref	
Present	837 (40.9)	507 (29.2)	0.60 (0.52 – 0.68)	
Cysts				<0.001
Absent	970 (47.4)	1339 (78.9)	Ref	
Present	1076 (52.6)	359 (21.1)	0.24 (0.21 – 0.28)	
Duct Ectasia				<0.001
Absent	1652 (80.7)	1546 (91.0)	Ref	
Present	394 (19.3)	152 (9.0)	0.41 (0.34 – 0.50)	
Fibrosis				<0.001
Absent	648 (31.7)	1031 (63.8)	Ref	
Present	1397 (68.3)	586 (36.2)	0.26 (0.23 – 0.30)	
Intraductal Papilloma				<0.001
Absent	1662 (81.2)	1629 (96.1)	Ref	
Present	385 (18.8)	66 (3.9)	0.18 (0.13 – 0.22)	
Sclerosing Adenosis				<0.001
Absent	1416 (69.2)	1404 (82.7)	Ref	
Present	630 (30.8)	294 (17.3)	0.47 (0.40 – 0.55)	
Columnar Alterations				<0.001
Absent	1302 (63.6)	1439 (84.7)	Ref	
Present	744 (30.8)	259 (15.3)	0.32 (0.27 – 0.37)	
Radial Scar				<0.001
Absent	1975 (96.5)	1665 (98.6)	Ref	
Present	71 (3.5)	23 (1.4)	0.39 (0.23 – 0.61)	
Dupont and Page criteria				<0.001
Nonproliferative disease	868 (42.4)	1325 (73.7)	Ref	
Proliferative disease without atypia	1054 (51.5)	450 (25.0)	0.28 (0.24 – 0.32)	
Proliferative disease with atypia	125 (6.1)	23 (1.3)	0.12 (0.08 – 0.19)	
Developed breast cancer				<0.001
No	1902 (92.9)	1722 (95.8)	Ref	
Yes	145 (7.1)	76 (4.2)	0.58 (0.43 – 0.77)	

[a]Numbers may not sum to the total number of patients if features could not be assessed on biopsy

[b]χ^2 test comparing distribution of features across absence or presence of fibroadenoma on biopsy

Table 3. Distribution of benign breast features and other characteristics by fibroadenoma status for African American women under the age 50 in the Detroit BBD cohort, 1997-2010

Characteristic	Fibroadenoma status, N (%)[a]		Odds ratio	*P* value[b]
	Absent	Present	(95% CI)	
	1079 (48.3)	1155 (51.7)		
Age at benign biopsy				<0.001
<40	387 (35.9)	573 (49.6)	Ref	
40-49	692 (64.1)	582 (50.4)	0.57 (0.48 – 0.67)	
Biopsy Type				<0.001
Excisional	450 (41.7)	371 (32.1)	Ref	
Core Needle	629 (58.3)	784 (77.9)	1.51 (1.27 – 1.80)	
Apocrine Metaplasia				<0.001
Absent	645 (59.8)	905 (83.7)	Ref	
Present	434 (40.2)	176 (16.3)	0.29 (0.24 – 0.35)	
Ductal Hyperplasia				<0.001
Absent	690 (63.9)	876 (81.5)	Ref	
Present	389 (36.1)	199 (18.5)	0.40 (0.33 – 0.49)	
Lobular Hyperplasia				0.072
Absent	1069 (99.2)	1065 (99.8)	Ref	
Present	9 (0.8)	2 (0.2)	0.24 (0.03 – 0.94)	
Calcifications				<0.001
Absent	743 (68.9)	960 (87.1)	Ref	
Present	336 (31.1)	142 (12.9)	0.33 (0.26 – 0.41)	
Cysts				<0.001
Absent	526 (48.7)	860 (79.9)	Ref	
Present	553 (51.3)	217 (20.1)	0.24 (0.20 – 0.29)	
Duct Ectasia				<0.001
Absent	853 (79.1)	977 (90.7)	Ref	
Present	226 (20.9)	100 (9.3)	0.39 (0.30 – 0.50)	
Fibrosis				<0.001
Absent	323 (30.0)	663 (64.9)	Ref	
Present	755 (70.0)	359 (35.1)	0.23 (0.19 – 0.28)	
Intraductal Papilloma				<0.001
Absent	896 (83.0)	1045 (97.1)	Ref	
Present	183 (17.0)	31 (2.9)	0.15 (0.10 – 0.21)	
Sclerosing Adenosis				<0.001
Absent	712 (66.0)	887 (82.4)	Ref	
Present	367 (34.0)	190 (17.6)	0.42 (0.34 – 0.51)	
Columnar Alterations				<0.001
Absent	705 (65.3)	931 (86.4)	Ref	
Present	374 (34.7)	146 (13.6)	0.30 (0.24 – 0.37)	
Radial Scar				0.003
Absent	1042 (96.6)	1061 (98.6)	Ref	
Present	37 (3.4)	15 (1.4)	0.40 (0.21 – 0.72)	
Dupont and Page criteria				<0.001
Nonproliferative disease	479 (44.4)	861 (74.5)	Ref	
Proliferative disease without atypia	549 (50.9)	290 (25.1)	0.29 (0.25 – 0.35)	
Proliferative disease with atypia	51 (4.7)	4 (0.3)	0.05 (0.01 – 0.11)	
Developed breast cancer				0.021
No	1027 (95.2)	1122 (97.1)	Ref	
Yes	52 (4.8)	33 (2.9)	0.58 (0.37 – 0.90)	

[a]Numbers may not sum to the total number of patients if features could not be assessed on biopsy

[b]χ^2 test comparing distribution of features across absence or presence of fibroadenoma on biopsy

Table 4. Distribution of benign breast features and other characteristics by fibroadenoma status for African American women aged 50 or older in the Detroit BBD cohort, 1997-2010

Characteristic	Fibroadenoma status, N (%)[a]		Odds ratio	*P* value[b]
	Absent	Present	(95% CI)	
	968 (60.1)	643 (39.9)		
Age at benign biopsy				0.658
50-59	577 (59.6)	374 (58.2)	Ref	
60-69	249 (25.7)	164 (25.5)	1.02 (0.80 – 1.29)	
70+	142 (14.7)	105 (16.3)	1.14 (0.86 – 1.51)	
Biopsy Type				<0.001
Excisional	376 (38.8)	165 (25.7)	Ref	
Core Needle	592 (61.2)	478 (74.3)	1.84 (1.48 – 2.29)	
Apocrine Metaplasia				<0.001
Absent	557 (57.5)	496 (79.9)	Ref	
Present	411 (42.5)	125 (20.1)	0.34 (0.27 – 0.43)	
Ductal Hyperplasia				<0.001
Absent	582 (60.1)	489 (79.0)	Ref	
Present	386 (39.9)	130 (21.0)	0.40 (0.32 – 0.51)	
Lobular Hyperplasia				0.201
Absent	943 (97.4)	597 (98.5)	Ref	
Present	25 (2.6)	9 (1.5)	0.58 (0.25 – 1.20)	
Calcifications				0.027
Absent	466 (48.2)	269 (42.4)	Ref	
Present	501 (51.8)	365 (57.6)	1.26 (1.03 – 1.54)	
Cysts				<0.001
Absent	444 (45.9)	479 (77.1)	Ref	
Present	523 (54.1)	142 (22.9)	0.25 (0.20 – 0.32)	
Duct Ectasia				<0.001
Absent	799 (82.6)	569 (91.6)	Ref	
Present	168 (17.4)	52 (8.4)	0.44 (0.31 – 0.60)	
Fibrosis				<0.001
Absent	325 (33.6)	368 (61.8)	Ref	
Present	642 (66.4)	227 (38.2)	0.31 (0.25 – 0.39)	
Intraductal Papilloma				<0.001
Absent	766 (79.1)	584 (94.3)	Ref	
Present	202 (20.9)	35 (5.7)	0.23 (0.15 – 0.33)	
Sclerosing Adenosis				<0.001
Absent	704 (72.8)	517 (83.3)	Ref	
Present	263 (27.2)	104 (16.7)	0.54 (0.42 – 0.69)	
Columnar Alterations				<0.001
Absent	597 (61.7)	508 (81.8)	Ref	
Present	370 (38.3)	113 (18.2)	0.36 (0.28 – 0.46)	
Radial Scar				0.013
Absent	933 (96.5)	604 (98.7)	Ref	
Present	34 (3.5)	8 (1.3)	0.37 (0.16 – 0.77)	
Dupont and Page criteria				<0.001
Nonproliferative disease	389 (40.2)	464 (72.2)	Ref	
Proliferative disease without atypia	505 (52.2)	160 (24.9)	0.27 (0.21 – 0.33)	
Proliferative disease with atypia	74 (7.6)	19 (3.0)	0.22 (0.13 – 0.36)	
Developed breast cancer				0.049
No	875 (90.4)	600 (93.3)	Ref	
Yes	93 (9.6)	43 (6.7)	0.68 (0.46 – 0.98)	

[a]Numbers may not sum to the total number of patients if features could not be assessed on biopsy

[b]χ^2 test comparing distribution of features across absence or presence of fibroadenoma on biopsy

Breast cancer risk within the BBD cohort

Adjusting for age at biopsy alone, the presence of fibroadenoma was associated with a reduced breast cancer risk (RR 0.64; 95% CI 0.45 – 0.85) compared to the absence of fibroadenoma within the BBD cohort (Table 5). After adjusting for age at biopsy and Dupont and Page criteria, no other variables were selected for model selection using BIC criteria. In the fully adjusted model including age at biopsy, proliferation, and atypia, fibroadenoma was still associated with a reduced risk (RR 0.67; 95% CI 0.48 – 0.93) of developing breast cancer.

Table 5. Relative risk of breast cancer by fibroadenoma status in African American women in the Detroit BBD cohort, 1997-2010

	Age-adjusted relative risk[a] (95% confidence interval)	*P* value[b]	Fully-adjusted relative risk (95% confidence interval)	*P* value[b]
No fibroadenoma on biopsy	Ref		Ref	
Fibroadenoma	0.64 (0.48, 0.85)[e]	0.003	0.67 (0.48, 0.93)[c,f]	0.017
Fibroadenoma				
No other lesions	0.63 (0.22, 2.32)[g]	0.435	0.59 (0.20, 2.16)[c,h]	0.367
One or more other lesions	0.67 (0.49, 0.91)[i]	0.013	0.70 (0.48, 0.99)[d,j]	0.047
Under age 50				
No fibroadenoma on biopsy	Ref		Ref	
Fibroadenoma	0.71 (0.45, 1.11)[k]	0.133	0.58 (0.34, 0.96)[c,l]	0.037
Age 50 or older				
No fibroadenoma on biopsy	Ref		Ref	
Fibroadenoma	0.68 (0.46, 0.98)[m]	0.042	0.79 (0.52, 1.19)[c,n]	0.275

[a]Multivariable logistic regression model adjusting for age at biopsy
[b]Wald test statistic
[c]Multivariable logistic regression models adjusting for age, proliferative disease, and cellular atypia at biopsy
[d]Multivariable logistic regression adjusting for age, columnar alterations, proliferative disease, and cellular atypia at biopsy
N at risk: [e]3845, [f]3761, [g]607, [h]607, [i]3238, [j]3000, [k]2234, [l]2071, [m]1611, [n]1536

Among biopsies without other benign breast lesions, presence of fibroadenoma was associated with a reduced breast cancer risk when adjusting for age at biopsy though this comparison did not reach statistical significance (RR 0.63; 95% CI 0.22 – 2.32) when compared to absence of fibroadenoma in the Detroit BBD cohort due to limited sample size for this

comparison. In a fully adjusted model including age at biopsy, proliferation and atypia, fibroadenoma was still associated with reduced breast cancer risk but not significantly so (RR 0.59; 95% CI 0.20 – 2.16). Among biopsies containing one or more other benign breast lesions, presence of fibroadenoma was associated with a reduced breast cancer risk when adjusting for age at biopsy when compared to the absence of fibroadenoma on biopsy in the Detroit BBD cohort (RR 0.67; 95% CI 0.49 – 0.91). In a fully adjusted model controlling for age at biopsy, columnar alterations, epithelial proliferation and cellular atypia on biopsy, fibroadenoma was still associated with a reduced breast cancer risk (RR 0.70; 95% CI 0.48 – 0.99).

