ASSOCIATIONS BETWEEN MODE OF DETECTION, IMAGING FEATURES, AND BREAST CANCER SUBTYPE IN THE CAROLINA BREAST CANCER STUDY

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ABSTRACT

Samantha Puvanesarajah; Associations between mode of detection, imaging features, and breast cancer subtype in the Carolina Breast Cancer Study
(Under the direction of Melissa A. Troester)

Purpose: Symptomatic cancers generally have poor prognosis compared to screen-detected cancers and likelihood of screen detection may vary as a function of biological subtype or imaging characteristics of the breast cancer. The aims of this study were to study the association between breast cancer subtype and 1) mode of detection and 2) radiologic/ imaging features.

Methods: In the first aim, we identified 1497 women diagnosed with primary invasive breast cancer from a linked data set between the Carolina Breast Cancer Study and the Carolina Mammography Registry. Among recently-screened (within 24 months) women (n=370, 25%), 45% of cancers were screen-detected (N=165), and 55% were interval-detected (N=205). Interval cancer was evaluated in association with clinical and genomic characteristics. In the second aim, 412 women with mammograms within 2 years before to 30 days after diagnosis were identified and associations between subtype and radiologic features were assessed.

Results: Interval cancer was associated with large tumors (>2 cm) (OR=2.3; 95% C.I.: 1.5, 3.7), positive nodal status (OR=1.8; 95% C.I.: 1.1, 2.8), and triple negative cancer (OR=2.5; 95% C.I.: 1.1, 5.5). Associations between interval detection and genomic characteristics were strong, and suggested that the vast majority of screen-detected cancers were indolent (96% were low risk of recurrence; 71% were Luminal A). Both young (<50) and African-American women showed higher relative frequency of masses and lower frequency of calcifications compared to older (≥ 50) and White women. Masses were less frequent among interval-detected vs. screen-detected

women (33% vs. 46%, p=0.04). Relative to Luminal A breast cancers (42% presenting as masses), PAM50 Basal-like and HER2-enriched subtypes were more likely to present as masses (59% and 72%, respectively). Few Basal-like and ROR-PT high cancers presented with calcifications (n=4/49 Basal-like and n=3/30 ROR-PT high).

Conclusions: Underlying cancer biology plays a role in screen detection; some interval cancers arise from aggressive tumor biology and distinct molecular and genomic subtypes of breast cancer present with distinct mammographic features. Results of this research add to our understanding of mammographic screening limitations and helps prioritize research questions in the context of evolving radiologic practices.

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LIST OF ABBREVIATIONS

ACOG	American Congress of Obstetricians and Gynecologists
ACS	American Cancer Society
AJCC/ UICC	American Joint Committee on Cancer/ International Union Against Cancer
BCSC	Breast Cancer Surveillance Consortium
BI-RADS	Breast Imaging Reporting and Data System
CBCS	Carolina Breast Cancer Study
CDC	Centers for Disease Controls and Prevention
CI	Confidence Interval
CIPHR	Cancer Information & Population Health Resource
CK 5/6	Cytokeratin 5/6
CMR	Carolina Mammography Registry
CT	Computerized Tomography
DBT	Digital Breast Tomography
DCIS	Ductal Carcinoma In Situ
DMIST	Digital Mammography Imaging Screening Trial
EGFR	Epidermal Growth Factor Receptor
ER	Estrogen Receptor
FDA	Food and Drug Administration
FFDM	Full Field Digital Mammography
FFPE	Formalin-fixed Paraffin-embedded
FISH	Fluorescent In Situ Hybridization
HER2	Human Epidermal Growth Factor Receptor 2

HR	Hormone Receptor
HRT	Hormone Replacement Therapy
IC	Interval Cancer
IHC	Immunohistochemistry
IRB	Institutional Review Board
MRI	Magnetic Resonance Imaging
OC	Oral Contraceptive
OR	Odds Ratio
PAM50	Prediction Analysis of Microarray 50
PD	Prevalence Difference
PR	Progesterone Receptor
RNA	Ribonucleic Acid
ROR-PT	Risk of Recurrence- Proliferation and Tumor size weighted
SDC	Screen-detected Cancer
SFM	Screen Film Mammography
SSN	Social Security Number
TMA	Tissue Microarray
TMIST	Tomosynthesis Mammography Imaging Screening Trial
TN	Triple Negative
UNC	University of North Carolina
US	Ultrasound
USPSTF	US Preventive Services Task Force

CHAPTER 1: BACKGROUND

1.1 Breast cancer epidemiology

Breast cancer is the second leading cause of cancer death among US women[1]. Though breast cancer survival has improved over the last two decades[2], breast cancer remains an important public health issue in the US. It is estimated that approximately 12% of women in the US will be diagnosed with breast cancer during their lifetime[3]. In 2013 alone, there were an estimated 232,340 new cases of breast cancer[4] and 39,260 breast cancer deaths[5]. Breast cancer mortality has declined over the past 25 years, by approximately 2% a year[6]; however, racial and ethnic disparities have increased due to a greater decline in mortality among white women compared to minority women[6]. Previous studies have suggested that mortality differences may be partially attributed to lower adherence to screening and more aggressive tumors at diagnosis, but tumors are also more aggressive in black women after conditioning upon screening initiation[7]. Better understanding of differences in prevalence of aggressive breast cancer subtypes requires resolution of how mammography use and mammographic detection contribute to tumor aggressiveness patterns overall, and also in black and white women.

1.2 Mammography

Mammography is the most common breast cancer screening method, and consists of a low dose x-ray image of the breast, which can be either recorded on film or digitally. In a mammographic image, adipose content, which is radiologically lucent, will appear dark while fibroglandular content, which is radiologically dense, will appear light. Tumors, which are radiologically dense, will also appear light on mammograms. In a national sample of US women,

the proportion of women over 40 who had a mammogram within the last two years increased from 29% in 1987 to 72.4% in 2003, and has remained fairly stable in both Whites and African Americans[8]. Newer screening techniques, such as tomosynthesis and ultrasound, are starting to become more utilized in the US, but are still far from reaching the widespread use of mammography.

1.2.1 Risks and benefits

The purpose of screening is to advance the time of diagnosis to an earlier more treatable cancer stage thereby reducing mortality[9,10]. Mammography has been shown to reduce breast cancer mortality in both randomized control trials[11,12] and population-based screening programs[13,14]. However, though it has been shown that breast cancer screening increases the proportion of early stage cancer, a lower decrease in incidence of advanced stage cancer has been observed, suggesting overdiagnosis of indolent cancers[15-17]. This has led to some of the controversy surrounding mammography with little agreement on screening strategies, risks and benefits, and the ideal target population, and with some questioning its true efficacy for screening[18]. A recent meta-analysis found that while mammography reduced breast cancer mortality, the magnitudes of effect were small (8 deaths prevented per 10000 women over 10 years for those aged 50-59)[19]. This, in addition to risk of false positive results[20] and their associated negative psychological effects, has led to the considerable debate around mammography use in asymptomatic women.

Disagreements are especially prevalent concerning women 40-49, a group for which the harms may outweigh the benefits, contributing to different screening guidelines among national organizations[21]. As summarized by the USPSTF, although slightly more cancers are detected when starting screening at age 40 vs. age 50, the number of unnecessary breast biopsies and

overdiagnosed breast tumors are also increased[22]. However, a meta-analysis of randomized trials shows a 15% reduction in mortality among women who were invited to begin screening from 40-49[23]. When coupled with the finding that screening mammography sensitivity is lower in younger women[24], it is understandable why there is a great deal of variability in screening recommendations for women in this younger age group. Disagreement also exists around screening regimens for women over the age of 75 as reviewed by Freedman et al.[25]. It appears that dissimilarity in recommendations arises because the risks and benefits of mammography differ for this older population compared to women < 75; even though older women have a higher probability of developing breast cancer[26], they may not experience as much of a survival benefit through early detection as younger women[19].

1.2.2 Mammography guidelines

Mammography guidelines during CBCS recruitment periods are shown in Appendix A. Until 1997, mammography recommendations were fairly consistent between national organizations such as the American Cancer Society (ACS) and the American Congress of Obstetricians and Gynecologists (ACOG) each calling for mammography every 1-2 years for women 40-49 and annual mammography for women 50 and older[27,28]. However, both the ACS and the United States Preventive Services Task Force (USPSTF) decreased age of initiation of annual mammography to 40 years in 1997 and 2002, respectively[28,29]. This is in contrast to the ACOG, who remained with their previous guidelines[30]. In 2009 further discordance developed when USPSTF updated their guidelines, increasing the age of initiation to 50 and recommending only biennial screens[31]. While it is difficult to determine how each of these strategies have individually affected breast cancer mortality rates in the US, models suggest that annual screening beginning at age 40 confers a greater reduction in breast cancer mortality (37.8 deaths

per 1000 women) relative to biennial screening after age 50 (25.8 deaths per 1000 women)[32]. Current screening recommendations in 2016 are still different from those in 2010. The USPSTF continues to recommend biennial screening after age 50[33], while the American Cancer Society suggests annual mammograms between ages 45-54, and biennial mammograms for women 55 and older, with screening continuing while a women has a life expectancy of 10 years or longer[34].

The lack of consensus in guidelines may have affected mammography screening rates; several studies have evaluated changes in screening behavior after the guidelines were announced. Three years after the 2009 USPSTF recommendations, using population-based data from the Behavioral Risk Factor Surveillance System (BRFSS), there was no significant change in age of screening initiation[35], while another study found decreased screening mammography after the guidelines were announced[36,37]. Still other studies based on self-reported data found no change[38] or increased screening since 2009[39,40]. Surveys administered to physicians 2-3 years after the 2009 USPSTF guideline change showed that the majority were not adhering to the new guidelines[41,42], which could lead to patients receiving conflicting recommendations. Confusion by health providers and among women[43,44], could have long-term effects on mammography initiation and adherence that remain to be seen, especially given that provider recommendation is a very strong predictor of mammography utilization[45,46].

1.3 Mode of detection

1.3.1 Definitions

Breast cancers can be categorized into three general groups based on mammographic mode of detection: screen-detected cancers (SDC), which are cancers that are detected by a screening mammogram; interval cancers (IC), which are cancers that are detected after a

negative mammogram in the interval between regular screenings; and clinically detected cancers (CDC), which we define as cancers that are neither screen nor interval-detected. The rate of interval cancers has been reported as being from 14% to 39%[47-54] (Appendix B, Table B1), and vary depending on screening interval.

1.3.2 Predictors of mode of detection

The factors that lead to missed mammographic detection of cancer are complex and encompass individual factors such as demographics and cancer characteristics, community factors such as screening facility availability and quality, and higher level characteristics such as national screening recommendations. All of these factors are often interrelated. One example of this is screening interval, which is the time between cancer screenings. Screening interval has been shown to be associated with mode of detection, with higher interval cancer rates measured with increasing screening interval[55-57]. Screening intervals can be determined using screening recommendations from national organizations. Facility distance can also determine screening interval; women who live a great distance from a facility may choose to screen less often compared to a woman who lives relatively near to a facility. In addition, women who have had a previous diagnosis of breast cancer and have chosen not to have a full mastectomy or women who have a strong family history of breast cancer may have shorter screening intervals.

For this project, we will be focusing on patient and tumor characteristics and how they are associated with mode of detection. Of the patient characteristics, age, race, mammographic density, and family history are predictors of interest in this study.

Mammographic density

One of the strongest risk factors for breast cancer is mammographic density[58-60]. Mammographic density is a measure of the epithelium and stroma, or fibroglandular, content of

the breast and can be determined using mammography. Women who have a higher relative proportion of fibroglandular content in their breast will have a higher mammographic density compared to women who have breasts that are predominantly fat tissue. Since mammographic density became used as a method to classify breasts, several different classifications methods have been used. The most commonly used classification for assessing mammographic density in the United States is the breast imaging and reporting data system (BI-RADS), developed by the American College of Radiology. BI-RADS is a semi-quantitative assessment, and is categorized from a (breasts are almost entirely fatty) to d (breasts are extremely dense.

Mammographic density is effected by several factors. Mammographic density is known to decrease with age and BMI[61]. In addition, hormone therapy is associated with increased density[62]. Several studies have described the relationship between mammographic density and mode of detection. Compared to screen-detected cancers, cancers that are non-screen-detected are more likely to occur in more dense breasts[63-65]. This relationship may be due in part to masking bias. Masking bias can occur in mammographic screening because both fibroglandular content and tumors have the same appearance on mammograms; this may cause some tumors to be missed in women in dense breasts. HRT use has been shown to be associated with interval cancers in several studies[66-70]; it is unknown if this relationship is due to the effect of HRT on mammographic density, though it is likely since a study within the BCSC found that HRT use was not an independent predictor of mammographic accuracy, but effects accuracy through its effect on breast density[71].

To assess the association between mammographic density and mode of detection in the absence of masking, studies have performed analyses stratified by density. Interval breast

cancers that arise in fatty breasts are more aggressive than interval cancers found in dense breasts[72-74].

Age

Younger age (age <50) has been reported to be associated with non-screen-detected cancers, including interval cancer[48-50,68,75-77]. The sensitivity of screening mammography increases with age[24], with one study showing an increase from 69.5% among women 30-39 to 87.7% in women 60-69[78]. Among women 50-69, the relationship between age and mode of detection may be confounded by hormone replacement therapy (HRT) use; after accounting for HRT use, age was not related to mode of detection among 60,000 women in the National Health Service Breast Screening Program[79] and 122,000 women in the Million Women Study[80], both aged 50-65.

Race

Racial disparities in breast cancer mortality could result from several factors, including mammography use, quality of mammography received, and breast cancer biology. Racial differences in mammography use have been well studied, and the racial disparity in mammography screening between Black and White women has diminished over the past two decades, with both races reporting similar mammography use over the last few years[26]. Although mammography usage is similar, there has been some research suggesting that Black women are more likely to receive screenings from facilities with less favorable characteristics such as lacking access to academic facilities, breast imaging specialists, and digital mammography[81].

The rates of interval cancer by race are less well studied. In a population of Chicago women, based on self-report, Black women were more likely to have an interval cancer

compared to White women; the authors concluded that the racial disparity was mostly accounted for by tumor and facility characteristics[82].

In a study conducted within the Breast Cancer Surveillance Consortium, African-American women were both more likely to have received inadequate screening and to present with larger and higher grade tumors than white women[7]. Among those that are screened, both digital and film-screen mammography perform equally well among white and black women[83,84], suggesting that mortality differences seen between the races beyond screening patterns may be due to tumor biology.

Family history/ BRCA status

The relationship between family history and mode of detection is inconclusive[47,63,70,74]. As mentioned previously, women with known BRCA mutations or have relatives with known BRCA mutations are often recommended to start screening earlier and to screen more often than woman with average risk, which is important to keep in mind when considering associations between BRCA status and mode of detection. Possessing a mutation in the BRCA gene is a strong predictor of developing breast cancer, with penetrance up to 88%[85,86]. Women with BRCA1 mutations are more likely to have triple negative cancer compared to women with no mutation[87]. There are also some differences in tumor biology and mode of detection with respect to which BRCA gene is mutated, which might explain why there are conflicting results for the association between family history and mode of detection, since most studies group BRCA1 and BRCA2 mutations together. Women with BRCA1 mutations are more likely to present with triple negative cancers and have lower mammographic detection rates, whereas women with BRCA2 mutations are more likely to have hormone receptor positive tumors and higher

mammographic detection rates[88]. In addition BRCA1 carriers were more likely to present with interval cancers compared to BRCA2 carriers[88,89].

1.3.3 Tumor characteristics by mode of detection

Compared to screen-detected cancers, clinically-detected and interval cancers generally have poorer survival[90-93] and more negative prognostic factors, including larger size, lymph node involvement, higher stage, higher grade, and are ER- and PR- [48,67,90,91,93-95]. In addition, lobular histology is more common among interval cancer compared to screen-detected cancers[48,53]. While differences between screen-detected and non-screen-detected cancers are marked, the differences between interval and clinically-detected cancer are mixed, with some studies reporting that they have similar clinical factors and survival [67,96-101], and others reporting that women with interval cancers have prognostic factors, such as grade and tumor size that fall between those of women with screen-detected and clinically-detected cancer[102,103]. Studies of interest are summarized in Table B2 (Appendix B).

1.4 Breast cancer subtype

Breast cancer is a heterogeneous disease. Characterizing heterogeneity has historically emphasized differences according to hormone receptor status, namely estrogen receptor (ER) and progesterone receptor (PR). However, there is additional heterogeneity within receptor-defined classes, necessitating a more fine-tuned approach when classifying breast cancers. As first reported by Perou in 2000[104], there are several subtypes of breast cancer based on RNA expression patterns, which have been confirmed in several populations[105,106]. These subtypes are luminal, HER2+/ enriched, basal, and normal-like. The luminal subtypes of breast cancer are ER positive and express genes that are similar to luminal mammary epithelial cells, while basal-like

tumors are ER negative and express genes associated with the myoepithelial cells of the outer layer of the breast duct[105,107].

In general, basal-like tumors have worse prognostic factors compared to luminal tumors; basal-like tumors are more likely to be invasive ductal cancers, high grade, and have a high proliferative index[108-110]. The basal-like subtype of breast cancer has been shown to have poor prognosis compared to the other intrinsic subtypes[92,111-113], and is more common among young and African-American women[111,114-116]. Subtype can be distinguished using immunohistochemical, RNA, or protein-based methods as described below.

1.4.1 IHC-based subtypes and mode of detection

Immunohistochemical (IHC) methods have been developed for subtype classification, and utilize formalin-fixed paraffin embedded tissues[117]. In studies using intrinsic subtyping, Luminal A tumors are generally those that are ER+/PR+/HER2- or ER+/PR-/HER2-. Luminal B tumors differ from Luminal A tumors in that they are positive for HER2; these tumors are ER+/PR+/HER2+ or ER+/PR-/HER2+. Basal-like tumors are triple negative (ER-/PR-/HER2-), and express either EGFR or CK5/6. IHC is the most commonly used classification scheme for molecular subtypes of breast cancer in epidemiologic studies.

Studies of interest examining associations between mode of detection and molecular subtype are summarized in Table B3 (Appendix B). Very few studies examining mode of detection have used basal-like breast cancer in their analyses[118,119], with the majority of studies using the triple-negative breast cancer phenotype[68,76,77,92,113,120]. Though triple-negative and basal-like breast cancers overlap, the two designations are not interchangeable[121-124]. Basal-like and triple negative breast cancers have differing tumor characteristics; in a study that reclassified triple negative tumors using gene expression profiling, basal-like tumors were found to be of a

higher grade and have a larger tumor size compared to non-basal-like triple negative tumors[121]. This emphasizes the importance of using 5-marker IHC subtyping to differentiate these two subtypes in future studies.

The studies that have examined IHC subtypes in association with mode of detection have tended to be small[68,77,118] (<200 participants) and studies with larger populations were demographically very different from CBCS[92,125,126]. Thus there is still more to be studied with respect to how mode of detection relates to IHC-defined intrinsic subtypes.

1.4.2 PAM50 subtypes and mode of detection

While some important advances in understanding the epidemiology of breast cancer have resulted from the use of IHC surrogates, new methods can better resolve distinct subtypes using tens to hundreds of genes. PAM50 is a multi-gene classification method, and is a gold standard for breast cancer subtyping, using the expression of 50 genes[127]. Using the expression of these genes, breast tumors can be classified into 5 intrinsic subtypes: luminal A, luminal B, HER2-enriched, basal-like, and normal-like. This method is more accurate in recapitulating subtypes based upon thousands of genes, and may be particularly useful in resolving epidemiologic differences between luminal A and luminal B breast cancers[128]. To our knowledge, only one study has reported associations between mode of detection and PAM50 subtypes[129], and no studies have examined associations with other PAM50 derived variables, such as the proliferation signature.

1.4.3 p53 and mode of detection

Wild type p53 is a tumor suppressor protein that plays a role in controlling the cell cycle and inducing apoptosis when a cell is damaged beyond repair[130,131]. Mutations in p53 are found in 20-30% of breast cancers[132]. The absence of p53 mutations is associated with longer disease

free and overall survival[133-135]. p53 status can be captured using IHC methods, or by application of an RNA-based gene signature.

Our interest in studying different molecular signatures in relation to mode of detection reflects the overarching hypothesis of this work: that the underlying cancer biology of screen-detected and interval cancers may be different. Previous lines of evidence have also supported this hypothesis. It has recently been hypothesized that cancers that grow large enough to be detected may harbor mutations that distinguish them from non-detectable cancers. In other words, certain mutations lead to the rapid expansion of a clonal population which contributes a large proportion of tumor mass, leading to detection[136]. Considering interval cancers, the majority of these cancers have increased cell proliferation[49,97] , and therefore interval cancers may harbor a similar or shared mutations that caused accelerated growth between the previous negative mammogram and detection. In line with this hypothesis, studies report that cancers with a p53 mutation are more prevalent among interval cancers compared to screen-detected cancers[70,138,139]. Although no study has specifically examined somatic mutations of interval vs. screen-detected cancers, beyond p53 and BRCA, one study reported copy number imbalances between screen-detected and clinically detected cancers in areas of the chromosome that are highly related highly malignant breast cancers[120], suggesting that tumor genetics may be useful in identifying women with indolent cancers. In the current study, we will revisit associations between p53 status and mode of detection using both IHC and RNA-based classification of p53 status.

1.5 Imaging features

There are several mammographic imaging features that are used in the detection and diagnosis of breast cancer including calcifications, masses, asymmetry, and architectural

distortion. Masses are the most common feature associated with cancers, followed by calcifications, architectural distortion, and asymmetry[140,141]. Documentation of each of these characteristics is highly associated with screening use.

1.5.1 Masses

Masses are a relatively common imaging feature for breast cancer; in a study using data from a prospectively collected hospital database, masses alone were present in 61% of detected breast cancers, while both masses and calcifications were present in 14% of cancers[141]. These proportions appear to change based on the population, as a series of patients from a hospital based in China found that masses, and masses along with calcifications, were each found in approximately 40% of cancers[142].

1.5.2 Calcifications

Calcifications can present with or without visible masses. Calcifications are non-palpable calcium deposits that can be found in breast tissue and are used in the detection and diagnosis of breast cancer. They can be visualized using mammography, appearing as bright spots on mammograms, and can present with both benign and malignant breast lesions. Calcifications have been found to be present in approximately 40% of non-palpable breast cancers[143] and up to 90% of DCIS cases[144]. Presence of calcifications predicts poor breast cancer survival[145], with women with casting-type calcifications having the worst prognosis[146]. Although the exact mechanism for how calcifications develop is unknown, they have been categorized into two categories based on composition, those made of hydroxyapatite and those made of calcium oxalate and it is believed that hydroxyapatite calcifications evolve more rapidly and may be the product of an active secretory process, while calcium oxalate calcifications are more likely to arise in benign lesions[147].

1.5.3 Breast asymmetry

Breast asymmetry occurs when asymmetrical breast density is present either within a breast or between two breasts. Though less common than calcifications and masses, it is still useful in cancer detection and shares similar positive predictive values at screening[140]. Cancers identified based on asymmetry are frequently false positives; it is posited that this may be because what was viewed as asymmetry may actually be the superimposition of normal breast structures[140]. However, upon a recall visit, additional views that are used to assess asymmetry more closely may lead to cancer detection.