Fibroadenoma diagnosed in women under the age of 50 was associated with a decrease in breast cancer risk after adjusting for age at biopsy, but not significantly so (RR 0.71; 95% CI 0.45 – 1.11). After additionally adjusting for proliferation and cellular atypia, fibroadenoma on biopsy was associated with a significant decrease in breast cancer risk in women under the age of 50 (RR 0.58; 95% CI 0.34 – 0.96). Fibroadenoma diagnosed in women aged 50 or older also show a reduction in breast cancer risk when adjusting for age at biopsy (RR 0.68; 95% CI 0.46 – 0.98); however, this reduction failed to reach statistical significance, likely due to limited sample size, after adjusting for age at biopsy, proliferation and cellular atypia (RR 0.79; 95% CI 0.52 – 1.19).

Breast cancer risk compared to population level risk

Overall, this cohort of women exhibited an increased incidence of approximately 20% (SIR 1.19; 95% CI 1.05 – 1.36) of breast cancer compared to the general African American population in Metropolitan Detroit (Table 6). Stratifying the cohort by presence of fibroadenoma on biopsy revealed that breast cancer incidence associated with fibroadenoma was indistinguishable from population level (SIR 0.93; 95% CI 0.75 – 1.17), but the breast cancer incidence associated with the absence of fibroadenoma on biopsy was significantly higher than population level (SIR 1.40; 95% CI 1.19 – 1.65).

Table 6. Risk of breast cancer in African American women in the Detroit BBD cohort compared to population level breast cancer risk in African American women in the Metropolitan Detroit Cancer Surveillance System between 1997 and 2015

	Total no	Observed cases	Expected cases	SIR[a] (95% CI)
Population rate	N/A	N/A	N/A	Ref
Entire BBD cohort	3845	221	185.02	1.19 (1.05 – 1.36)
Biopsy without fibroadenoma	2047	145	103.56	1.40 (1.19 – 1.65)
Fibroadenoma	1798	76	81.46	0.93 (0.75 – 1.17)
Biopsies without fibroadenoma				
Nonproliferative disease	868	47	44.42	1.06 (0.80 – 1.40)
Proliferative disease	1054	81	52.45	1.54 (1.24 – 1.92)
Proliferative disease with atypia	125	17	6.69	2.54 (1.58 – 4.09)
Biopsies with fibroadenoma				
Nonproliferative disease	1325	55	59.52	0.92 (0.71 – 1.20)
Proliferative disease	450	16	20.73	0.77 (0.47 – 1.26)
Proliferative disease with atypia	23	5	1.21	4.14 (1.72 – 9.94)
No other lesions	546	17	22.62	0.75 (0.47 – 1.21)
One or more other lesions	1252	59	58.84	1.00 (0.78 – 1.29)

[a]Standardized incidence ratio (SIR) compares the observed number of breast cancers that developed in the study to the number expected on the basis of the Detroit surveillance, epidemiology, and end results data for African American women of a similar age and calendar period

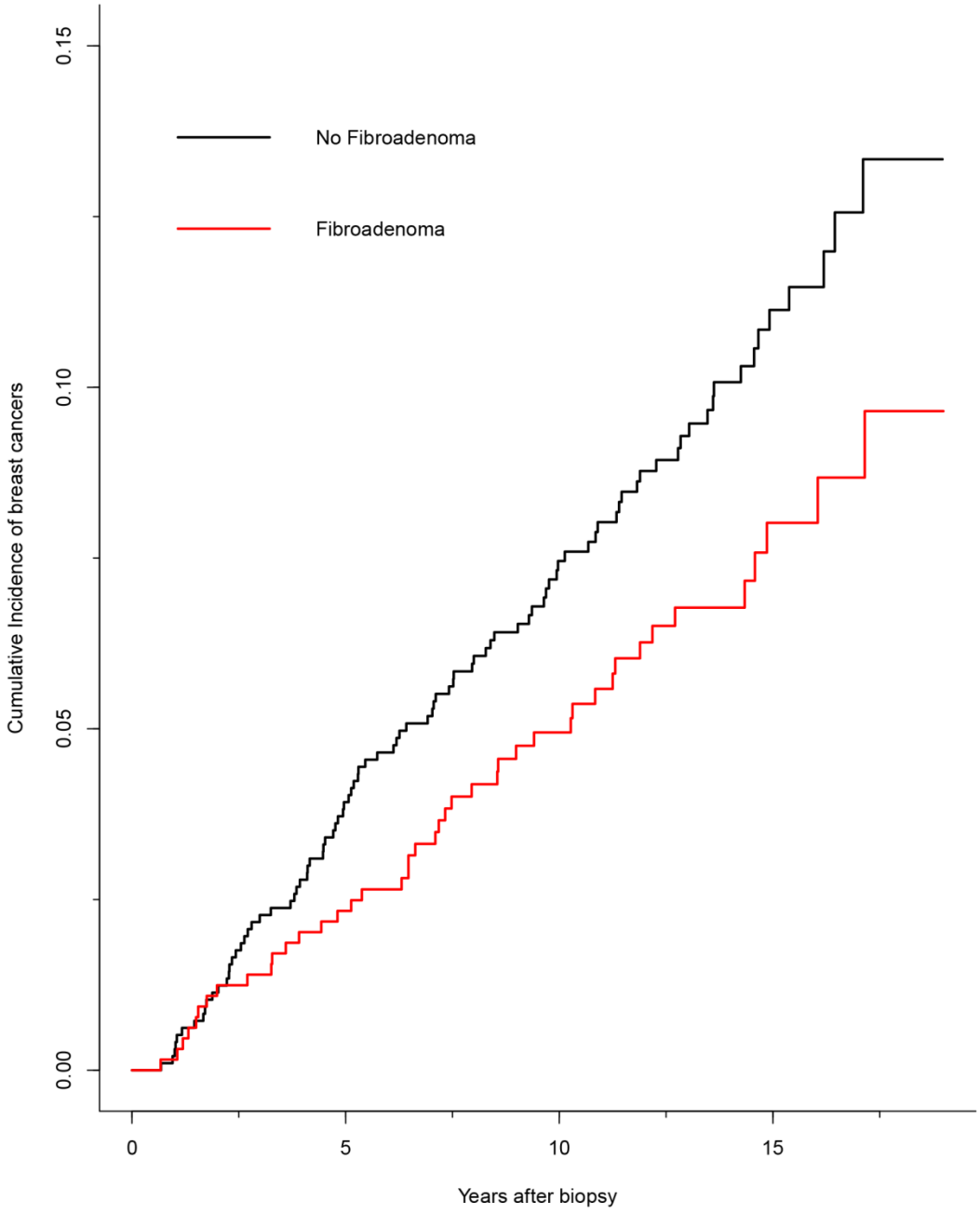
In biopsies that did not indicate fibroadenoma presence, stratifying by Dupont and Page criteria showed that compared to population level breast cancer incidence: nonproliferative disease was associated with slight but not significantly increased incidence (SIR 1.06; 95% CI 0.80 – 1.40), proliferative disease was associated with an increased incidence (SIR 1.54; 95% CI 1.24 – 1.92), and proliferative disease with atypia was associated with an increased incidence (SIR 2.54; 95% CI 1.58 – 4.09).

In biopsies that indicated fibroadenoma presence, stratifying by Dupont and Page criteria showed that compared to population level breast cancer incidence: nonproliferative disease was associated with slight but not significantly decreased incidence (SIR 0.92; 95% CI 0.71 – 1.20), proliferative disease was also associated with a slight but not significantly decreased incidence (SIR 0.77; 95% CI 0.47 – 1.26), and proliferative disease with atypia was associated with an increased incidence (SIR 4.14; 95% CI 1.72 – 9.94). In biopsies that indicated fibroadenoma

presence, stratifying by the presence of one or more benign breast biopsy showed that compared to population level breast cancer incidence: no other breast lesions was associated with a reduced, but not significantly so, incidence (SIR 0.75; 95% CI 0.47 – 1.21); one or more other benign breast lesions was not associated with a different incidence (SIR 1.00; 95% CI 0.78 – 1.29).

Cumulative incidence of cancers in subgroups

Women with fibroadenoma on biopsy accumulated fewer breast cancers over the study period than women without fibroadenoma on biopsy (Figure 2; Fine and Gray test $p<0.001$). Stratifying by likely menopausal status by age indicated the incidence of breast cancers was lower in women under the age of 50 than in women aged 50 or older (Figure 3). In both strata, women with fibroadenoma on biopsy accumulated fewer cancers over the study period than women without fibroadenoma on biopsy (Fine and Gray test $p=0.014$ for under age 50; $p=0.059$ for age 50 and older).



Number at Risk

No Fibroadenoma	2,047	2,003	1,943	1,751	1,409	1,100	571	157
Fibroadenoma	1,798	1,772	1,739	1,516	1,259	1,023	526	171

Fig. 2 Cumulative incidence of *in situ* and invasive breast carcinomas over study period in African American women in the Detroit BBD cohort, 1997-2010. Women with biopsies that indicated fibroadenomas accumulated fewer breast cancers over the study period than women whose biopsies did not indicate fibroadenomas. Fine and Gray test $p<0.001$

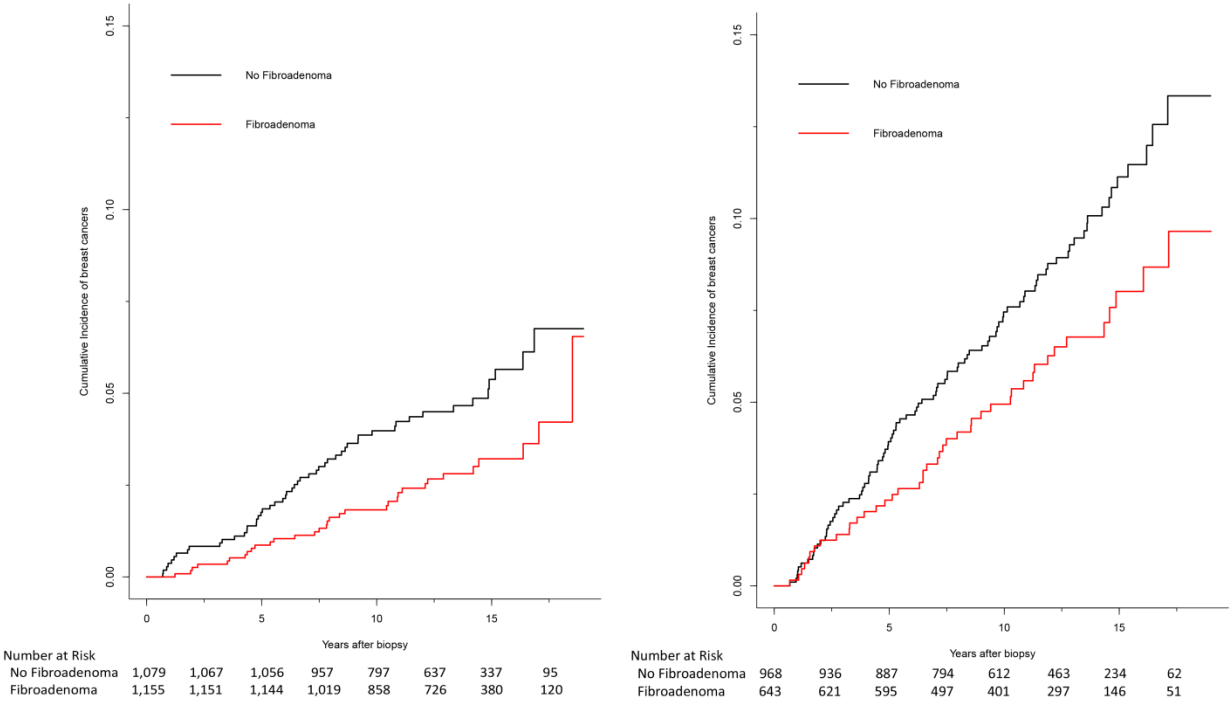


Fig. 3 Cumulative incidence of *in situ* and invasive breast carcinomas over study period by likely menopausal status by age in African American women in the Detroit BBD cohort, 1997-2010. (A) Women under the age of 50 with biopsies indicating fibroadenomas accumulated fewer breast cancers over the study period than women under the age of 50 whose biopsies did not indicate fibroadenomas. Fine and Gray test p=0.014. (B) Women aged 50 or older with biopsies indicating fibroadenomas accumulated fewer breast cancers over the study period than women aged 50 or older whose biopsies did not indicate a fibroadenoma. Fine and Gray test =0.059

IV. Discussion

Here we report findings from a contemporary cohort of African American women who have had a breast biopsy that show that biopsies that indicated a fibroadenoma were associated with a reduced risk of breast cancer compared to biopsies with other BBD lesions even after adjusting for age, proliferative disease and atypia. Additionally, we found that women with a fibroadenoma observed on biopsy were not at increased risk of subsequent breast cancer compared to the general population of African American women. These findings suggest that current breast cancer risk models that incorporate benign biopsies without considering the pathological lesion overestimate risk in African American women who have fibroadenomas on biopsy. Given that fibroadenomas were identified in nearly half of all breast biopsies in this population, and were the

only lesion identified in 19% of all biopsies, these findings represent a significant clinical population. In comparison, fibroadenomas were identified in only 23.5% of the biopsies of the primarily European American Mayo Clinic cohort[59].

Our investigation suggests that biopsies indicating fibroadenoma exhibit a reduced risk of breast cancer compared to all other BBD biopsies, contrary to most other studies' estimates of increased risk of breast cancer[56]. Discordant risk estimates between our investigation and those from other studies may reflect differences in race, age, and period of cohorts utilized. The Nashville group[60], which found a significant increase in breast cancer risk with fibroadenoma (SIR 1.61; 95% CI 1.30 – 2.00) compared to the Connecticut Tumor Registry, studied European American women diagnosed with a fibroadenoma between 1950 and 1968. The Mayo Clinic benign breast disease (BBD) cohort[59] studied European American women diagnosed with fibroadenoma between 1967 and 1991 and found modest increases breast cancer risk with fibroadenoma (SIR 1.60; 95% CI 1.38 – 1.85) compared to biopsies without fibroadenoma (SIR 1.50; 95% CI 1.39 – 1.62). A BBD cohort from Henry Ford Health System (HFHS), where women with fibroadenomas on biopsy had a decreased odds (OR 0.55; 95% CI 0.39 – 0.77) of developing breast cancer compared to women without fibroadenoma on biopsy, more closely approximates our risk estimates[42]. Worsham et al[42] studied a mixed cohort of European American and African American women in metropolitan Detroit diagnosed between 1981 and 1994. However, it is unlikely that the differences in risk estimates are due solely to race: the HFHS group tested an interaction factor between race and BBD and did not find statistical significance[42].

Period effects may also contribute to variation in risk estimates. Inclusion criteria for BBD studies span from 1950 to 2010; thus, differences in risk estimates may also reflect the endogenous and exogenous exposures that varied over this period. Exogenous hormone use, including hormone replacement therapy and contraceptive use have changed in frequency, dose, and formulation. Changes in exogenous hormone use can alter total estrogen exposure, a strong breast cancer risk factor, and influence risk estimates of tissue-based markers[61,62]. Environmental

exposures that vary over time and/or geographic areas can further add to risk estimate variation. Changes in the indication for biopsy is perhaps the most pertinent shift over these study periods: physicians are more likely to biopsy now than in the 1950s. Population uptake of mammography began in the 1970s[63] and screening technology has continued to improve since[64,65], leading to an increase in breast biopsy incidence. The adoption of core needle biopsies, which are less invasive than excisional biopsies, further increased the likelihood of a breast biopsy, especially in what are considered high-risk populations.

The strengths of our study stem from the cohort study design where all breast biopsies were re-examined for benign lesions in a centralized and standardized manner by WSU pathologists, and identification of breast cancers occurred through institution medical records and then standardized for the region through use of the population-based SEER registry. This allowed for the identification of breast cancers among women who sought care outside of the hospitals served by the University Pathology Group. In addition, linkage to MDCSS allowed for identification of other causes of death, so that competing risk analyses could be performed.

It should be noted there are limitations to our study. First, the population estimates used in the SIR analysis includes women who have been diagnosed with benign breast disease in the metropolitan Detroit area, thus the SIR may slightly underestimate the risk associated with breast cancer. We are also limited by the passive follow-up for *in situ* and invasive breast carcinoma incidence in this study; women who move out of metropolitan Detroit would be missed, also underestimating breast cancer risk in this study. Next, our assessment was limited to the presence or absence of fibroadenomas on breast biopsy, but there may be added value in assessing whether these fibroadenomas exhibit other BBD lesions, data that were not collected in the original study design. There are conflicting reports on the breast cancer risk associated with complex fibroadenomas, or fibroadenomas that contain cysts, calcifications, sclerosing adenosis, and/or apocrine metaplasia[59,60]. Because of the high prevalence of fibroadenomas in this population, breast cancer risk associated with complex fibroadenoma should also be

independently reviewed in African American women. We are also limited by behavioral risk factor information we were unable to collect or unable to measure in the original study design that may confound these risk estimates.

Currently, a diagnosis of fibroadenoma requires no further intervention, and is followed by a primary care physician or gynecologist unless the patient elects to have to mass removed, usually due to size of the tumor, recurrence, or pain[66,67]. As previous investigations of fibroadenoma on biopsy estimated an elevated risk of breast cancer that persists for 20 years[60], physicians may currently screen women with fibroadenomas frequently. Our study suggests that fibroadenomas do not increase risk of subsequent breast cancers. Ultimately, examining specific features of BBD will improve risk estimates used in breast cancer risk models, reduce patient anxiety, and improve management of fibroadenoma in the clinic by reducing overscreening and overtreatment of this population, both associated with potential patient harms and excessive resource allocation.

CHAPTER 3: BREAST DENSITY AND PARENCHYMAL PATTERNS IN AFRICAN AMERICAN WOMEN

I. Introduction

Breast density, whether measured qualitatively or quantitatively, has been consistently associated with increased breast cancer risk[44,68]. Mammograms exhibiting more than 75% density are associated with about a four-fold increase in breast cancer risk compared to those in the least dense category of under 25% density[44,68]. High breast density is thought to increase breast cancer risk as it equates to increased connective and epithelial tissue, tissues highly associated with breast carcinogenesis. Increased breast density may also mask small growths on film, leading to delayed cancer detection, but not the primary mechanism by which breast density contributes to breast cancer risk.

Despite the increased rate of aggressive breast cancer incidence and breast cancer mortality in African American women compared to European American women[2,9,10], few studies have assessed breast density and subsequent risk of breast cancer in African American women. Tice et al.[49] included over 2800 African American women from the multi-center Breast Cancer Screening Consortium, but this analysis only adjusted for race and did not report race-specific estimates. Race-specific estimates are necessary as African American women present differently in BBD[53], breast density[50,51,69], and breast cancer subtype[9,12]. After adjusting for age and BMI, African American women are more likely to have dense breasts than European American women on quantitative density measures[51].

Recent analyses showed that qualitative assessments by radiologists were similar, if not better, than quantitative assessments of breast density at discriminating between future breast cancer cases and controls[70,71]. This work suggests that there are features other than density on mammogram associated with breast cancer that radiologists are able to detect but are missed by automated measures. Other radiologic classification methods including the Tabár classification[72], which describes the appearance of the breast tissue or parenchyma on mammogram, are not

routinely used as breast density has been more strongly linked to breast cancer, but this classification may have renewed potential as contrast resolution and image quality have greatly improved with the switch from film to digital mammography[73]. High-risk Tabár patterns include nodular or extremely dense breasts, which are at 2.5 times higher odds of developing breast cancer than other, low-risk patterns[74]. Qualitative density and parenchymal patterns of the breast may be particularly important for women with benign breast disease (BBD), a higher-risk population which may deserve additional surveillance and assessment on mammogram. Here we sought to investigate the association between qualitative density and parenchymal patterns with breast cancer in African American women with BBD.

II. Methods

Study design

Here we conducted a case/control study nested within the Detroit BBD cohort to examine whether qualitative breast density and parenchymal patterns are associated with breast cancer in African American women with BBD. Our main outcome of interest was the presence or absence of *in situ* or invasive breast carcinoma. The main exposures of interest include the BI-RADS density score, Tabár classification, and a complexity indicator score created by our study radiologists.

We also examined whether the association between BI-RADS density and breast cancer differed among women with BBD or the population by additionally examining this association in African American women undergoing routine screening in Detroit over a similar time period using the Detroit Screening cohort. Utilizing this cohort allows us to evaluate potential differences that may stem from race, period, or site differences that may limit our direct comparisons to other studies.

Study populations

Study participants included all breast cancer cases (n=214) and controls (n=214) matched on five-year age groups and year at biopsy from the Detroit BBD cohort. The Detroit BBD cohort

comprises African American women diagnosed with BBD between 1997 and 2010 and followed for breast cancer current through December 2015; additional study details can be found in Chapter 2. For this study, cases and controls were eligible if a screening or diagnostic mammogram within five years of the BBD diagnosis and before the *in situ* or invasive breast carcinoma diagnosis was available for review in film or digital format. Mammographic films were digitized for review. This study protocol was approved by the Institutional Review Board of Wayne State University. Individual consent was not required as this study was retrospective in design and carried minimal risk to participants.

To examine breast density differences between women with or without BBD, the Detroit BBD cohort was compared to the Detroit Screening cohort. The Detroit Screening cohort is a retrospective cohort of all European American and African American women who underwent routine mammographic screening at Karmanos Cancer Institute (KCI) from January 1, 2012 to December 31, 2016. Patients were identified from the KCI Image Department database where medical record numbers, date of birth, mammogram type and date, age and race were available. Women with a prior history of breast cancer or a breast cancer within six months of the initial mammogram were excluded. Breast cancer diagnosis and vital status were ascertained using MDCSS linkage.