1.5.4 Architectural distortion

Architectural distortion is a distortion of the normal breast architecture and is the third most common mammographic feature of non-palpable breast cancer[148]. Although only representing 6% of abnormalities detected by screening[148], it has a high positive predictive value for cancer at both screening and diagnosis[140], and both this feature and asymmetry present for breast cancers that were missed at screening mammography[149-151]. As with calcifications, architectural distortions can occur due to both benign (e.g., fat necrosis or radial scars) and malignant causes (e.g., DCIS or breast cancer). In a study that reclassified false negative mammograms, those that could have had a prognostic gain (been diagnosed at a lower stage) with early detection presented with a higher proportion of architectural distortion compared to cancers with no prognostic gain[152].

1.5.5 Relationship between imaging features and subtype

There have been a small number of studies published that have examined the association between breast cancer subtype and imaging features. HER2+ cancers are more likely to present with calcifications than other subtypes of breast cancer[141,153,154], while luminal and basal cancers

are more likely to present with masses[154,155]. A review by Gao et al., showed that while triple negative breast cancers typically presented with masses, they were less likely to also present with calcifications, asymmetry, and architectural distortion than ER+ or HER2+ cancers[142]. There have been no studies that examined associations between p53 or PAM50 subtype and mammographic features.

1.5.6 Relationship between imaging features and mode of detection

The relationship between mode of detection and imaging features is mixed. In a study conducted within a Spanish breast cancer screening program, a similar proportion of screen-detected and interval cancers appear to present with masses (63.3 vs. 60.5) and distortions (11.7 vs. 11.1); however screen-detected cancers had more calcifications (12.7 vs. 4.6)[155]. Similar patterns were seen with respect to mass and architectural distortion in a study conducted within the British National Public Health Service Breast Screening Program, except calcifications were equally as likely to be present between screen and interval-detected cancers[156].

1.6 Misclassification of interval cancers

Interval cancers can be further divided based on retrospective review into true interval cancers (cancers that present with normal/benign features on previous screening mammogram), false negatives (cancers that were detectable on previous mammogram based on retrospective review), minimal-sign (cancers that show detectable but non-specific features at previous screening), and occult tumors (cancers that show clinical signs of disease but no mammographic abnormalities)[72]. Studies that have done this retrospective review have found that about 50% of interval cancers are true interval cancers[72,157,158]. True interval cases have similar phenotype distributions to minimal sign cancers, whereas false negative and occult tumors were more similar to screen-detected cancers[72].

1.7 Digital vs. film mammography

Full field digital mammography (FFDM) has increasingly replaced screen film mammography (SFM) due to the technological advances that it provides, including images of higher resolution, the ability to adjust contrast, and increased efficiency of image storage. FFDM was approved by the FDA in 2000 with 98% of certified mammography facilities having FFDM units as of June 1, 2017[159]. While some studies have shown an increased rate of breast cancer detection using FFDM[52,160], the majority of studies, including the large DMIST trial[161], have reported no difference in cancer detection rate using FFDM vs. SFM[162-168] among the general screening population; increased cancer detection rates with FFDM may be due to higher rates of DCIS detection by this modality[169-171]. DMIST also showed that FFDM performed better among premenopausal women and women with dense breasts[161,172]. Studies using data from both European population-based screening programs[163,173,174] and an American mammography registry[51] have seen no difference in interval cancer rates when comparing the two screening technologies, although the Oslo II clinical trial found a lower interval cancer rate at FFDM vs. SFM[175].

With respect to subtype, among screen-detected cancers, higher rates of ER+, PR+, and HER2- cancers were detected using FFDM vs. SFM[176]; the authors also recorded increased detection of smaller, node-negative cancers using FFDM. Microcalcifications appears to be the radiologic feature that has the most potential to differ between cancers detected through FFDM vs. SFM. Recall rate, the percent of screening mammograms that necessitate diagnostic follow-up, is increased when using FFDM, with women most often recalled due to microcalcifications, some of which proved to be benign[176,177]. In addition, more interval cancers presented with

microcalcifications at the diagnostic mammogram following screening with SFM than with FFDM[178,179].

1.8 Future/ alternate screening methods

There are several supplemental/ alternate breast cancer screening methods that are in use, including ultrasound (US), magnetic resonance imaging (MRI), digital breast tomosynthesis (DBT), and molecular breast imaging (MBI). Of the alternate screening methods, US and MRI are the most common and are often used to supplement mammography. Both of these screening modalities do not involve radiation, allowing for increased use of these methods. While mammography results in a two dimensional image of the breast, tomosynthesis provides a quasi-3D image that is able to bypass one major drawback to mammography, which is tumors being hidden by overlapping tissue. There are several studies that are currently in progress to assess the efficacy of DBT in cancer detection compared to mammography. Studies have shown that compared to mammography, DBT is more effective in classifying both architectural distortion[180,181] and masses[181]. It remains to be seen if, compared to mammography, use of these alternate screening methods conclusively decreases the rate of interval cancers and/or results in increased cancer detection among women with dense breasts.

1.9 Summary

The goal of any cancer screening program is to be able to detect a cancer at a point in its natural history where it is treatable. Although mammography has been used for the past forty years, it remains somewhat divisive; this controversy may in part arise due to the confusion of the risks and benefits of mammography. There is some concern about mammography efficacy in subsets of women or for some tumor subtypes. It is established that mammography is less accurate in women with dense breasts; the sensitivity of mammography decreases from 87% in

women with almost entirely fatty breasts to 63% in women with extremely dense breasts[71] and that higher mammographic density is more often associated with interval breast cancers [63,64,182]. It has also been noted that mammography itself may contribute to lead time bias, a spurious survival benefit that is seen due to the time period between screening detection of a cancer and clinical presentation of the cancer, and length time bias, when screening preferentially detects indolent tumors[183] that may have never clinically manifested, leading to over-treatment. This comes at the price of potentially missing more aggressive, faster growing cancers that evade screening and have a large impact on mortality because they are detected at a more advanced stage than a screen-detected cancer.

Interval cancers are a group of cancers where screening may have failed and since these cancers have been shown to present with worse prognostic factors than screen-detected cancers, they may signify a circumstance where mammographic detection can be improved. Mammographic density is not the only factor that can affect mode of detection; molecular characteristics of a cancer such as intrinsic subtype or p53 status, which can be used to describe cancer agressivity, may also be associated with mode of detection. Understanding the tumor biology of screen vs. interval vs. clinically detected cancers is therefore important as it can provide information on the utility of mammography and enable a better understanding of its benefits and limitations.

The radiologic features of cancers [inclusive of both detection features (screen vs. interval-detected) and imaging features (calcifications, mass, etc.)] can potentially be used as a means to predict breast cancer subtype. Studies have shown that this is possible when categorizing cancers into broad subtypes, but it has not been used as widely with molecular subtypes defined using IHC and never using PAM50 derived subtypes. The population-based

study sample, with its racial diversity and well-characterized tumor biology, sets this study apart from similar studies. To better understand the limitations and public health opportunities surrounding breast cancer screening, it is essential to better characterize cancers that are detected outside of mammography.

CHAPTER 2: SPECIFIC AIMS

Mammography is the most widely used breast cancer screening method with approximately 70% of US women > 50 having had a mammogram within the past 2 years[184]. Among a regularly screening population, breast cancers can be categorized into two groups based on mammographic mode of detection: screen-detected cancers (SDC) and interval cancers, which are cancers that are detected symptomatically between regular screenings. Compared to SDCs, interval cancers generally have poor survival and many adverse prognostic factors[91-93,185]. Current literatures suggests that screening mammography may detect indolent cancers, and miss more aggressive cancers that have the greatest impact on mortality. Biologic characteristics of screen-detected vs interval cancers have been reported, but most previous studies with well-characterized tumors subtyped using IHC have relatively few subjects[68,118,125,138]. After a cancer has been detected through screening or otherwise, it may be further possible to identify cancer subtype based on mammographic features. Some studies suggest that triple negative cancers are more likely to present mammographically with rounder masses and fewer calcifications compared to ER+ cancers[142,154,186-188], although studies of these features have been small (<200 cases) and there remains important uncertainty about the relationships between imaging features and subtype.

In this study, we used a linked dataset of the Carolina Breast Cancer Study (CBCS) and the Carolina Mammography Registry (CMR) to study mammographic and radiologic characteristics by breast cancer subtype. Identification of these associations is important as it highlights limitations of mammographic screening.

Aim 1. **To identify molecular and genomic characteristics of screen vs. interval-detected cancers in the Carolina Breast Cancer Study.**

Tumor characteristics vary according to mode of detection, with interval cancers showing higher grade, larger size, and lower rates of hormone receptor positivity. However, there is limited data on how interval cancers relate to molecular subtype of breast cancer. Among linked invasive CBCS-CMR linked cases, patients were classified as screen vs. interval-detected using a two year screening interval. Associations between molecular and genomic characteristics (p53 status, 3- and 5-marker IHC subtyping, PAM50 subtype and risk of recurrence score) and mode of detection were assessed. We hypothesized that with high mammographic density and aggressive tumor characteristics such as larger size, higher grade, and more aggressive molecular subtype (Basal, p53 positive) will be at higher risk of having an interval-detected cancer.

Aim 2. To estimate associations between imaging features (mass and calcifications) and breast cancer subtype among women with invasive breast cancers with mammograms recorded in CMR (N=412).

Previous small studies (generally, N <200) have used broad categories (i.e., ER+, HER2+, triple negative vs. non-triple negative) to show that different tumor types present with different imaging features, which may affect probability of screen vs. interval detection. We hypothesized that calcifications, are more likely to present in screen-detected cases and in Luminal breast cancers and that interval cancers are more likely to present as a mass and are more likely to be basal-like.

CHAPTER 3: METHODS

3.1 Data Sources

3.1.1 Carolina Breast Cancer Study

The Carolina Breast Cancer Study (CBCS) is a population-based epidemiological study designed to identify both genetic and environmental risk factors for breast cancer among North Carolina women. The current study will use data from all three phases of CBCS. The CBCS has a high proportion of both African-Americans and young women, allowing for a more thorough assessment of factors affecting mammography uptake and cancer outcomes in these groups with a larger sample size compared to previous studies[68,73,100,118,119,189]. This research within the CBCS resource has been approved by the University of North Carolina at Chapel Hill School of Medicine Institutional Review Board (IRB).

The first two phases of CBCS recruited both cases and controls. Phases 1 and 2 recruited from 24 counties of eastern and central NC[190]. Cases were eligible women between the ages of 20 and 74 diagnosed with a primary invasive breast cancer May 1, 1993 and December 31, 2000. These women were identified through rapid case ascertainment from the North Carolina Central Cancer Registry. Controls were obtained from NC Division of Motor Vehicles lists for women aged 20-64; for women 65-74, the US Health Care Financing Administration lists were used. Controls were frequency matched to cases by race and 5-year age group. There were 2311 cases and 2022 controls enrolled in both of these phases. Randomized recruitment was used to oversample both African American and younger cases (under age 50)[114]. The sampling proportions differed between the two phases; in Phase 1, which recruited from 1993-1996, 100% of younger African Americans, 75% of African Americans over the age of 50, 67% of younger

non-African Americans, and 20% of non-African Americans over the age of 50 were sampled[190]. In Phase 2, which recruited from 1996-2001, all African Americans, 50% of younger non-African American, and 20% of older non-African American cases were sampled. The overall cooperation rate for invasive cases was 78%, with 84% for younger White cases, 80% for younger African American women, and 76% and 72% for older White and African-American women, respectively[191]. Overall cooperation for controls was 70%.