Mammographic breast features

Screening or diagnostic mammograms from the Detroit BBD cohort were independently assessed by breast radiologists at KCI blinded to case/control status. Study radiologists reviewed mammographic density and parenchymal patterns on mediolateral-oblique and cranio-caudal views together and a final density or pattern score were given after the radiologists came to a consensus. If density or pattern scores were discordant between right and left breasts, the most suspicious score was utilized for analysis. If only one breast was available for viewing, the density or pattern score for this breast was utilized for analysis. Only one density or parenchymal pattern

was scored for mammographic images at a time; sessions focused on reading one parameter to increase speed (by not switching measures).

Breast density was evaluated qualitatively using the American College of Radiology (ACR) Breast Imaging Reporting and Data System (BI-RADS) density score[75]. This density score classifies breast tissue into four categories by the amount of fatty and radiolucent tissue to fibroglandular and radio-opaque tissue seen on film including: (A) predominantly fat, (B) scattered fibroglandular densities, (C) heterogeneously dense, and (D) extremely dense tissue. BI-RADS density for the Detroit screening cohort was abstracted from PACS imaging reports.

Study radiologists also categorized mammograms by the Tabár classification of parenchymal patterns[72,74]. This classification sorts mammograms into five patterns based on four features with anatomic significance: radiolucent areas (adipose), linear densities (ducts), nodular densities (terminal duct lobule units (TDLU)), and homogenous densities that lack structure (fibrosis). Patterns include: (I) breasts that include all four features equally represented, scalloped contours, and oval fatty areas. (II) Predominately fatty tissue with linear densities. (III) Predominately fatty but with significant densities, often linear (prominent ductal pattern), in the retroareolar region. (IV) Predominately enlarged nodular densities and linear densities (indicating proliferating TDLUs and periductal fibrosis). (V) Predominately homogenous density with ground-glass-like appearance.

Mammographic images were also scored for a novel complexity indicator described by our study radiologists. This indicator reflected the complexity and suspicion raised (categorized as uncomplicated, borderline and complicated) for all study mammograms. Complexity for an image increased with the presence of multiple interfaces or changes in breast tissue density, which would necessitate additional time to sufficiently review. Areas of density that were patchy, nodular or diffuse on mammogram raised suspicion that a developing tumor may be missed and contributed to this complexity indicator. For each image, study radiologists made an overall call

(uncomplicated, borderline, or complicated) followed by the components that contributed to the overall call (multiple interfaces as well as patchy, nodular, or diffuse areas of density).

C. Statistical approaches

Our first objective was to examine the distribution of clinicopathologic characteristics among the cases and controls in the final study population from the Detroit BBD cohort. Clinicopathologic characteristics were described in percentages and included age, likely menopausal status by age (pre-, peri-, and postmenopausal), year of biopsy, mammogram type, biopsy type, Dupont and Page criteria for epithelial proliferation and cellular atypia on biopsy, vital status. We evaluated potential differences in clinicopathologic characteristics among cases and controls using Pearson chi-square tests. Differences in mean age was tested using a one-way ANOVA. Median time to cancer diagnosis was determined by a Kaplan Meier estimator; median time of follow-up for controls was determined by the reverse Kaplan Meier method.

Our second objective was to examine whether mammographic density and parenchymal patterns were associated with breast cancer in African American women with BBD. We described the distribution of mammograms in the nested study from the Detroit BBD cohort by BI-RADS density, Tabár classification, and complexity indicator status using percentages. We evaluated the odds of having breast cancer by density or parenchymal pattern status with conditional logistic regression adjusting for epithelial proliferation and cellular atypia on biopsy (likely confounders for BBD studies Dupont 1985) and stratifying for 5-year age and biopsy groups. Although BMI is an important variable to adjust for in this analysis, we were unable to ascertain the BMI for all women in the final study population from the Detroit BBD cohort.

Our third objective was to examine the association between mammographic density and breast cancer in the larger population of African American women in Detroit undergoing routine screening. We evaluated this association by estimating the odds of having breast cancer in the Detroit Screening Cohort by BI-RADS density using logistic regression adjusting for age and BMI.

III. Results

Clinicopathologic characteristics

The final nested case/control study from the Detroit cohort consisted of 126 cases and 151 controls. The mean age of the study population was 53.6 years and did not differ between cases and controls (p=0.358). Likely menopausal status by age was 36.8% premenopausal and 63.2% postmenopausal; likely menopausal status did not differ by cases and controls (Table 7, p-value=1). Year of biopsy did not differ between cases and controls (p=0.823); 33.9% of biopsies were conducted between 1997-2000, 40.0% between 2001-2005, and 26.0% between 2006-2010. The majority of images assessed were screening mammograms (53.4%); mammogram type did not differ between cases and controls (p=1). Indication for an excisional biopsy may indicate a larger area of suspicious tissue; however, the majority of biopsies were core-needle (63.9%) and did not significantly differ by case status (p=0.079). Cases showed a larger proportion of proliferative disease with atypia (PDWA) than controls (11.1% versus 4.0%; p=0.044). 16.6% of the study population is deceased, and this proportion did not significantly differ among cases and controls (p=0.4036).

Table 7. Distribution of clinicopathologic characteristics among African American women in a case/control study nested in the Detroit BBD cohort, 1997-2010

	Controls (N=151)	Cases (N=126)	p-value[a]
Age			1
Premenopausal (< 50)	56 (37.1%)	46 (36.5%)	
Premenopausal (≥ 50)	95 (62.9%)	80 (63.5%)	
Year of biopsy			0.8233
1997-2000	52 (34.4%)	42 (33.3%)	
2001-2005	62 (41.1%)	49 (38.9%)	
2006-2010	37 (24.5%)	35 (27.8%)	
Mammogram type			1
Screening	81 (53.6%)	67 (53.2%)	
Diagnostic	70 (46.4%)	59 (46.8%)	
Biopsy type			0.0792
Excisional	62 (41.1%)	38 (30.2%)	
Core Needle	89 (58.9%)	88 (69.8%)	
Histological impression			**0.0440**
Non-proliferative disease	71 (47.0%)	62 (49.2%)	
Proliferative disease	74 (49.0%)	50 (39.7%)	
Proliferative disease with atypia	6 (4.0%)	14 (11.1%)	
Vital Status			0.4036
Alive	129 (85.4%)	102 (81.0%)	
Deceased	22 (14.6%)	24 (19.0%)	

[a]Pearson chi-square tests

Median time to cancer diagnosis in cases was 6.3 years (range 0.7 – 17.2 years). Median time of follow up for controls was 12.6 years (range 3.0 – 18.9 years). Median time between biopsy and mammogram date was 0.10 years for controls (range 4.96 years prior to 4.16 years post biopsy) and 0.13 years for cases (range 4.7 years prior and 2.16 years post biopsy).

Density and parenchymal patterns

Table 8 summarizes the distribution of ACR BI-RADS density scores, Tabár classifications, and Complexity categories across cases and controls from the Detroit BBD cohort. 79% of mammograms were considered density categories B (scattered densities) and C (heterogeneously dense) regardless of case/control status. The odds of having breast cancer were elevated and increased with density categories B, C, and D (extremely dense) compared to the odds of having breast cancer in fatty breasts (BI-RADS category A), consistent with prior findings from other studies (OR 1.83, 1.70, 2.69, respectively), but these estimates did not reach statistical significance. The odds of having breast cancer with dense breasts (categories C & D) were elevated but not significant compared to non-dense breasts (categories A & B; OR 1.15, 95% CI 0.69 – 1.90). Associations with BI-RADs categories did not reach statistical significance, likely due to sample size limitations. With 80% power, we are able to detect a minimum odds ratio for dense breasts (category C & D) of 2.05.

The majority of controls were considered Tabár patterns II (32.7%) and I (28.7%); the majority of cases were considered Tabár patterns I (29.6%) and IV (26.4%). Compared to mammograms classified as Tabár pattern II or primarily fatty replacement, mammograms with a Tabár pattern IV or primarily nodular densities conferred a 2.83 times higher odds of breast cancer (95% CI: 1.35-5.91). Mammograms with Tabár patterns I, III and V conferred very modestly increased odds of having breast cancer compared to Tabár pattern I, but not significantly so (p=0.309, 0.434, and 0.701, respectively).

The majority of controls had mammograms categorized as uncomplicated (45.0%); the majority of cases were categorized as complicated (46.8%). About 20% of mammograms from cases and controls were categorized as borderline. A complicated mammogram conferred an approximate 2-fold increase in breast cancer risk (OR 1.92, 95% CI: 1.07-3.43) compared to uncomplicated mammograms. A borderline mammogram conferred an odds increase in breast cancer by 1.16 (OR 1.16, 95% CI: 0.58 – 2.29); though not statistically significant, this association indicates an increasing trend in breast cancer risk similar to the qualitative density categories.

Table 8. Distribution of parenchymal density or patterns on mammogram and associations with breast cancer among African American women in a case/control study nested in the Detroit BBD cohort, 1997-2010

Parenchymal pattern	Controls (N=151)	Cases (N=126)	Odds ratio (95% CI)	p-value[a]
ACR BI-RADS				
A. Fatty	24 (15.9%)	12 (9.5%)	Ref	--
B. Scattered densities	56 (37.1%)	52 (41.3%)	1.83 (0.81 – 4.14)	0.146
C. Heterogeneously dense	61 (40.4%)	50 (39.7%)	1.70 (0.74 – 3.89)	0.210
D. Extremely dense	10 (6.6%)	12 (9.5%)	2.69 (0.85 – 8.51)	0.091
Non-dense (A & B)	80 (53.0%)	64 (50.8%)	Ref	--
Dense (C & D)	71 (47.0%)	62 (49.2%)	1.15 (0.69 – 1.90)	0.083
Tabár classification				
I. Equal	43 (28.7%)	37 (29.6%)	1.42 (0.73 – 2.75)	0.309
II. Fatty	49 (32.7%)	28 (22.4%)	Ref	--
III. Retroareolar	10 (6.7%)	10 (8.0%)	1.52 (0.53 – 4.31)	0.434
IV. Nodular	20 (13.3%)	33 (26.4%)	2.83 (1.35 – 5.91)	**0.006**
V. Dense	28 (18.7%)	17 (13.6%)	1.18 (0.50 – 2.78)	0.701
Missing	1	1		
Complexity indicator				
Uncomplicated	68 (45.0%)	44 (34.9%)	Ref	--
Borderline	31 (20.5%)	23 (18.3%)	1.16 (0.58-2.29)	0.678
Complicated	52 (34.4%)	59 (46.8%)	1.92 (1.07-3.43)	**0.028**

[a]P-value from conditional logistic regression adjusting for histologic impression

Table 9 shows the distribution of all mammograms by the four BI-RADS density and the five Tabár classification categories. There is some overlap between patterns, but no categories or patterns completely overlap. All images considered BI-RADS density A or fatty breasts were categorized as the predominantly fatty Tabár II pattern; in contrast, images considered Tabár II

pattern were categorized as either BI-RADS A (46.8%) or BI-RADS B (53.2%). Images considered BI-RADS density B or scattered densities were categorized as Tabár patterns I (37.4%), II (38.3%), III (13.0%), and IV (11.2%). Images considered BI-RADS density C or heterogeneously dense were categorized as Tabár patterns I (36.4%), III (5.5%), IV (33.6%), and V (24.5%). Images considered BI-RADS density D or extremely dense were categorized as either Tabár pattern IV (18.2%) or V (81.8%). Images considered Tabár pattern I or the equal pattern were categorized as either BI-RADS density B (50%) or C (50%). Images considered Tabár pattern III or primarily retroareolar densities were categorized as either BI-RADS density B (70%) or C (30%). Images considered Tabár pattern IV or primarily nodular densities were categorized as BI-RADS density B (22.6%), C (69.8%), or D (7.5%). Images considered Tabár pattern V or diffusely dense were categorized as either BI-RADS density C (60%) or D (40%).