Women were interviewed at baseline by a nurse, at which point they also provided written consent for medical record requests. At baseline, nurse-administered interviews were used to collect demographic and risk factor data (described below). The median time between diagnosis and interview for cases was 3 months, with 80% being interviewed within 5 months of diagnosis. For controls, median time between selection and interview was 2 months, also with 80% being interviewed within 5 months of selection.

Phase 3 of CBCS enrolled 3000 participants from 2008-2013. The design is similar to that of the previous phases except that it enrolled invasive breast cancer cases only (no controls), and recruited from 44 counties in NC, a larger recruitment area[192]. Like Phases 1&2, randomized recruitment was used to achieve oversampling of African Americans. The sampling fraction for African Americans less than 50 years old, and greater than 50 were 100% and 60% respectively. The sampling fractions for non-African Americans less than 50, and greater than 50 years old were 40% and 15%, respectively.

3.1.2 Carolina Mammography Registry

The CMR[193] is a large community-based mammography registry that has studied the performance and outcomes of mammography in North Carolina since 1994. Data from the CMR comes from 39 practices and 65 facilities across North Carolina and is collected from

both patients and radiologists/technologists. Of the registries associated with the Breast Cancer Surveillance Consortium, the CMR has historically has the highest proportion of African American women. The age range of women in the CMR is 18-95 years. As of 2013, there were over 20,000 women diagnosed with breast cancer in the CMR[194]. Mammography records are linked to the North Carolina State Death Tapes to ascertain cause and date of death.

In CMR, at the time of mammography, reason for visit, the type of any screening or diagnostic studies performed, and imaging findings are recorded; this is done at each imaging visit. Radiologists choose from one of the following options when recording the reason for the patient's visit: 1) clinically detected (screening), 2) clinically detected, problem solving, diagnostic work-up, 3) continued work-up following abnormal mammogram or ultrasound, 4) short-term follow-up (mostly 6 month follow-up), 5) post-cancer follow-up, 6) biopsy, or 7) other. Next, the radiologist records the type of screening or diagnostic study that was performed: 1) mammogram, 2) tomosynthesis, 3) ultrasound, 4) MRI, 5) CT, 6) other. This information will be used when assigning women to categories of initiation, adherence, and mode of detection.

Mammographic density is recorded at each mammogram. Radiologists associated with the CMR visually assess mammograms and assign mammographic density. The mammographic density categories used in this study will be based on the Breast Imaging-Reporting and Data System (BI-RADS) breast composition categories, a standardized visual assessment metric that is published by the American College of Radiology[195]. The four BI-RADS categories, going from least dense to most dense are: almost entirely fatty (BI-RADS a), scattered fibroglandular (BI-RADS b), heterogeneously dense (BI-RADS c), and

extremely dense (BI-RADS d). Though this measure is subjective, it has been shown to have high interobserver and intraobserver agreement for the two most extreme categories, though some misclassification exists between the two intermediate categories[196,197]. There has also been variability in mammographic density classification reported in the presence of cancer[198]. Though the potential for misclassification exists, the BI-RADS classification measures will be utilized for this study because of its clinical relevance. BI-RADS is the only mammographic density classification method currently in clinical use in the US[199], making our study results more applicable to current clinical practice. In the CMR, mammographic density is not recorded for each breast, but per woman. This is acceptable for this study as it has been shown that mammographic density is highly correlated between breasts within a woman[200]. Because this study is concerned with how breast density is associated with breast cancer detection, mammographic density will be recorded using the mammogram closest in time to the diagnosis date, with priority being given to mammograms before diagnosis. For all analyses, mammographic density with be categorized as non-dense (BI-RADS 1 and 2) and dense (BI-RADS 3 and 4).

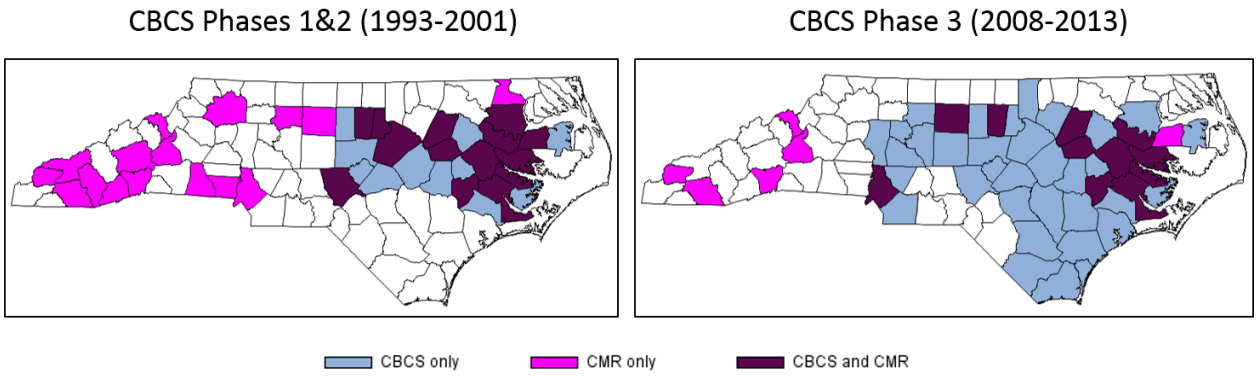
The CMR is reviewed annually by the University of North Carolina Chapel Hill School of Medicine IRB. CMR data undergo quality control checks: missing and incongruous data are flagged and reports are sent to practices for verification. The major advantage of linkage to CMR data is the detail of mammographic data that can be obtained from this source; this level of detail is useful for classification of women based in their mammographic screening behavior. During the recruitment time period for CBCS were recruited, with screen-film mammography being used for participants from Phases 1&2 and

digital mammography primarily being used over the last decade when CBCS Phase 3 was in recruitment[201].

3.1.3 Carolina Mammography Registry- Carolina Breast Cancer Study Linkage

Phases 1, 2 and 3 of CBCS (N=7331) were linked to the all participants enrolled in CMR from 1994-2014 inclusive (N=657,060), with a final dataset of 2,614 women (871 controls and 1,743 cases). Figure 3.1 shows the overlapping coverage of CBCS and CMR. Due to data security concerns, the linkage did not include women from one large CMR facility in eastern North Carolina. IRB approval was obtained before data merging.

Figure 3.1 Overlap of CBCS and CMR



The linkage was performed by experienced programmers from the Cancer Information & Population Health Resource (CIPHR) at UNC using the following identifiers: last four digits of social security number (SSN), first names, last name, middle initial, date of birth, and address. There were some limitations with regards to using SSN for linkage. Full security numbers were not available for all women in CMR and only the last 4 digits of SSN were available, so linkage was done using the last 4 digits. In addition, because some women in CBCS Phase 3 did not give permission for their SSNs to be used in any other data analysis, Phase 3 of CBCS had to be linked in two stages: once for those with SSN information available, and once for those without. The sensitivity of linkage for both stages was 100%. The specificity was 95.2% for women

without SSN information and 97.1% for women with SSN information. Matches and non-matches were determined using thresholds set based on linking probabilities of the identifiers chosen.

Selection bias

Selection bias was assessed in several different ways. First, selection bias between linked and unlinked women was first assessed (Table 3.1). Women who were linked were more likely to be cases, from Phase 2, older, post-menopausal, and had any hormone replacement therapy. No differences were seen by any other demographic variables. We also assessed if there was any bias related to whether social security information was available for use in linkage, and saw no differences by any of the variables studied (Table 3.2). Because only invasive cases were used in this study, we also evaluated differences in frequencies of demographic and cancer clinical variables comparing linked vs. unlinked invasive cases (Table 3.3). Among the linked invasive cases, there was a higher frequency of women over the age of 50, postmenopausal women, participants from Phase 2 of CBCS, and women who had ever used hormone replacement therapy. With respect to clinical characteristics, there was a higher frequency of higher stage (Stage III & IV) cancers and cancers with larger (>2 cm) tumors among the unlinked invasive cancers. Taking all of these selection bias analyses together, it appears that the linked women in our study display characteristics of an older population. This is expected as women captured in CMR are those who are getting mammography screening, which is generally recommended for women 50 and above.

Table 3.1 Assessment of selection bias: linked vs. unlinked women, CMR-CBCS linkage

	Linked (N=2614)	**Unlinked (N=4717)**	**X^2 p**
	N (%)	N (%)	
Case/control			
Control	871 (33.3)	1151 (24.4)	
Case	1743 (66.7)	3566 (75.6)	<0.0001
Phase of study			
Phase 1	583 (22.3)	1068 (22.6)	
Phase 2	1148 (43.9)	1534 (32.5)	
Phase 3	883 (33.8)	2115 (44.8)	<0.0001
Race			
White	1497 (57.3)	2657 (56.3)	
Black	1117 (42.7)	2060 (43.7)	0.4
Age at selection/ diagnosis			
<35	87 (3.3)	266 (5.6)	
35-54	1361 (52.1)	2698 (57.2)	
55-64	658 (25.2)	918 (19.5)	
65-74	508 (19.4)	835 (17.7)	<0.0001
Menopausal status			
Pre	1037 (39.7)	2211 (46.9)	
Post	1577 (60.3)	2506 (53.1)	<0.0001
Marital status			
Never married	255 (9.8)	461 (9.8)	
Married	1613 (61.7)	2750 (58.3)	
Widowed	277 (10.6)	504 (10.7)	
Separated, divorced	468 (17.9)	1001 (21.2)	0.01
Missing	1	1	
Education			
< High school	365 (14.0)	614 (13.0)	0.2
High school & Post High school	1430 (54.7)	2531 (53.7)	
≥ College	819 (31.3)	1569 (33.3)	
Missing	0	3	
Family income			
<15K	420 (17.3)	891 (20.2)	0.03
15-30K	537 (22.1)	926 (21.0)	
30-50K	552 (22.8)	950 (21.5)	
>50K	916 (37.8)	1645 (37.3)	
Missing	189	305	
Family history			
No	2102 (83.0)	3815 (83.2)	

Yes	431 (17.0)	768 (16.8)	0.8
Missing	81	134	
Any hormone replacement therapy			
Never	1753 (67.1)	3488 (74.1)	
Ever	858 (32.9)	1217 (25.9)	<0.0001
Missing	3	12	

Table 3.2 Assessment of selection bias among linked invasive cases from Phase 3 of CBCS: linkage with SSN vs. linkage without SSN

	Social security information available (N=399)	**Social security information not available (N=461)**	**X^2 p-value**
	N (%)	N (%)	
Race			
White	214 (54)	221 (47)	
Black	185 (46)	248 (53)	0.06
Age			
<50	247 (62)	275 (59)	
≥50	152 (38)	194 (41)	0.3
Education			
< High school	201 (50)	249 (53)	
≥ High school	198 (49)	220 (47)	0.4
Income			
< 30K	145 (38)	174 (40)	
>30K	26 (62)	261 (60)	0.6
Missing	18	34	
Family history			
No	294 (76)	366 (81)	
Yes	95 (24)	88 (20)	0.08
Missing	10	15	
Menopausal status			
Pre	138 (35)	179 (38)	
Post	261 (65)	290 (62)	0.3

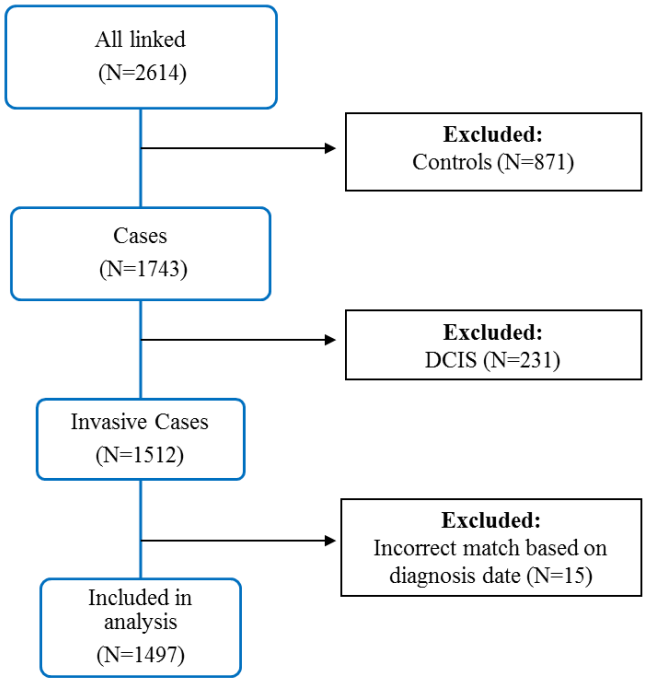
Table 3.3 Assessment of selection bias of clinical cancer characteristics of linked vs. unlinked invasive cases.

	Linked invasive cases (N=1497)	**Unlinked invasive cases (N=3309)**	**X^2 p-value**
	N (%)	N (%)	
Age at diagnosis			
<35	62 (4)	201 (6)	<0.0001
35-44	276 (18)	882 (27)	
45-54	489 (33)	1053 (32)	
55-64	386 (26)	640 (19)	
65-74	284 (19)	533 (16)	
Race			
White	788 (53)	1735 (52)	0.9
African-American	709 (47)	1574 (48)	
Phase of study			
Phase 1	252 (17)	609 (18)	<0.0001
Phase 2	377 (25)	570 (17)	
Phase 3	868 (58)	2130 (64)	
Menopausal status			
Premenopausal	590 (39)	1627 (49)	<0.0001
Postmenopausal	907 (61)	1682 (51)	
Marital status			
Never married	178 (12)	367 (11)	0.1
Married	881 (59)	1867 (56)	
Widowed	143 (10)	318 (10)	
Divorced	295 (20)	756 (23)	
Missing	0	1	
Family income			
<15K	237 (17)	628 (20)	0.06
15-30K	308 (22)	628 (20)	
30-50K	289 (21)	649 (21)	
>50K	568 (41)	1211 (39)	
Missing	95	193	
Education			
< HS	189 (3)	380 (12)	0.3
HS & Post HS	801 (54)	1746 (53)	
College+	507 (34	1182 (36)	
Missing	0	1	
First degree family history of breast cancer			
No	1174 (81)	2624 (82)	0.5

Yes	277 (19)	583 (18)	
Missing	46	102	
Any hormone replacement therapy			
Never	1059 (71)	2574 (78)	<0.0001
Ever	435 (29)	723 (22)	
Missing	3	12	
Tumor size			
<=2 cm	799 (55)	1623 (51)	0.02
>2-5 cm	507 (35)	1165 (37)	
>5 cm	143 (10)	381 (12)	
Missing	48	140	
AJCC/UICC Stage Grouping			
Stage I	645 (45)	1289 (40)	0.0007
Stage II	611 (42)	1360 (42)	
Stage III	159 (11)	436 (14)	
Stage IV	34 (2)	126 (4)	
Missing	48	98	
IHC subtype			
Present	427 (29)	722 (22)	<0.0001
Missing	1070 (72)	2587 (78)	
IHC subtype			
Basal-like	72 (17)	133 (18)	0.9
Luminal A	241 (56)	384 (53)	
Luminal B	39 (9)	73 (10)	
HER2+/ER-	27 (6)	46 (6)	
Unclassified	48 (11)	86 (12)	
Missing	1070	2587	
ER Status			
Positive	915 (63)	2088 (66)	0.1
Negative	496 (34)	999 (31)	
Borderline	36 (3)	92 (3)	
Missing	50	130	
PR Status			
Positive	762 (53)	1746 (55)	0.005
Negative	614 (43)	1214 (38)	
Borderline	67 (5)	203 (6)	
Missing	54	146	
HER2 Status			
Positive	206 (15)	480 (16)	0.4
Negative	1146 (85)	2474 (84)	
Missing	145	355	

The exclusion criteria applied in this study are shown in Figure 3.2. As a secondary quality control measure for the linkage, information from one commonly collected variable between the two data sets, date of diagnosis, was compared. Both CBCS and CMR collected data for this variable from the NC Central Cancer Registry, so date of diagnosis should therefore be the same if the match from the linkage was correct. There were 15 women where dates of diagnosis did not match, and these women were excluded from analysis. The final data set that was used for this dissertation contained 1497 women.

Figure 3.2 Flowchart showing exclusion criteria.



3.1.4 Data Acquisition

Letters of intent were filed with both CBCS and CMR before the linkage was done. The linkage was approved by the Institutional Review Board (IRB) at the University of North Carolina at Chapel Hill (IRB# 14-2263). A separate proposal for this study was approved by the UNC IRB (IRB# 16-2104).

3.2 Data Analysis

3.2.1 Mode of detection categorization

Mode of detection was constructed using both CMR and CBCS data and was used to classify how breast cancer was detected. We initially categorized mode of detection into three groups: screen-detected, interval-detected, or clinically detected, based on standard

definitions for mode of detection. However, due to likely missing data and heterogeneity within the clinically-detected group, this group was later renamed as "unknown" mode of detection and excluded from all analyses. In this section mode of detection categorization will be defined as it was originally planned.

The date of the last screening mammogram before diagnosis in combination with the date of breast cancer diagnosis was used to assign mode of detection. The date of breast cancer diagnosis was taken from CBCS data. A screening mammogram was defined using the definition constructed by the BCSC. The BCSC considered a mammogram to be screening if the indication for the exam is routine screening, a mammogram exam was done, the first exam sequence of the day, the woman was 18 or older, had no breast implants or prior mastectomy, bilateral screening views were done, there was no history of breast cancer cased on self-report or in the analytic cancer file, there was no imaging in the previous 9 months in the database or based on self-report, radiologist report, or comparison film, and the overall assessment code was not BI-RADS 6.

The mammogram findings are recorded in CMR, using BI-RADS assessment categories[202], as shown in Table 3.4; it is important to note that these categories are different from the BI- RADS categories that are used to describe mammographic density. Our definitions for classifying how breast cancer was detected use the outcome of a screening mammogram, more specifically, whether it was positive or negative. A positive screening mammogram will be defined as a screening mammogram with a BI-RADS assessment code of 4 (suspicious abnormality), 5 (highly suggestive of malignancy) or 0 (incomplete) or 3 (probably benign finding) with a recommendation for biopsy, fine needle aspiration (FNA),

or surgery. A negative screening mammogram will be defined as a screening mammogram with a BI-RADS assessment category of 1, 2, or 3 with no recommendation for biopsy, fine needle aspiration (FNA), or surgery.

Table 3.4 BI-RADS assessment categories.

Category	Description	Likelihood of Malignancy
0	Incomplete	Unknown
1	Negative	0
2	Benign finding	0
3	Probably benign finding	<2%
4	Suspicious abnormality	12-25%
5	Highly suggestive of malignancy	>95%
6	Known biopsy-proven malignancy	100%

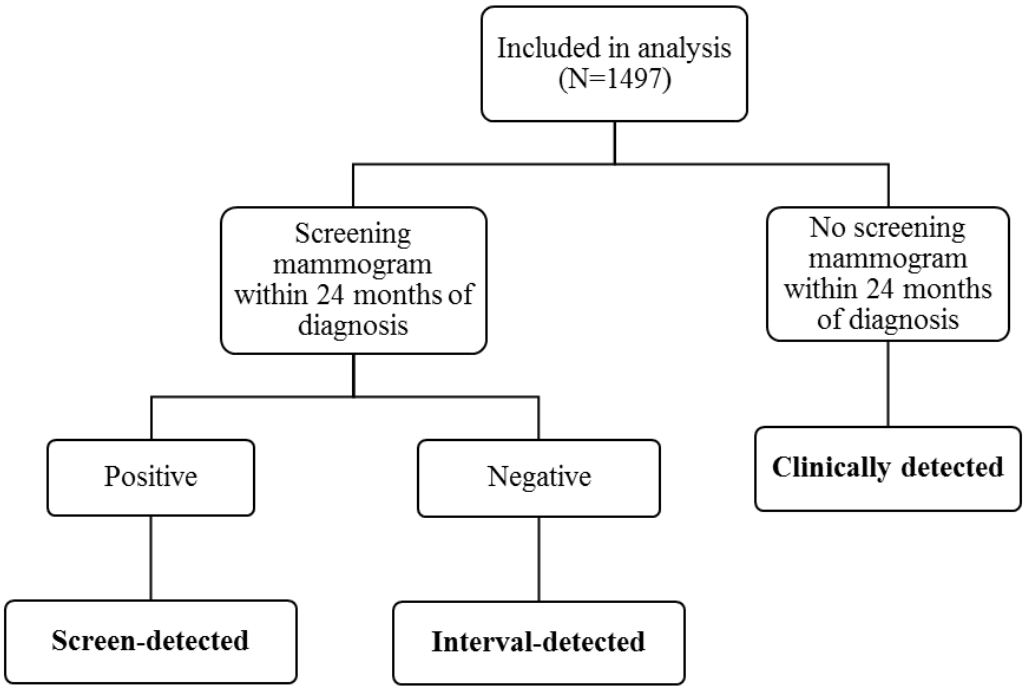
As described earlier and as shown in Appendix A, screening recommendations greatly varied from organization to organization and from year to year over all 3 phases of CBCS; recommendations were for 1 year, 2 year, and 1-2 year screening intervals. The 2-year interval was chosen for constructing the main mode of detection variable for this analysis in order to increase comparability with other studies and to reflect current screening recommendations, although a 1 year interval was used to construct the mode of detection variable that was used in sensitivity analyses. Figure 3.3 visually demonstrates the classification scheme that was originally used to categorize women by mode of detection. The following definitions were used to classify cancers:

Screen-detected. Cancer diagnosed within 24 months after a positive screening mammogram.

Interval-detected. Cancers diagnosed within 24 months after a negative screening mammogram and prior to the next screening mammogram, among women with no self-reported symptoms at time of screening mammogram.

Clinically detected. Women in this group are women who did not have a screening mammogram within 24 months of breast cancer diagnosis, and were not classified as screen-detected or interval-detected. This category includes women whose breast cancers were detected by themselves or by a clinician. This group was renamed "unknown" mode of detection and excluded in final analyses.

Figure 3.3 Mode of detection classification flowchart



To check the coding of the mode of detection variable, the variable constructed for this study was compared against the BCSC computed variable. The classification algorithm that the BCSC used is shown below, in Figure 3.4. One notable difference between the classification schemes used to construct the main mode of detection variable in this study vs. in BCSC is BCSC's use of a "peri-cancer" mammogram for further classification of some interval and clinically detected cancers. In addition, the BCSC classification schema also includes an "unknown" group. Table 3.5 shows the frequencies of the BCSC variable along with frequencies

for the mode of detection variable constructed using the 1 year screening interval and the 2 year screening interval. The frequency of screen-detected cancers is similar for all 3 variables (11-12%). The interval cancer counts from the 2 year interval variable are very similar to the BCSC variable, which is based on a 1 year interval, but they should in fact be approximately double the BCSC count since the time interval is twice as long. The BCSC definition includes an additional way to classify interval cancers, using the peri-cancer mammogram; when examining the full breakdown of the BCSC "interval" group, the interval cancers that were classified using the peri-cancer mammogram accounted for about 50% of interval cancers. Since the peri-cancer mammogram was not used for the variable used in this study, this would account for why the study variable interval cancer counts are approximately half of those from the BCSC.