Table 9. Distribution of ACR BI-RADS density by Tabár classification categories among African American women in a case/control study nested in the Detroit BBD cohort, 1997-2010

Parenchymal pattern	**ACR BI-RADS density**			
	A. Fatty	B. Scattered densities	C. Heterogeneously dense	D. Extremely dense
Tabár classification				
I. Equal	0	40	40	0
II. Fatty	36	41	0	0
III. Retroareolar	0	14	6	0
IV. Nodular	0	12	37	4
V. Dense	0	0	27	18

Table 10 shows the distribution of all mammograms by the four BI-RADS density and the three complexity indicator categories. Similar to Table 9, there is some overlap between categories, but no categories completely overlap. All images considered BI-RADS density A or fatty breasts were categorized as the uncomplicated pattern. All images considered BI-RADS density B or scattered densities were categorized into all three complexity indicator categories uncomplicated, borderline, and complicated (66.7%, 25.9%, and 7.4%, respectively). All images considered BI-RADS density C or heterogeneously dense were categorized into all three complexity indicator categories uncomplicated, borderline, and complicated (3.6%, 21.6%, and

74.8%, respectively). All images considered BI-RADS density D or extremely dense were categorized as either borderline or complicated (9.1% and 90.1%). All images considered uncomplicated by the complexity indicator were categorized into BI-RADS densities A (32.1%), B (64.3%), or C (3.6%). All images considered borderline were categorized into BI-RADS densities B (51.9%), C (44.4%), or D (3.7%). All images considered complicated were categorized into BI-RADS densities B (7.2%), C (74.8%), or D (18.0%).

Table 10. Distribution of ACR BI-RADS density by Complexity indicator categories among African American women in a case/control study nested in the Detroit BBD cohort, 1997-2010

Parenchymal pattern	**ACR BI-RADS density**			
	A. Fatty	B. Scattered densities	C. Heterogeneously dense	D. Extremely dense
Complexity indicator				
Uncomplicated	36	72	4	0
Borderline	0	28	24	2
Complicated	0	8	83	20

Table 11 shows the distribution of all mammograms by the three complexity indicator and five Tabár pattern categories. Images classified as uncomplicated were categorized as Tabár classification I (20.5%), II (65.2%), III (9.8%), and IV (4.5%). Images classified as borderline were categorized as Tabár classification I (58.5%), II (5.7%), III (15.1%), IV (11.3%), and V (9.4%). Images classified as complicated were categorized as Tabár classification I (23.6%), II (0.9%), III (0.9%), IV (38.2%), and V (36.4%). Images classified as Tabár classification I were categorized as uncomplicated (28.8%), borderline (38.8%), and complicated (32.5%). Images classified as Tabár classification II or primarily fatty were categorized as uncomplicated (94.8%), borderline (3.9%), and complicated (1.3%). Images classified as Tabár classification III or primarily retroareolar densities were categorized as uncomplicated (55%), borderline (40%), and complicated (5%). Images classified as Tabár classification IV or nodular densities were categorized as uncomplicated (9.4%), borderline (11.3%), and complicated (79.2%). Images classified as Tabár classification V or diffusely dense were categorized as either borderline (11.1%) or complicated (88.9%).

Table 11. Distribution of Complexity indicator by Tabár classification categories among African American women in a case/control study nested in the Detroit BBD cohort, 1997-2010

Parenchymal pattern	Complexity indicator		
	Uncomplicated	Borderline	Complicated
Tabár classification			
I. Equal	23	31	26
II. Fatty	73	3	1
III. Retroareolar	11	8	1
IV. Nodular	5	6	42
V. Dense	0	5	40

Complicated mammograms

The characteristics on mammogram that contribute to the complexity and raised suspicion are summarized in Table 12. Virtually all mammograms considered borderline or complicated show multiple interfaces of density changes within the breast parenchyma; only 35.7% of mammograms considered uncomplicated show these interfaces. The proportion of features that raise suspicion, including patchy areas of densities, nodular densities or general diffuse density on mammogram, also show clear increases with complexity indicator categories.

Table 12. Distribution of parenchymal characteristics contributing to the Complexity indicator score among African American women in a case/control study nested in the Detroit BBD cohort, 1997-2010

Overall (N=277)	Uncomplicated (N=112)	Borderline (N=54)	Complicated (N=111)
Multiple interfaces	40 (35.7%)	53 (98.1%)	107 (96.4%)
Patchy	3 (2.7%)	34 (63.0%)	87 (78.4%)
Nodular	6 (5.4%)	18 (33.3%)	51 (45.9%)
Dense	3 (2.7%)	35 (64.8%)	105 (94.6%)

Table 13 summarizes the distribution of characteristics that contribute to complexity indicator among cases and controls. Multiple interfaces, patchy areas, and diffusely dense areas were similarly distributed among cases and controls. Nodular densities were seen more frequently in cases than controls (35.6% versus 19.9%) and the presence of this feature was associated with a 2.33 higher odds of having breast cancer (95% CI: 1.33 – 4.09, p-value=0.003) compared to the absence of this feature on mammogram. After adjusting for Dupont and Page criteria and the presence of all of the complexity indicator characteristics, nodular densities on mammogram

is associated with a 2.47 higher odds of having breast cancer (95% CI; 1.34 – 4.56, p-value=0.004), suggesting that the nodular densities drives the statistically significant association between breast cancer and the complexity indicator.

In this study, 36 mammograms were considered both pattern IV on Tabár and nodular by the complexity indicator; this correlates to 68% of all mammograms considered pattern IV and 49% of all mammograms considered Tabár. As breast cancer risk factors, pattern IV on Tabár shows higher specificity than the nodular characteristic on our complexity indicator (86.7% versus 80.1%), but lower sensitivity (26.4% versus 35.6%).

Table 13. Distribution of parenchymal characteristics contributing to the Complexity indicator score associations with breast cancer among African American women in a case/control study nested in the Detroit BBD cohort, 1997-2010

	Controls (N=151)	Cases (N=126)	Odds ratio[a] (95% CI)	p-value[a]	Odds ratio[b] (95% CI)	p-value[b]
Multiple interfaces	106 (70.2%)	94 (74.6%)	1.30 (0.74 – 2.29)	0.368	1.01 (0.49 – 2.08)	0.970
Patchy	62 (41.1%)	62 (49.2%)	1.49 (0.88 – 2.52)	0.142	1.83 (0.90 – 3.73)	0.095
Nodular	30 (19.9%)	45 (35.6%)	2.33 (1.33 – 4.09)	**0.003**	2.47 (1.34 – 4.56)	**0.004**
Dense	77 (51.0%)	66 (52.4%)	1.05 (0.64 – 1.73)	0.847	0.57 (0.27 – 1.19)	0.1360

[a]Conditional logistic regression adjusting for histologic impression
[b]Conditional logistic regression adjusting for histologic impression and all other complexity indicator characteristics.

Density for women in Detroit

To compare effect estimates for the association between density categories and breast cancer between women with BBD versus the routine screening population, we also examined this association in the Detroit Screening Cohort. The distribution of ACR BI-RADs density scores among African American women in the Detroit Screening Cohort is summarized in Table 14. The majority (81.5%) of control mammograms were classified as BI-RADS density A fatty or B scattered densities. The majority (81.8%) of case mammograms were classified as BI-RADS density B scattered densities or C heterogeneously dense. Compared to mammograms showing

a BI-RADs density A or fatty pattern, mammograms with density B, C, and D all conferred significantly increased odds of breast cancer compared to BI-RADS density A or fatty breasts (OR 2.59, 5.05, and 2.89, respectively). Though the estimates from the Detroit Screening Cohort differed slightly from estimates from the Detroit BBD Cohort, the 95% confidence intervals for the odds ratios for each BI-RADS density category overlapped between cohorts (Table 8 and 14). This suggests that the differences in odds ratio estimates may stem from the limited sample size or lack of BMI adjustment of the nested case/control study from the Detroit BBD Cohort rather than a biological difference in the associations between density and breast cancer in women with or without BBD.

Table 14. Distribution of ACR BI-RADs density and associations with breast cancer among African American women in the Detroit Screening Cohort, 2012-2016

	Controls (N=14314)	Cases (N=275)	Odds ratio (95% CI)	p-value[a]
A. Fatty	4384 (30.6%)	45 (16.4%)	Ref	--
B. Scattered densities	7287 (50.9%)	154 (56.0%)	2.59 (1.85 – 3.70)	**<0.001**
C. Heterogeneously dense	2318 (16.2%)	71 (25.8%)	5.05 (3.35 – 7.66)	**<0.001**
D. Extremely dense	325 (2.3%)	5 (1.8%)	2.89 (0.98 – 6.86)	**0.029**

[a]P-value from logistic regression adjusting for Age and BMI

IV. Discussion

Here we report findings from a case/control study nested in a contemporary cohort of African American women with BBD examining qualitative breast density and parenchymal patterns and their associations with breast cancer. Although ACR BI-RADS was not a significant risk factor in this study, we found similar effect estimates for this categorical variable and breast cancer found in prior studies; with a limited sample size we did not have the power needed for statistical significance. We found statistically significant OR estimates from using the Tabár classification and our described Complexity indicator. The strength of these risk factors is strongly driven by nodular patterns on breast parenchyma. This evidence suggests that among women with BBD, parenchymal patterns may be a strong predictor of subsequent breast cancer risk.

Other studies of the Tabár classification have indicated that patterns IV and V are “high risk” while patterns I, II, and III are low risk[72,74]. In our study we were only able to recapitulate the finding that pattern IV was associated with breast cancer[72,74,76]; we found a smaller proportion of pattern V in our cases than controls (13.6% versus 18.7%). Our complexity indicator showed significant overlap with the Tabár classification, particularly for the nodular densities on breast parenchyma. One key difference between our complexity indicator and the Tabár classification is that our indicator does not require a reviewer to choose a predominating parenchymal pattern. If several features exist on mammogram, these will be preserved by the complexity indicator but may be lost in the Tabár classification.

Our study suggests that African American women with BBD exhibit denser breasts on mammography than the wider metro-Detroit population: 48% of women from our case/control study nested in the Detroit BBD cohort were considered to have dense breasts, or BI-RADS density C and D, while only 16.4% of Detroit screening cohort were considered to have dense breasts. This finding mimics those from predominately white studies: 64% of women with BBD from the Mayo clinic’s cohort[52] were considered dense, while 43.3% of women from Breast Cancer Screening Consortium (BCSC) [77] were considered dense. It is important to compare our study to a screening cohort of primarily African American women as breast density measurements differ by race[51]. As breast density is inversely correlated with BMI[50] and obesity prevalence is higher in African American women[34], directly comparing our estimates to BCSC would bias the effect of BBD on breast density towards null. The lower prevalence of extremely dense breasts in both African American and European American women in the Detroit Screening cohort compared to the prevalence in BCSC suggests that factors beyond race contribute to density differences including differences in population or radiology practice by site. Prevalence of extremely dense breasts in the Detroit Screening cohort more closely resemble prevalence estimates from screening studies conducted in Pennsylvania[51,78] and Vermont[79].