Figure 3.4 BCSC mode of detection classification.

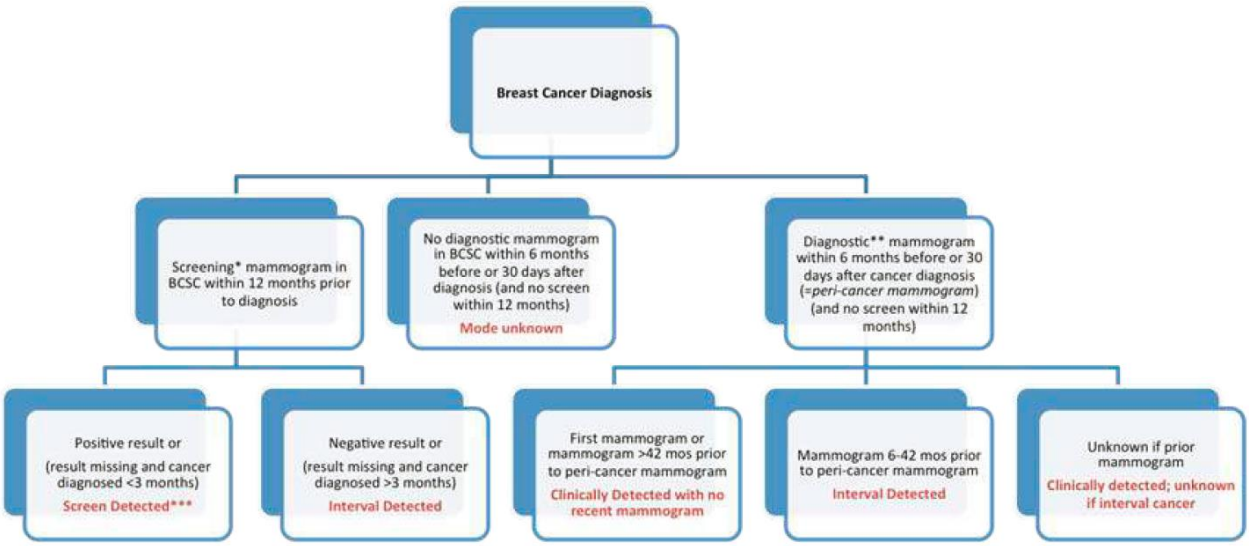


Table 3.5 Comparison of frequencies of mode of detection variable.

	1 year interval N (%)	**2 year interval N (%)**	**BCSC (1 year) N (%)**
Screen-detected	161 (11)	165 (11)	176 (12)
Non-screen-detected	1336 (89)	1332 (89)	295 (20)
Interval	107 (7)	205 (14)	196 (13)
Clinically detected	1229 (82)	1127 (75)	99 (7)
Unknown			1026 (69)

CBCS

3.2.2 Clinical and molecular variables

All clinical tumor variables that were used are described in Table 3.6. Histological grade was determined by a CBCS study pathologist. All variables in the table below are available in all 3 phases of CBCS. Tumor size, nodal status, and stage were abstracted from medical records. ER and PR status were determined from medical record abstract and from IHC staining; women with values that were borderline had their status set to missing. HER2 status was determined using IHC only for Phases 1&2. In Phase 3 of CBCS, HER2 status was determined using IHC and FISH. Women who were positive, negative, or borderline by IHC and were missing FISH status were classified using IHC HER2 status. Women who were either missing or borderline for IHC HER2 status, but had FISH results were assigned the FISH status. Women who were either positive for both IHC and FISH or negative for both IHC and FISH were assigned the IHC status. When women did not have an IHC HER2 status that matched FISH status, but FISH status was positive, these women were classified as HER2 positive; otherwise HER2 status was set to missing.

Table 3.6 CBCS clinical tumor variables

Variable	Description/ Code in statistical analysis
Tumor size	Categorized as ≤ 2 cm and >2 cm
Nodal status	Categorized as positive and negative. Positive is defined as either having at least one node positive for malignancy or lymph node metastasis.
Stage	Based on AJCC/UICC Stage grouping, categorized as: 1) Stage I & Stage II 2) Stage III & Stage IV
ER status	Categorized as positive/negative.
PR status	Categorized as positive/negative.
HER2 status	Categorized as positive/negative.

Subtype definitions

IHC

Because CBCS data is more comprehensive, all breast cancer subtype data came from the CBCS dataset, despite the availability of limited histologic and molecular data in the Carolina Mammography Registry. Approximately 64% (N=1149) of enrolled Phase 1&2 CBCS women had sufficient tissue for IHC analysis[114], with a similar proportion of women in CBCS Phase 3 (1888/2998=63%). Among women in Phases 1&2, there are a few significant differences between women with and without sufficient tissue: women with sufficient tissue had a higher proportion of African-American women, later stage at diagnosis[114] and larger tumors[111]. In CBCS Phases 1&2, the tumor tissue was sectioned and stained at the Immunohistochemistry Core Laboratory (ICL) at the University of North Carolina. A single pathologist reviewed all slides to confirm diagnosis of breast cancer and to assign tumor histology [203]. The following IHC markers were used to distinguish intrinsic subtype: estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor-2 (HER2), human epidermal growth factor-1 (HER1), and cytokeratin 5/6 (CK5/6). Previously described assays were used for these IHC markers[111,204,205]. ER and PR status were determined from medical records for the 80% of women who had this

data available from medical records[204]; for the remaining cases with paraffin-embedded tissue available, IHC analysis was performed at the Immunohistochemistry Laboratory. Positivity for ER and PR status were defined as having more than 5% of cells showing nuclei-specific staining[111]. Tumors with HER2 staining in more than 10% of cells were considered HER2 positive[205]. Positivity of EGFR was defined as any HER1 staining and positivity for CK 5/6 was defined as any cytoplasmic and/or membranous staining[117]. Previously identified IHC profile proxies for intrinsic subtypes are shown in Table 3.7[111,206].

For Phase 3, paraffin-embedded tumor blocks were used for tissue microarray (TMA) construction[128]. These TMAs were stained for ER, PR, HER2, Ki67, CK5/6, and EGFR by IHC, and digitally quantified using digital image analysis as described by Allott et al[128].

Table 3.7 IHC markers used to classify subtypes

Intrinsic subtype	**IHC profile for clinical subtype (3 marker)**	**IHC profile for Phases 1 & 2**	**IHC profile for Phase 3**
Luminal A	HER2-, ER+ and/or PR+	HER2-, ER+ and/or PR+	ER+, PR ≥ 20%, HER2-, AND Ki67 <10%
Luminal B	HER2+, ER+ and/or PR+	HER2+, ER+ and/or PR+	ER+, PR≤20%, HER2-, AND Ki67 ≥10% OR ER+, PR ≥10%, HER2-, AND Ki67 ≥10%
Triple negative	HER2-, ER-, PR-	HER2-, ER-, PR-	HER2-, ER-, PR-
Basal-like		HER2-, ER-, PR-, EGFR+ and/or CK 5/6+	(ER- AND PR- AND HER2-) AND (EGFR ≥ 1% OR CK5/6 ≥ 1%)
HER2+/ER-	HER2+, ER-, PR-	HER2+, ER-, PR-	ER- AND HER2 positive
Unclassified	N/A	ER-, PR-, HER2-, EGFR-, CK 5/6-	Equivocal HER2 or missing biomarker status for one or more markers

PAM50

PAM50 subtyping was performed on a subset (n=2007) of samples from CBCS Phases 1-3; 32% of these women (N=644) were among the invasive cases in the linked CBCS-CMR data set. For samples from CBCS 1&2 (N=188), RNA was extracted from two unstained 10-µM FFPE slides per patient. For women in CBCS Phase 3, RNA was extracted from cores (N=377) and slides (N=79). As described previously, for women with cores available, RNA was extracted from two flash frozen 1.0-mm cores taken from paraffin-embedded tumor blocks that were pooled for analysis[128]. For women who did not have cores available, two unstained 4-µM FFPE biopsy slides were used per patient for RNA extraction. Extracted RNA was isolated using the RNEasy FFPE Kit (Qiagen) and Nanostring analyses were performed in the Rapid Adoption Molecular laboratory at UNC.

All CBCS Phase 1&2 samples were run using a Nanostring probe set of 417 genes and the majority of CBCS Phase 3 samples were run using a probe set of 200 genes. Both code sets contained the 50 genes that make up the PAM50 group of genes. Tumors were classified as luminal A, luminal B, HER2-enriched, basal-like, and normal-like using the PAM50 predictor[127].

RNA gene expression for p53 mutation status was determined using a previously published 52-gene p53 signature[207]. A different subset of the PAM50 genes were also used to construct the risk of recurrence score, taking into account proliferation and tumor size (ROR-PT)[208]. The ROR-PT score is the research correlate of the clinically used Prosigna assay (NanoString Technologies Inc., Seattle, WA, USA), which has been clinically validated[209]. The ROR-PT is a continuous score, but can be categorized (Low/Medium/High) using

published protocols[127]. In this data set, the ROR-PT score was correlated with both PAM50 subtype and p53 status.

Selection bias analysis was done among the linked invasive cases that were included for analysis (N=1497) to ascertain any differences between the group of women that had RNA data available (N-644) vs. those who did not (N=853) (Table 3.8). The only difference that was seen was with respect to CBCS recruitment phase, with a smaller proportion of women from the early phases of CBCS 1&2 having genomic data available.

Table 3.8 Assessment of selection bias among women with and without genomic data available

	Genomic data available (N=644)	**No genomic data (N=853)**	**X^2 p**
CBCS Phase			
1&2	188 (29)	441 (52)	
3	456 (71)	412 (48)	<0.0001
Race			
White	323 (50)	465 (55)	
Black	321 (50)	388 (45)	0.1
Age			
<50	369 (57)	474 (56)	
≥50	275 (43)	379 (44)	0.5

p53

p53 status was assigned based on both IHC data (Phases 1-3 of CBCS) and RNA (Phase 3) data. p53 positivity for IHC was defined as dark nuclear protein staining present in 10% or more of invasive cells, all other cases were considered p53 negative[210]. While an IHC-based method for p53 classification is more widely used due to its relative ease, it cannot detect all of the types of p53 mutations that RNA-based methods are able to. For Phase 3, we will have both p53 data derived from both IHC and RNA. p53 classification was compared using both methods in CBCS3 (Williams et al., in preparation) and it was found

that there was increased misclassification of p53 mutant status when using IHC methods compared to RNA; 20% of cases were found to be p53 mutant using IHC methods whereas 41% were mutant according to RNA-based methods.

3.2.3 Imaging feature categorization

All mammography data used in this analysis came from CMR. The indication for the study (screening/diagnostic/ follow-up), breast composition, important findings (imaging features), and final assessment (negative, benign, etc.) are recorded for each imaging exam. BI-RADS classifications, which can be used to predict malignancy[211-213], for each imaging feature are noted by the radiologist for each imaging exam performed. Imaging features used in this analysis were mass and calcifications. Architectural distortion and asymmetry were not used due to low prevalence in our study sample. All data on these features were extracted from the most recent diagnostic exam (recorded within two years before to 30 days after diagnosis) when possible. Data from the most recent (within two years before diagnosis) screening mammogram was used for women who did not have diagnostic exam data available. Due to power considerations, imaging features were categorized dichotomously. A feature was considered "absent" when BI-RADS=1; a feature was categorized as "present" when BI-RADS=2, 3, 4, or 5. Imaging features with BI-RADS=0 were excluded. The imaging feature variables used in analysis were any mass (mass ± calcifications), any calcifications (calcification ± mass), and mass only (mass without calcifications). Presence of any mass was the most common (49%), followed by mass only (42%), and any calcifications (20%).