We did not detect a statistically significant association between BI-RADs density and breast cancer in our nested study of the Detroit BBD cohort, though we were likely limited by sample size as the effect estimates were within the range of prior studies. Most screening studies in primarily European American or European women show strong associations between BI-RADS density and breast cancer that are monotonically increasing, where the extremely dense category increases odds of having breast cancer 4-6 times compared to the odds of entirely fatty breasts[45,46]. We were able to detect statistically significant associations between BI-RADS density and breast cancer in the Detroit Screening cohort. Surprisingly in the Detroit Screening cohort the strongest increase in odds of breast cancer was associated with BI-RADS density C or heterogeneously dense breasts rather than density D or extremely dense breasts; however, this finding most likely reflects the limited sample (less than 3%) of extremely dense breasts in the Detroit Screening cohort as the 95% confidence intervals for these odds ratios overlap. A similar pattern where BI-RADS density C conferred the largest increase in odds of breast cancer was estimated for African American women in the Carolina Breast Cancer Study (CBCS) [69] where less than 10% had BI-RADS density D or extremely dense breasts.

The strengths of our study include a centralized assessment of all mammograms by our radiologists for BI-RADS density, Tabár classification and our Complexity indicator. This is the first study we know of that examines breast density and parenchymal patterns in African American women with BBD. There are a few important limitations to this study including a restricted sample size. We also primarily use qualitative measures in this study which are more likely to suffer from low reproducibility due to inter- and intra-observer variability; however, BI-RADS and Tabár reproducibility is relatively high[74]. We are also limited by risk factors we were unable to capture or measure during data collection including body mass index (BMI). The missing information on BMI in the Detroit BBD cohort limits the validity of comparisons between BI-RADS density and breast cancer risk between the Detroit BBD and Detroit Screening cohorts.

Our findings suggest there is clinical utility to assessing structural features on mammogram for women with a history of BBD. The Breast Cancer Risk Assessment Tool[15] considers women with a prior biopsy at increased breast cancer risk and these women subsequently undergo increased surveillance, receiving mammograms every six months for two years post biopsy. Improving risk assessment from these mammograms can limit further biopsies that may result in unnecessary patient harms including stress, anxiety and pain. This study warrants further study in a larger sample.

CHAPTER 4: ADIPOSE INFLAMMATION AND THE RISK OF BENIGN AND MALIGNANT BREAST DISEASE IN AFRICAN AMERICAN WOMEN

I. Introduction

Breast cancer incidence has been rising in African American women, who suffer a 40% higher cancer specific mortality compared to European American women[2,80]. Despite this burden, African American women are poorly represented in cohorts studying breast cancer risk factors. Resulting breast cancer risk models, which physicians can use to determine patient surveillance and preventative needs, underestimate risk in African American patients[17,19]. Among women of all races, commonly used risk models cannot discriminate between women who will develop breast cancer from women who will not with a high degree of accuracy at the individual level[16]. Risk model accuracy improves with the inclusion of risk factors that capture biological information associated breast cancer risk and carcinogenesis such as gene risk scores, breast density, and benign breast disease (BBD)[16].

Another risk factor that has not been widely incorporated into risk models is obesity, likely because of its complex relationship with breast cancer risk. Obesity is associated with increased breast cancer incidence in postmenopausal women, and is associated with triple negative breast cancer in premenopausal women[23,25]. Several mechanisms may contribute to this increase, including the peripheral aromatization of androgens to estrogens in adipose tissue depots which increases postmenopausal estrogen exposure significantly and thus breast cancer risk[24,25]. Obesity has also been associated with increased breast adipose inflammation, exhibiting adipocytes surrounded by macrophages, or crown-like structures of the breast (CLS-B) on light microscopy[81]. Adipose inflammation may be independently associated with increasing breast cancer incidence; inflammation has been associated with incidence of other cancers[82,83]. Breast cancer risk models, including the Breast Cancer Risk Assessment Tool (BCRAT), currently do not capture obesity and may improve by including this important risk factor[16]. While easy to ascertain, body mass index (BMI) is a poor indicator of obesity[84] and can misclassify African American

women who have lower body fat and higher lean muscle mass than European American women at a given BMI[85,86]. Adipose inflammation measures such as CLS-B may serve as better indicators of a metabolically obese state.

Prior studies examining CLS-B in mastectomy tissue of breast cancer patients show that adipose inflammation is associated with increasing BMI and post-menopausal status. In a study of Caucasian, Latino and African American women with breast cancer, African American women had the highest CLS-B counts, and increased CLS-B density was associated with poorer progression-free survival[87]. Further studies utilizing breast cancer mastectomy tissue revealed that CLS-B is also associated with mechanisms that increase estrogen in the breast microenvironment including increased aromatase expression, activity, and an elevated local estrogen to androgen ratio[81,88]; these results highlight a potential mechanism where adipose inflammation may increase breast cancer risk. Another potential mechanism may stem from wound healing responses that may occur with or after tissue inflammation; wound healing can remodel tissue[89]. Remodeling of breast tissue could potentially lead to benign lesions seen on non-malignant breast biopsies that increase subsequent breast cancer risk[38]. Carter et al[90] is the first study to examine whether CLS-B is associated with breast cancer risk by studying CLS-B presence in non-malignant breast tissue from women with benign breast biopsies whose subsequent breast cancer status was ascertained. This study found that the presence of five or more CLS-B on biopsy was a significant risk factor for subsequent breast cancer[90].

Adipose inflammation or CLS-B has not been well-described in normal tissue not from surgery (reduction mammoplasty, prophylactic mastectomy or mastectomy tissue adjacent to a tumor) or tissue from benign breast biopsies; furthermore, CLS-B has never been described in such tissue from African American women. Studies of CLS-B in mastectomy tissue suggest CLS-B presence differs in frequency by race[87,91] thus characterizing adipose inflammation in diverse populations is critical. Here we examined whether CLS-B is associated with risk of benign breast

disease and breast cancer in African American women who suffer a higher incidence of breast cancer, poorer breast outcomes, and a higher prevalence of obesity[2,92].

II. Materials and Methods

Study design

We conducted a study utilizing a subset of the nested case/control study described in Chapter 3 and additional age-matched controls from the Komen Normal Tissue Bank (KTB). Our subset included women who developed invasive breast carcinoma and their age and year of biopsy matched controls. Our main outcome of interest was breast cancer risk, where we considered women from KTB as low risk, BBD controls as medium risk, and BBD cases as high risk (as these women have developed invasive breast cancer). Our main exposure of interest was adipose inflammation as assessed by CLS-B on breast biopsy; we identified CLS-B on histology using a CD68 stain to mark the presence of macrophages. Associations between CLS-B and breast cancer risk were then examined.

Study population

Study participants included three age-matched cohorts of African American women from the Susan G. Komen Normal Tissue Bank (KTB) at the Indiana University Simon Cancer Center and the Detroit BBD cohort[53,93]. KTB collects percutaneous needle biopsy breast tissue, blood, and questionnaire data including BMI from healthy volunteer donors at collection events around the country; 5% of KTB donors are African American[93]. Further details on the Detroit BBD cohort are located in Chapter 2.

Three groups for this study in order of decreasing breast cancer risk included the BBD cases, BBD controls, and Komen population controls. 55 African American women from the Detroit BBD cohort without cellular atypia on benign biopsy, developed a subsequent invasive breast cancer, and available BBD and tumor tissue were classified as BBD cases. 47 African American women from the BBD cohort who had not developed invasive or *in situ* breast cancer as of December 2016 and matched to the BBD cases on age and year of biopsy were classified

as BBD controls. An additional 50 African American women from the KTB with no self-reported history of BBD or breast cancer were matched to BBD cases on age were classified as Komen population controls. Komen population control biopsies were reviewed by our study pathologist for Dupont and Page criteria[39] and subsequently further divided by BBD presence on biopsy into Komen Normal and Komen BBD groups for analysis. Komen BBD and BBD controls were grouped together for several analyses as this group showed similar histological abnormalities that confer increased breast cancer risk from normal tissue. Any Komen BBD or BBD controls that showed cellular atypia on biopsy, a strong breast cancer risk factor, were excluded from analyses to avoid bias. BMI close to the BBD date and prior to breast cancer diagnosis was ascertained via medical record review for the Detroit BBD cases and controls. This study was approved by the Institutional Review Board of Wayne State University.

Laboratory methods

Formalin-fixed paraffin embedded biopsy tissue from each study participant were serially sectioned and deparaffinized in a xylene-ethanol series. Endogenous peroxides were removed with a methanol/1.2% hydrogen peroxide incubation at room temperature for thirty minutes. HIER antigen removal was completed with a pH 6 citrate buffer and the BIOCARE Decloacking Chamber. A 40-minute blocking step with Super Block Blocking buffer (Thermo Scientific) was performed prior to adding the primary antibody for CD68, DAKO #M0876, 1:100 dilution overnight. Detection was obtained using GBI Labs DAB chromagen kit (#D41-18) and counterstained with Mayer's Hematoxylin. Sections were then de-hydrated through a series of ethanol to xylene washes and cover slipped with Permount. Stained slides were assessed by pathologists for CLS-B presence (see Figure 4). CLS-B number was assessed on digital images of slides. Adipose area was calculated from digital images of Hematoxylin & Eosin slides using the Adiposoft plugin from ImageJ[94,95].

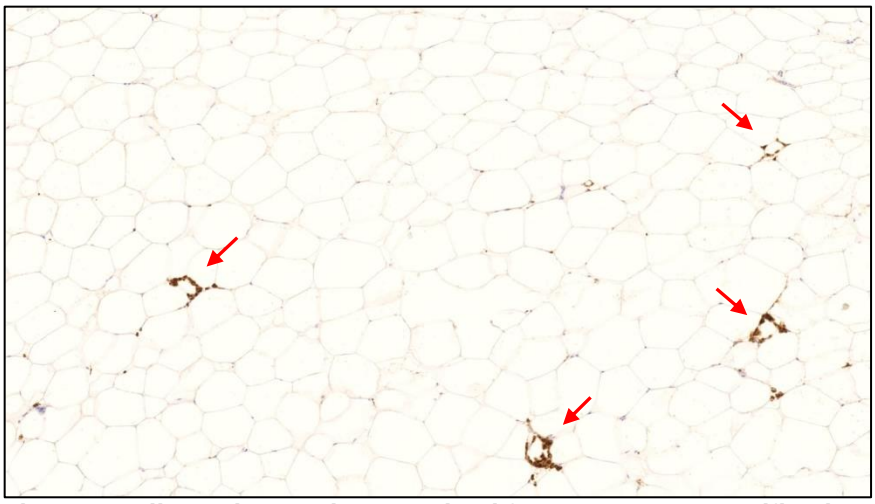


Figure 4. Adipose breast tissue stained for CD68, 5.0x magnification. 4 crown-like structures of the breast, adipocytes surrounded by macrophages, are indicated by red arrows.