Selection bias analysis was done among the linked invasive cases that were included for analysis (N=1497) to ascertain any differences between the group of women that had imaging data available (N=412) vs. those who did not (N=1085) (Table 3.9). The only

difference that was seen was with respect to CBCS recruitment phase, with the majority of women that had imaging data available being from the first 2 phases of CBCS.

Table 3.9 Assessment of selection bias among women with and without imaging feature data

	Missing data N (%)	**Included in analysis N (%)**	**p**
Age			
≥ 50	621 (57)	222 (54)	
< 50	464 (43)	190 (46)	0.2
Race			
White	584 (54)	204 (50)	
Black	501 (46)	208 (50)	0.2
CBCS Phase			
1&2	358 (33)	271 (66)	
3	727 (67)	141 (34)	<0.0001

3.2.4 Demographics/ confounders

The demographic information that was used in analyses are presented in Table 3.10. These variables were chosen based on the literature and the data available in the data set. Though the CMR collected demographic information, all demographic data to be used in analyses, was taken from the CBCS dataset for consistency. This information was collected during the nurse administered in-person interviews. All measures were self-reported, but BMI was nurse-measured. Women who are not White or African-African American will be excluded, as we will not have enough power to detect any associations in these smaller racial groups.

Table 3.10 Description of demographic variables.

Variable	Description/ code in statistical analysis
Age at diagnosis	<50 50-74
Race	White African-American
BMI	Underweight (BMI < 18.5) Normal weight (18.5 ≤ BMI < 25) Over weight (BMI ≥ 25)
First degree family history of breast cancer	Yes No
Highest level of education completed	< High school ≥ High school
Family income	< $30,000 ≥ $30,000
Menopausal status	Premenopausal Postmenopausal
Marital status	Married Single Widowed/ divorced
Oral contraceptive use	Ever (current or former) or never
Hormone replacement therapy use	Ever (current or former) or never

Because mammographic density can change for a variety of reasons (e.g., age, parity, HRT use), a sensitivity analysis was performed to see how changing the time interval used to assign mammographic density status affected results. Four different density variables were made using different time intervals before/after diagnosis. These time intervals are: ≤ 5 years before diagnosis, ≤ 10 years before diagnosis, ≤ 5 years before or after diagnosis, and ≤ 10 years before or after diagnosis. Univariate analyses were done comparing associations between each MD variable and mode of detection (Table 3.11). All four definitions of mammographic density yielded similar distributions and associations with mode of detection (Table 3.12). Using this information, mammographic density using Definition 4 was used for all analyses.

Table 3.11 Definitions used to construct mammographic density variables.

	Definition 1	Definition 2	Definition 3	Definition 4
	Women with MD info < 5 years before diagnosis (N=642)	Women with MD info < 10 years before diagnosis (N=884)	Women with MD info ± 5 years of diagnosis (N=962)	Women with MD info ± 10 years of diagnosis (N=1241)
Density				
1	32 (5)	39 (4)	43 (4)	52 (4)
2	233 (36)	299 (34)	378 (39)	468 (38)
3	311 (48)	451 (51)	455 (47)	608 (49)
4	66 (10)	95 (11)	86 (9)	113 (9)

Table 3.12 Associations between each density variable and mode of detection.

		Definition 1	Definition 2	Definition 3	Definition 4
Interval vs. screen-detected	1 year	1.9 (1.1, 3.2)	2.0 (1.2, 3.3)	2.1 (1.2, 3.4)	2.1 (1.3, 3.5)
	2 year	1.7 (1.1, 2.7)	1.8 (1.2, 2.8)	1.7 (1.1, 2.7)	1.9 (1.2, 2.9)

3.2.5 Statistical methods

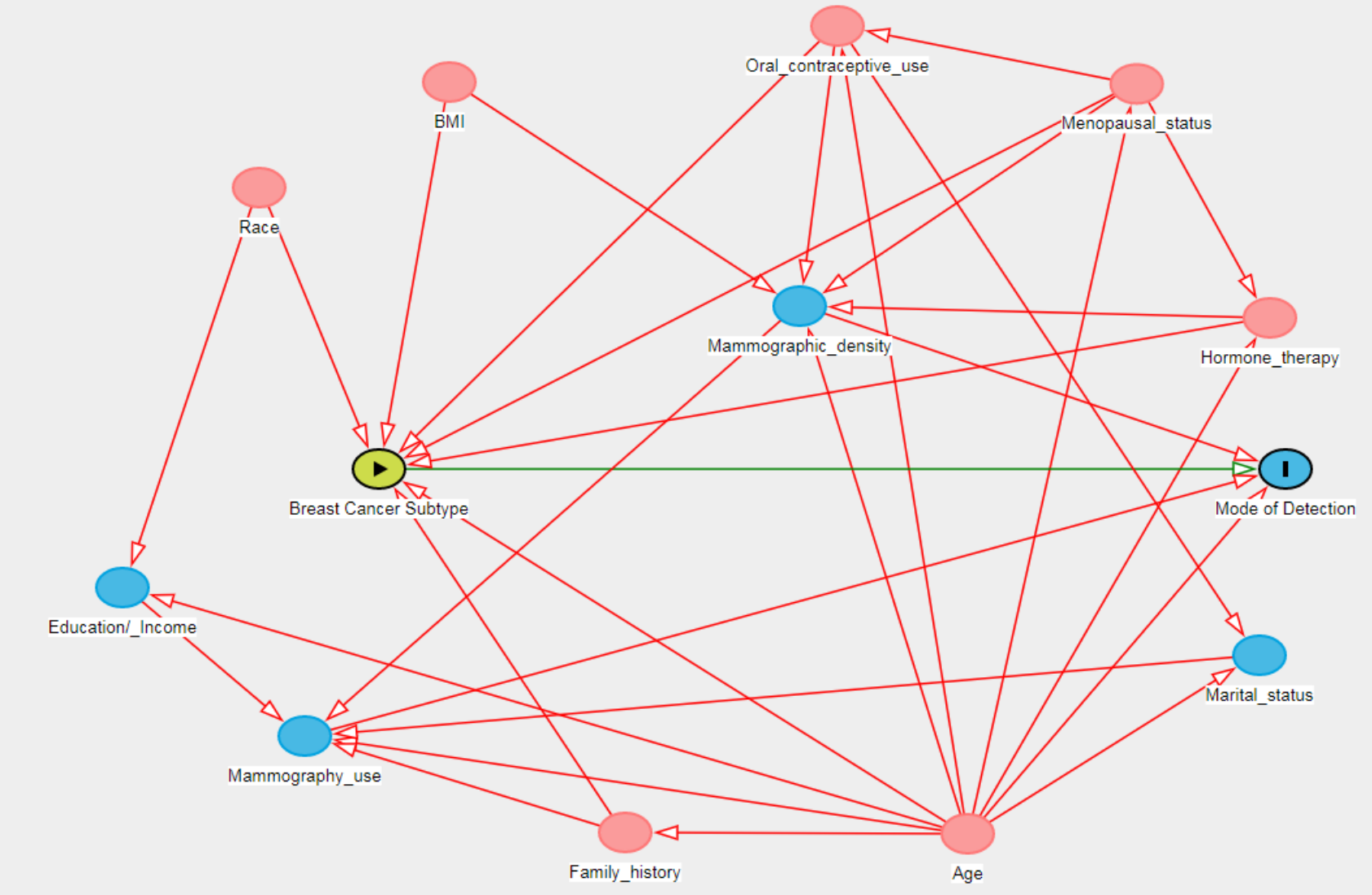
Clinically detected women were excluded from all analyses because this group is likely to be heterogeneous due to misclassification. Imaging facility participation in the CMR is voluntary. We do not have full mammography records for all women in this study owing to the fact that not all imaging facilities in NC participate. This missing information would likely lead to misclassification of mode of detection, with estimates of clinically detected cancers likely to be inflated by including both screen and interval-detected women. Due to the study definition of clinically detected cancers, women for whom we are missing screening information were classified as clinically detected because any cancer detected will appear to occur more than two years after screening. Therefore, clinically detected women were excluded since associations derived using this group most likely will not reflect true clinically detected cancers (cancers diagnosed among mammography non-imitators, or irregular screeners).

In Aim 1, t-tests were used to compare mammography usage characteristics by demographic characteristics. Potential confounders were chosen based on a review of the literature and a directed acyclic graph (Figure 3.5). Logistic regression was used to calculate univariate odds ratios for associations for each of the demographic variables (e.g. age, race, mammographic density) with mode of detection, with screen-detected cancers being used as the referent group. Adjusted odds ratios were then calculated for the association between clinical and molecular variables and mode of detection; odds ratios were adjusted for patient variables found to be significant in the univariate analysis.

Because mammographic density was considered a potential effect measure modifier of the relationship between patient and cancer characteristics and mode of detection, all analyses were repeated stratifying for mammographic density. Due to the large proportion of African-American women in our study sample, we also stratified analysis in Aim 1 by race, since race-stratified analyses are not commonly reported. Odds ratios whose 95% confidence intervals did not contain the null value of 1 were considered to be statistically significant.

For Aim 2, chi-square tests were used to study differences in mammographic feature presentation by patient, clinical, molecular, and genomic factors. Prevalence differences and their associated 95% confidence intervals were calculated using generalized linear models. Because differences in frequencies of imaging features were observed by CBCS phase of study, all prevalence difference analyses were adjusted for CBCS phase. All analyses in both aims were performed using SAS version 9.3 (SAS Institute, Cary, NC).

Figure 3.5 Directed acyclic graph



CHAPTER 4: MOLECULAR AND GENOMIC CHARACTERISTICS OF INTERVAL BREAST CANCERS

4.1 Overview

Introduction: Breast cancers detected after a negative breast screening exam and prior to the next scheduled screening are referred to as interval cancers. These cancers generally have poor clinical characteristics compared to screen-detected cancers, but associations between interval cancer and genomic cancer characteristics are not well understood.

Methods: Mammographically-screened women who were diagnosed with a primary invasive breast cancer from 1993-2013 (n=370) were identified by linking the Carolina Breast Cancer Study and the Carolina Mammography Registry. Among women with a registry-identified screening mammogram 0-24 months before diagnosis, cancers were classified as screen-detected (N=165) or interval-detected (N=205). Using logistic regression, we examined the association of mode of detection (interval- or screen-detected) with cancer characteristics (tumor size, stage, and clinical, IHC, and genomic biomarkers), overall, and in analyses stratified on mammographic density and race.

Results: Interval cancer was associated with large tumors > 2 cm) (OR=2.3; 95% C.I.: 1.5, 3.7), positive nodal status (OR=1.8; 95% C.I.: 1.1, 2.8), and triple negative cancer (OR=2.5; 95% C.I.: 1.1, 5.5). Associations between interval detection and genomic characteristics were strong, with interval cancers more likely to have non-Luminal A subtype (OR=2.9; 95% C.I.: 1.5, 5.7). Results suggested that the vast majority of screen-detected cancers were indolent (96% had low risk of recurrence genomic scores; 71% were PAM50 Luminal A). When stratifying on race and mammographic density, associations between interval detection and poor prognostic

features were somewhat stronger among women with low mammographic density and among black women, although there were no significant interactions.

Conclusions: Strong associations between interval cancers and both non-Luminal A subtype and high risk of recurrence score provide genomic evidence supporting that aggressive tumor biology is an important contributor to interval cancer rates.