Statistical methods:

Our first objective was to examine whether the three risk groups (KTB, BBD Controls and BBD Cases) differed by distribution of age, BMI, epithelial proliferation and cellular atypia at biopsy. We described the distribution of these clinicopathologic characteristics using percentages and evaluated potential differences with Pearson chi-square tests. Our second objective was to examine whether breast cancer risk was associated with adipose inflammation. We evaluated this association by estimating the odds of having breast cancer by CLS-B presence (or the presence of 5 or more CLS-B, similar to study at Mayo Clinic[90]) using univariable and multivariable ordinal logistic models using the logarithm of adipocyte area on biopsy as an offset variable. Our third objective was to examine the association between breast cancer risk and adipose inflammation in clinically-indicated biopsies (BBD Cases and Controls) in order to understand how this marker could be used in future patient management this subgroup is more likely to reflect women biopsied in the future. To evaluate this association, we estimated the odds of having breast cancer by CLS-B presence (or 5 or more CLS-B) among BBD cases and controls using univariable and multivariable conditional logistic regression models using the logarithm of adipocyte area on biopsy as an offset variable and age as a stratification variable. Our last objective was to

understand how the association between breast cancer risk and adipose inflammation may be modified by BMI or epithelial proliferation, a risk factor in benign breast disease. We evaluated these associations using Cochrane-Mantel-Haenzel trend tests.

III. Results

Clinicopathologic characteristics

The mean age of the study population was 54.0 years; mean age did not significantly differ between study groups (p=0.52). Menopausal status by age did not differ significantly by group; 14.5% were categorized as premenopausal (age < 45), 43.4% were categorized as perimenopausal (aged 45 to 55), and 42.1% were categorized as postmenopausal (age > 55, Table 15). The mean BMI of the study population was 32.0; mean BMI did not differ between study groups significantly (p=0.39). Over half of the study participants were considered obese with a BMI greater than 30, so we examined the distribution among normal, overweight and obesity classes I to III as defined by the World Health Organization[96]. Distribution of BMI classes did not differ significantly by study group; 22% were normal weight, 24% overweight, 20% obese class I, 18% obese class II, and 16% obese class III.

Histological review of the KTB Population Control biopsies revealed that 54% of the biopsies did not show histologic abnormalities, or KTB Normal. Of the biopsies that showed histologic abnormalities, or KTB BBD, 70% showed non-proliferative disease, 26% showed proliferative disease and 4% showed proliferative disease with atypia. BBD controls and BBD cases did not significantly differ in distribution of Dupont and Page criteria; 51% of the BBD tissue showed non-proliferative disease. Distribution of epithelial proliferation was significantly lower among KTB BBD than the BBD cases and controls (p<0.001), suggesting that asymptomatic BBD is less likely to include proliferative disease on biopsy.

Table 15. Clinicopathologic characteristics of African American women with breast biopsy tissue from the Detroit BBD Cohort (1997-2010) and the Komen Normal Tissue Bank examined for the presence of CLS-B

	Overall	KTB Normal	KTB BBD	BBD Controls	BBD Cases
N (%)	**N = 152**	**n = 27**	**n = 23**	**n = 47**	**n = 55**
Age					
<45	22 (14.5%)	6 (22.2%)	1 (4.3%)	7 (14.9%)	8 (14.5%)
45-55	66 (43.4%)	12 (44.4%)	10 (43.5%)	19 (40.4%)	25 (45.5%)
>55	64 (42.1%)	9 (33.3%)	12 (52.2%)	21 (44.7%)	22 (40.0%)
BMI					
<25	32 (22%)	2 (7%)	7 (30%)	12 (26%)	11 (22%)
25 – 29	35 (24%)	7 (26%)	4 (17%)	8 (17%)	16 (31%)
30 – 34	30 (20%)	4 (15%)	4 (17%)	13 (28%)	9 (18%)
35 – 39	27 (18%)	11 (41%)	4 (17%)	6 (13%)	6 (12%)
40 +	23 (16%)	3 (11%)	4 (17%)	7 (15%)	9 (18%)
BBD					
No histologic abnormalities	27 (17.8%)	27	--	0	0
Non-proliferative disease	68 (44.7%)	--	16 (69.6%)	24 (51.1%)	28 (50.9%)
Proliferative disease without atypia	55 (36.2%)	--	6 (26.1%)	22 (46.8%)	27 (49.1%)
Proliferative disease with atypia[a]	2 (1.3%)	--	1 (4.3%)	1 (2.1%)	0

[a]Biopsies containing proliferative disease with atypia were excluded from further analyses

CLS-B and in breast tissue of varying risk

Overall, CLS-B were found in 61 of 143 (42.7%) of all study slides assessed. CLS-B were more likely to be identified in tissue with higher breast cancer risk (12.5% KTB normal controls, 33.3% KTB BBD and BBD controls, 68.6% BBD cases, Table 16, unadjusted p-value<0.001). KTB BBD and BBD controls were combined as these slides show histologic abnormalities on biopsy that would indicate increased breast cancer risk from population level risk. The median number of CLS-B in CLS-B positive slides did not differ significantly between groups (3, 2, and 5 for KTB normal, KTB BBD and BBD controls, and BBD cases, respectively), but the upper bound of range varied widely among groups (4, 33, 109). Higher breast cancer risk was associated with tissue that exhibited five or more CLS-B on biopsy (0% KTB normal controls, 9.1% KTB BBD and BBD controls, 37.3% BBD cases, p-value<0.001).

Table 16. Crown-like structures of the breast and breast cancer risk among all breast biopsy tissue from African American women in the Detroit BBD Cohort (1997-2010) and the Komen Normal Tissue Bank

	KTB Normal	KTB BBD and BBD Controls [a]	BBD Cases	OR (95% CI) [b]	OR (95% CI) [c]
CLS-B (any)	3 (12.5%)	22 (33.3%)	35 (68.6%)	5.87 (2.94 – 12.1)	3.34 (1.58 – 7.38)
CLS ≥5	0	6 (9.1%)	19 (37.3%)	8.82 (3.45 – 25.9)	6.59 (2.27 – 21.7)
Median (range)	3 (1-4)	2 (1-33)	5 (1-109)		

[b]KTB BBD and BBD controls were combined as these show histologic abnormalities that indicate increased breast cancer risk

[b]*P*-value from ordinal logistic regression unadjusted for other factors.

[c]*P*-value from ordinal logistic regression adjusted for the logarithm of adipocyte area and BMI.

The presence of CLS-B on biopsy was associated with a 5.87 times increased odds of being in a higher-risk tissue category in this study (95% Confidence Interval (CI): 2.94 – 12.1, Table 16). After adjusting for total adipocyte area and BMI, this association was attenuated with a 3.34-fold increased odds of being in a higher-risk tissue category that was statistically significant (95% CI: 1.58 – 7.38). The presence of five or more CLS-B on biopsy was associated with a 8.82-fold increased odds of being in a higher-risk tissue category (95% CI: 3.45 – 25.9). Similarly, once adjusting for total adipocyte area and BMI, the association between the presence of five or more CLS-B on biopsy was attenuated to a 4.81-fold increased odds of being in a higher-risk tissue category (95% CI: 2.27 – 21.7).

Among the subset of women with a clinical indication for biopsy, or the BBD controls and BBD cases from the Detroit cohort, CLS-B were more likely to be identified in BBD case than BBD control tissue (68.6% versus 37.8%, Table 17, p-value = 0.004). BBD cases were also more likely to have five or more CLS-B on tissue slides than BBD controls (37.3% to 11%, p-value = 0.007).

Table 17. Crown-like structures of the breast and breast cancer risk among benign breast biopsies from African American women from the Detroit BBD Cohort, 1997-2010

	BBD Controls	BBD Cases	OR (95% CI)[b]	OR (95% CI)[d]
CLS-B (any)	17 (37.8%)	35 (68.6%)	3.50 (1.50 – 8.19)	2.94 (1.12 – 7.73)
CLS ≥5	5 (11.1%)	19 (37.3%)	4.45 (1.51 – 13.1)	3.78 (1.17 – 12.2)
Median (range)	2 (1-33)	5 (1-109)		

[a]*P*-value from conditional logistic regression unadjusted for other factors.

[d]*P*-value from conditional logistic regression adjusted for the logarithm of adipocyte area, proliferative disease, and BMI

Among clinically-indicated biopsies, the presence of CLS-B on biopsy conferred a 3.50-fold increased odds of breast cancer (95% CI: 1.50 – 8.19; Table 17); adjusting for total adipocyte area, BBD and BMI attenuated this increase to 2.94 times increased odds (95% CI: 1.12 – 7.73). The presence of five or more CLS-B on clinically-indicated biopsy conferred a 4.45-fold increase in odds of breast cancer (95% CI: 1.51 – 13.1); adjusting for total adipocyte area, BBD and BMI attenuated this association to a 3.77-fold increased odds of breast cancer (95% CI: 1.17 – 12.20).

CLS-B and breast cancer risk, adjusting for BMI and/or BBD

CLS-B was not associated with BMI in the overall study; the proportion of CLS-B positive biopsies varies slightly between BMI categories but with no apparent pattern. CLS-B positive biopsies were found in 40% of normal weight women, 41% of overweight women, 33% of obese class I women, and 49% of obese class II & III women (Table 18, p-value=0.2082). CLS-B presence was associated with increasing breast cancer risk among each BMI group (p-value<0.001). The proportion of CLS-B positive biopsies remained consistent among BMI groups in both the KTB Normals and BBD Cases. The proportion of CLS-B positive biopsies increased in KTB BBD/BBD controls with increasing BMI (22% in normal weight to 64% in obese class III).

Table 18. Crown-like structures of the breast and breast cancer risk adjusting for BMI among breast biopsy tissue from African American women from the Detroit BBD Cohort (1997-2010) or the Komen Normal Tissue Bank

	Overall	KTB Normal	KTB BBD and BBD Controls	BBD Cases
N (%) with any CLS-B	N = 139	n = 24	n = 66	n = 49
BMI <25	12/30 (40%)	0/1	4/18 (22%)	8/11 (73%)
BMI 25 – 29	13/32 (41%)	1/6 (17%)	3/11 (27%)	9/15 (60%)
BMI 30 – 34	10/30 (33%)	0/4	5/17 (29%)	5/9 (56%)
BMI 35+	23/47 (49%)	2/13 (15%)	11/20 (55%)	10/14 (71%)

CLS-B was present more frequently in biopsies with proliferative disease compared to biopsies with non-proliferative disease, but this difference was not statistically significant (59% versus 41%, p-value>0.1, Table 19). CLS-B presence was associated with increasing breast

cancer risk among each proliferative group (p-value=0.002). CLS-B frequency differed more between BBD cases and KTB BBD/BBD controls in biopsies showing non-proliferative disease (64% versus 26%) than biopsies with proliferative disease (70% versus 48%).

Table 19. Crown-like structures of the breast and breast cancer risk adjusting for BBD among breast biopsy tissue from African American women with breast biopsy tissue from the Detroit BBD Cohort (1997-2010) or the Komen Normal Tissue Bank

	Overall	KTB BBD and BBD Controls	BBD Cases
N (%) with any CLS-B	N = 117	n = 65	n = 52
Non-proliferative disease	26/63 (41%)	10/38 (26%)	16/25 (64%)
Proliferative disease without atypia	32/54 (59%)	13/27 (48%)	19/27 (70%)

IV. Discussion

Our findings demonstrate that CLS-B is associated with risk of both BBD and breast cancer. After adjusting for BMI and BBD, CLS-B was independently associated with breast cancer risk in this study of biopsy tissue from African American women with benign breast disease. These data suggest that CLS-B is a candidate biomarker on histology for breast cancer risk among women with benign breast disease, and these lesions may provide additional insight into early events to carcinogenesis.

Our study found a stronger association between presence of CLS-B and breast cancer risk among clinically-indicated biopsies (p-value = 0.028) than a prior study published by Carter et al[90] (p-value = 0.11). Carter el al found similar trends between CLS-B presence between normal and BBD tissue, but a more specific metric of five or more CLS-B on biopsy was necessary to discriminate between BBD controls and cases. The difference in association may be due to differences between study cohorts. The cohort described in Carter et al primarily consists of European American women, while ours consists of African American women. Prevalence of obesity is higher in African American women compared to European American women[92], and a recent study of CLS-B in mastectomy tissue indicates that CLS-B presence is also elevated in

African American women compared to Hispanic and European American women[87]. Additionally, the study cohort in Carter et al was diagnosed with BBD between 1967 and 2001, while the Detroit cohort is a more contemporary cohort diagnosed between 1997 and 2010. Obesity prevalence has steadily increased since the 1960s[97] and other changes in reproductive or hormonal exposures over time may contribute to the stronger results in our study.

Contrary to other studies[81,88,90,91,98–100], CLS-B was not associated with BMI in our study. This discrepancy may arise because of the reduced utility of BMI as a measure of adiposity in this population: African American women have lower body fat compared to European American women at the same BMI[85,86]. Only one other CLS-B study had a sample size large enough for race-specific estimates in African American patients, but Koru-Sengul et al was unable to test for an association with BMI as patient heights were not collected[87]. Our study design may contribute to this null finding; we may have been unable to detect an association with the limited sample size of this study. We also examined one CD68-stained slide per patient to determine CLS-B status, while several other CLS studies[81,91,98,99] examined five slides per patient. From a clinical perspective, it is more feasible to examine a single representative slide, so future studies should consider this protocol. Increases in adipose tissue area assessed most likely increases the likelihood of finding a CLS-B. A majority of CLS-B studies[81,87,88,91,98–100] utilize mastectomy or prophylactic mastectomy tissue, which reflects extremely high-risk tissue that may be enriched for CLS-B compared to our study tissue of normal and BBD tissue from the Komen Normal Tissue Bank and Detroit Cohort. Other CLS-B studies were able to utilize reduction mammoplasty tissue[101], but this tissue is more likely to exhibit benign breast lesions and less likely to exhibit lobular involution on microscopy than KTB normal population-level risk tissue[102].

Strengths of our study include the use of population-level controls and a contemporary cohort of African American women diagnosed with BBD and subsequently followed for breast cancer to assess the relationship between CLS-B and breast cancer risk. Additionally, our study included the use of normal controls by identifying tissue from KTB free from histologic

abnormalities as well as a contemporary cohort of African American women diagnosed with BBD and subsequently followed for breast cancer to assess the relationship between CLS-B and breast cancer risk. Another strength is the ease of translating this approach to the clinic. Our results suggest that staining only one additional slide for CD68 can provide valuable information on breast cancer risk. Limitations of our study include the limited sample size and tissue area assessed, but these did not hinder us from detecting a relationship between CLS-B and breast cancer risk independent of age, BMI and BBD. Another potential limitation is that we do not have information on several other breast cancer risk factors including age at menarche, parity, BRCA status, and family history that may confound the relationship between CLS-B and breast cancer risk. Examination of the tissue may compensate somewhat for this limitation, as the tissue represents the totality of exposures.

Currently, the standard of care for women with biopsies that contain cellular atypia requires a more extensive surgical excision and consideration of chemopreventative efforts and increased surveillance because these lesions are more likely to be near a synchronous breast cancer and are associated with the greatest risk of a subsequent breast cancer[38]. However, approximately 95% of women with BBD who develop breast cancer have non-proliferative or proliferative disease without atypia on biopsy[38]. Refining our understanding of breast cancer risk can allow us to personalize surveillance and prevention efforts. CLS-B could be used to better identify patients exhibiting metabolic obesity who are poised to benefit greatly from behavioral changes or surveillance.

Additional studies must be completed before CLS-B can be used as a histological marker of breast cancer risk. This risk associated with this structure needs to be validated in a larger cohort, and while quite distinct, formal studies of pathologic reproducibility are also warranted. Our study and a few others in mastectomy tissue[87,91] suggest there may race-specific nuances to the relationship between CLS-B and risk of a subsequent breast cancer, and point to the importance of diverse and contemporary cohorts to characterize breast cancer risk factors.

CHAPTER 5: CONCLUSIONS

Summary of Findings

The results of this dissertation show that breast cancer risk factors vary by race. In Aim 1 fibroadenomas were associated with a reduced risk of breast cancer compared to all other BBD on biopsy in the Detroit BBD cohort. When compared to other African American women in Detroit, women with fibroadenomas on biopsy were not at elevated breast cancer risk. In Aim 2 we found similar associations between breast density and breast cancer in both the Detroit BBD cohort and Detroit Screening cohort to previous reports. We found that African American women with BBD were more likely to have dense breasts than African American women without BBD, consistent with prior studies in European American women. Nodular patterns, assessed by Tabár and our radiologists's complexity indicator, were strongly associated with breast cancer in the Detroit BBD cohort. In Aim 3, CLS-B was independently associated with breast cancer risk, and the effect size was greater in our study of African American women than a prior report of European American women.

Future directions

Each chapter in this dissertation has clear next steps or subsequent lines of thought to examine. In Aim 1, fibroadenomas could be further examined for features that would make these benign tumors complex such as cysts, apocrine metaplasia, calcifications and sclerosing adenosis. The presence of these features indicates that the fibroadenoma is complex and may confer increased risk compared to fibroadenomas uncomplicated by the lesions[59].

In Aim 2, quantitative measures of breast density from our study mammograms at the time of biopsy could examined for an association with breast cancer risk. Serial mammograms spanning the time of biopsy to breast cancer diagnosis or end of follow-up could also be examined for women in our study to assess whether there are any changes in temporal trends of quantitative or qualitative density or parenchymal patterns. Further gene expression study from the nested

case/control study could also be used to assess whether there are gene expression patterns that are associated with density or parenchymal patterns.

In Aim 3, other outcomes could be assessed in our study, including inflammation on biopsy measured by lymphocyte infiltration and adipocyte size on H&E slides. These outcomes could be tested for associations with BBD, BMI, and CLS-B on biopsy. The design of Aim 3 sampled women from the nested case/control study in Aim 2, so associations between CLS-B and breast density or parenchymal patterns could be assessed. Similarly, gene expression patterns associated with CLS-B on biopsy could be examined.

Conclusions

Before the risk factors described in this publication can be incorporated into risk models, these findings must be replicated in prospective cohorts of African American women as well as women of other ethnicities to validate for subsequent use. Consistent, replicable risk factors are critical to inform appropriate clinical management. Risk model utility can be improved by creating subtype-specific breast cancer risk models. Current models describe risk of ER positive tumors which respond well to treatment and have better prognosis that ER negative tumors[31]; yet it is the more aggressive, often ER negative, cancers that would likely benefit most from risk prediction. These tumors that rapidly progress and quickly prove fatal tend to occur more frequently in African American women[9].

The studies included in this dissertation showed that breast cancer risk estimates may vary with time, race, and site of the population studied. These studies also illustrated the potential for novel markers of risk on mammogram and biopsy, suggesting that we are not effectively using current screening tools. Annually, about 40 million women undergo screening mammography and 1.6 million women undergo a breast biopsy in the United States[37]. Precision medicine has great potential to improve population health, but current screening tools and technology also have great untapped utility, at potentially at far less cost. Current screening methods must continually to be assessed as predictive and prognostic markers across the continuum.

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ABSTRACT

CHARACTERIZING NOVEL RADIOLOGIC AND PATHOLOGIC TISSUE-BASED RISK FACTORS FOR BREAST CANCER IN AFRICAN AMERICAN WOMEN WITH BENIGN BREAST DISEASE

by

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Major: Cancer Biology

Degree: Doctor of Philosophy

African American women (AAW) suffer a higher breast cancer mortality burden than women of other ethnicities in the US. More likely to be diagnosed with aggressive subtypes resistant to therapy and with rapidly fatal course than European American women (EAW), AAW may benefit greatly from earlier detection of breast cancers. However, it remains difficult to predict with a high degree of accuracy which women will develop breast cancer. Current risk assessment is especially poor for AAW, where models consistently underestimate risk in the subset of women with a prior biopsy. Risk assessment can be improved with the inclusion of new risk factors and, for AAW, race-specific estimates of risk factors. Here we characterized current and novel radiologic and pathologic tissue-based risk factors to improve risk assessment in an understudied population.

We utilized the Detroit BBD cohort to examine several risk factors. We first assessed subsequent breast cancer risk associated with fibroadenomas, a previously-described risk factor. In a nested case/control study, we assessed whether previously-described BI-RADS density scores and Tabár patterns were associated with breast cancer. We also examined whether a complexity indicator, summarizing features routinely described on mammogram but not yet examined as a risk factor, was associated with breast cancer. Finally, in a subset of the nested case/control study additionally age-matched to population-level controls, we examined whether

crown-like structures of the breast (CLS-B) were associated with breast cancer risk. We used several uni- and multivariable logistic, ordinal logistic, and conditional logistic models to estimate associations between risk factors and breast cancer.

In Aim 1, fibroadenomas on biopsy were not associated with a breast cancer risk increase over population level risk, unlike prior studies in EA women. In Aim 2, nodular patterns on mammogram, assessed by Tabár classification or our complexity indicator, were strongly associated with breast cancer. These findings suggest that AAW with BBD may benefit from additionally assessing parenchymal patterns on mammography. In Aim 3, we found that CLS-B was associated with breast cancer independent from BMI and BBD and may serve as a histologic marker of risk. These findings suggest differences in risk by race, though we cannot rule out secular differences between our contemporary cohort and other cohorts. These dissertation results, once replicated in other studies, can inform risk assessment tools to better identify women at increased breast cancer risk who may benefit from increased surveillance or chemoprevention.

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Publications (selected)

1. **Shaik AN**, Ruterbusch JJ, Abdulfatah E, Shrestha R, Daaboul F, Pardeshi V, Visscher DW, Bandyopadhyay S, Ali-Fehmi R, Cote ML. Breast fibroadenomas are not associated with increased breast cancer risk in an African American contemporary cohort of women with benign breast disease. *Breast Cancer Research.* 2018 Aug 9;20(1):91.

Abstracts (selected)

1. **Shaik AN**, Kiavash K, Stark K, Boerner JL, Ruterbusch JJ, Ali-Femi R, Cote ML. Adipose inflammation and the risk of benign and malignant disease in African American women, Oral presentation at AACR Annual Meeting, Chicago, Illinois April 2018.

2**. Shaik AN**, Boerner JL, Cote ML, Dyson G, Ali-Fehmi R, Bandyopadhyay S, Purrington KS. Immune cell infiltrates differ by obesity in tumor and adjacent normal breast tissue in African American women with triple negative breast cancer, Poster at AACR Special Meeting on Breast Cancer Research, Los Angeles, California. October 2017.

3. **Shaik AN**, Ruterbusch JJ, Abdulfatah E, Ghanim M, Daaboul F, Pardeshi V, Ali-Fehmi R, Visscher DW, Bandyopadhyay S, Cote ML. Fibroadenomas on benign biopsy and subsequent breast cancer risk in an African American cohort, Poster at AACR Special Meeting on Improving Cancer Risk Prediction for Prevention and Early Detection, Orlando, Florida. November 2016.