4.2 Introduction

The purpose of screening is to diagnose cancer at an earlier more treatable stage, thereby reducing mortality[9,10]. Mammography, the most widely used breast cancer screening method, has been shown to reduce breast cancer mortality in both randomized control trials[11,12] and population-based screening programs[13,14]. However, mammography remains controversial. Interval cancers, which represent a failure of mammographic screening, are defined as cancers detected after a negative mammogram in the interval between regular screenings. These cancers tend to be higher stage and grade at the time of diagnosis whereas screen-detected cancers have been reported to have more indolent molecular characteristics[91-93,185]. The proportion of interval cancers in screened populations varies from 14% to 38%[47-54], depending on screening interval and underlying population breast cancer incidence rates[214].

Interval cancers are believed to arise from multiple scenarios. First, interval cancers may be cancers that existed but were missed at screening (false negatives). Some missed tumors are believed to be caused by masking bias, wherein high mammographic density can conceal a tumor from being detected, leading to false negative interval cancers[72,215]. Second, interval cancers may represent cancers that possess aggressive cancer characteristics that enable them to grow to detectable levels between screenings. Understanding how biologic characteristics and

masking contribute to the rate of interval cancer could help in understanding the limitations of mammography, particularly in light of emerging new technologies, like 3D-mammography.

In this study, we used a population-based study sample to examine the molecular characteristics (immunohistochemical and RNA-based) of interval cancers. Previous studies have shown that interval cancers have a more aggressive profile with respect to clinical factors such as estrogen receptor, progesterone receptor, or HER2-status[48,67,91], but only one study has reported associations between interval cancer and RNA-based genomic subtype such as the PAM50 intrinsic subtype[129]. No study has reported associations for the genomic risk of recurrence (ROR-PT) score based on PAM50. Given that genomic tests are increasingly utilized in clinical settings, it is important to understand the relationship of interval detection to these genomic characteristics.

4.3 Methods

4.3.1 Data Sources

Carolina Breast Cancer Study

The Carolina Breast Cancer Study (CBCS) is a population-based study designed to identify both genetic and environmental risk factors for breast cancer among North Carolina women[190]. The current analysis uses data from all three study phases of CBCS (Phase 1, 1993-1996; Phase 2, 1996-2001; and Phase 3, 2008-2013). Randomized recruitment was used to oversample both African American and younger cases (under age 50)[114,192] in all phases. The first two phases of CBCS recruited both cases and controls from 24 counties of eastern and central NC[190]. Cases were women aged 20 to 74 diagnosed with a primary invasive breast cancer between May 1, 1993 and December 31, 2000 and identified through rapid case ascertainment from the North Carolina Central Cancer Registry. Cases of *in situ* cancer were also enrolled in

Phase 2. There were a total of 2311 cases (1803 invasive cases, 508 *in situ* cases) enrolled in Phases 1&2. Phase 3 recruited cases only (N=3000) from 44 counties in NC[192].

CBCS Variables

Women in CBCS were interviewed at baseline by a nurse, at which point they also provided written consent for medical record requests. All measures were self-reported, except BMI, which was nurse-measured. All demographic (age at diagnosis, race, menopausal status, education, income, first degree family history of breast cancer, marital status, and hormone replacement (HRT) use), clinical (tumor size, nodal status, and stage), and molecular data used in this study came from CBCS.

The following IHC markers were used to distinguish intrinsic subtype: estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor-2 (HER2), human epidermal growth factor-1 (HER1), and cytokeratin 5/6 (CK5/6), and tumor suppressor p53 (p53). Previously described assays were used for these IHC markers[111,204,205]. ER and PR status were determined from medical records for the 80% of women who had this data available from medical records[204]; for the remaining cases with paraffin-embedded tissue available, IHC analysis was performed at the University of North Carolina Translational Pathology Laboratory. Positivity for ER and PR status were defined as having more than 5% of cells showing nuclei-specific staining[111]. Tumors with HER2 staining in more than 10% of cells were considered HER2 positive[205]. Positivity of EGFR was defined as any HER1 staining and positivity for CK 5/6 was defined as any cytoplasmic and/or membranous staining[117]. Previously identified IHC definitions for intrinsic subtypes were used[111,206]. p53 positivity for IHC was defined as dark nuclear protein staining present in 10% or more of invasive cells, all other cases were considered p53 negative[210].

PAM50 gene expression subtyping was performed on a subset (n=2007) of samples with available formal-fixed paraffin embedded cores or unstained slides from CBCS Phases 1-3. For samples from CBCS 1&2 (N=188), RNA was extracted from two unstained 10-µm FFPE slides per patient. For women in CBCS Phase 3, RNA was extracted from 2 1-mm cores (N=377) or 2-10 um slides (N=79) as described previously[128]. RNA was isolated using the RNEasy FFPE Kit (Qiagen) and Nanostring analyses were performed in the Rapid Adoption Molecular laboratory and the Translational Genomics laboratory at UNC. Tumors were classified as Luminal A, Luminal B, HER2-enriched, Basal-like, and normal-like using the PAM50 predictor[127]. RNA gene expression for p53 mutation status was determined using a previously published 52-gene p53 signature[207]. A subset of the PAM50 genes were also used to construct the risk of recurrence score, taking into account proliferation and tumor size (ROR-PT)[208]. The ROR-PT is the research correlate to the clinically used Prosigna assay (NanoString Technologies Inc., Seattle, WA, USA), which has been clinically validated[209]. The ROR-PT is a continuous score, but can be categorized (Low/Medium/High) using published protocols[127].

Carolina Mammography Registry

The Carolina Mammography Registry (CMR)[193] is a large community-based mammography registry that has studied the performance and outcomes of mammography in North Carolina since 1994 and participates in the Breast Cancer Surveillance Consortium (BCSC)[216]. The CMR collects data from breast imaging facilities across North Carolina. Data from patients and radiologists include patient demographics, prior screening history, breast cancer risk factors including family history of breast cancer, radiologist reported breast density using BI-RADS, reason for the visit, screening and diagnostic procedures performed, and

radiologists' interpretation of the exam using BI-RADS assessment categories and the recommend follow-up.

CMR Variables

All mammography data used in this analysis, including mammographic density, type of exam, screening dates, and screening outcomes came from the CMR. Mammographic density is recorded at each mammogram by CMR. For all analyses, mammographic density will be categorized as non-dense (BI-RADS 1 and 2) and dense (BI-RADS 3 and 4)[217].

Mammogram findings were reported by the radiologists in CMR using BI-RADS assessment categories, which are different from BI-RADS density categories[202]. Screening mammograms and results were defined using BCSC definitions[218]. A mammogram is considered to be screening if: the woman was 18 or older, had no breast implants or prior mastectomy, no history of breast cancer, the indication for the exam was routine screening, it was the first exam sequence of the day, bilateral screening views were done, there was no imaging in the previous 9 months, and the overall assessment code was not BI-RADS 6. A positive screening mammogram is defined as a screening mammogram with a BI-RADS assessment code of 4 (suspicious abnormality) or 5 (highly suggestive of malignancy). Screening mammograms with a BI-RADS assessment code of 0 (incomplete) with a recommendation for biopsy, fine needle aspiration (FNA), or surgery were also considered positive or 3 (probably benign finding) with a recommendation for biopsy, fine needle aspiration (FNA), or surgery were also considered positive. A negative screening mammogram is defined as a screening mammogram with a BI-RADS assessment category of 1, 2, or 3 with no recommendation for biopsy, FNA, or surgery.

CBCS-CMR Linkage

All cases and controls from Phases 1, 2 and 3 of CBCS (N=7331) were matched to all women in CMR from 1994-2014 inclusive (N=657,060) using probabilistic linkage. The following identifiers were used: last four digits of social security number (SSN), first name, last name, middle initial, date of birth, and address. Because some women in CBCS Phase 3 did not consent to use of SSNs, Phase 3 of CBCS was linked separately for those with and those without SSN.

Matches (women that were in both CBCS and CMR) were determined using thresholds set based on linking probabilities of the identifiers chosen. The final linked dataset included 2,614 women (871 controls and 1,743 cases of DCIS or invasive breast cancer). The sensitivity of linkage (100%) was the same for women linked with SSN information and those linked without, but specificity was higher (97.1% vs. 95.2%) for those with SSN information. Linkage was performed by the Integrated Cancer and Information Surveillance System (ICISS) at the University of North Carolina[219]. Consistent with screening patterns in the general population, CBCS women with records in the CMR were more likely to be cancer cases, older, post-menopausal, and have used hormone replacement therapy.

Eligibility criteria

The eligibility criteria applied in this study are shown in Figure 4.1. As a secondary quality control measure for the linkage, information from one commonly collected variable between the two data sets, date of diagnosis, was compared. Both CBCS and CMR collected data for this variable from the NC Central Cancer Registry; date of diagnosis should therefore be the same if the match from the linkage was correct. There were 15 of 1512 (0.1%) women where dates of diagnosis did not match. After manual review, it was determined that these women

represented false matches and these women were excluded from analysis. The final data set that was used for this study contained 1497 women. 43% of these women (N=644) had genomic data available.

4.3.2 Defining interval vs. screen-detected cases

Invasive breast cancer cases were classified as interval or screen-detected based on the date of the most recent pre-diagnostic screening mammogram and the date of breast cancer diagnosis. Screening interval recommendations varied from 1-2 years[28-31] during the study period (1993-2013). Mode of detection was defined using both a 12 and 24 month screening interval (Figure 4.2). For example, using the 24 month screening interval, if a positive screening mammogram was recorded in the 24 months before the diagnosis date, the cancer was classified as screen-detected. If a negative screening mammogram was recorded in the 24 months before diagnosis, cancers were defined as interval cancers. The 24 month interval was chosen for the main analysis to reflect current screening recommendations and to enhance comparability with other studies[47,72,77,91,138].

Of the 1,497 women with a primary invasive breast cancer in the CMR-CBCS data set, we identified 165 women who were screen-detected and 205 women who were interval-detected within one year of a negative screening mammogram. Sensitivity analyses that decreased the screening interval to 12 months were also performed; using this interval, 161 women were screen-detected and 107 women were interval-detected. Women who meet neither screen-detected nor interval-detected definitions were classified as "unknown". Compared to screen-detected women, women with unknown mode of detection had less screening history in the linked dataset, were more likely to be <50 and premenopausal. Women with unknown mode of detection were excluded from all analyses.

4.3.3 Statistical analysis

Logistic regression was used to calculate univariate odds ratios for associations for each of the demographic/patient variables (age, race, BMI, CBCS Phase, menopausal status, education, marital status, income, family history, hormone replacement therapy use, and mammographic density) with mode of detection, with screen-detected cancers being used as the referent group. Potential confounders were chosen *a priori* based on a review of the literature. Adjusted odds ratios were calculated for the association between clinical and molecular variables (tumor size, nodal status, cancer stage, ER, PR, and HER2 positivity, 3-marker subtype, 5-marker subtype IHC p53, PAM50 subtype, genomic p53) and mode of detection; odds ratios were adjusted for demographic/personal variables found to be strongly associated in the previous analysis. All analyses were done in SAS version 9.3 (SAS Institute, Cary, NC).

Because mammographic density and race are potential effect measure modifiers of the relationship between patient and cancer characteristics and mode of detection, all analyses were repeated stratifying for mammographic density and race separately.

4.4 Results

The final analytic population contained 370 women. As described in Table 4.1, the majority of women were ≥ 50 (60%), White (53%), postmenopausal (64%), and had no first degree family history of breast cancer (79%). In addition, the majority of women were never users of hormone replacement therapy (68%) and had low (BI-RADS 1 or 2) breast density (55%). To assess patterns of mammography use, we evaluated mean number of mammography visits, mammographic exams (screening and diagnostic exams), and screening mammograms among all participants with at least one screening mammogram recorded during a prediagnostic screening interval (defined as more than two years before diagnosis, Table 4.1). Of

demographic/personal factors assessed, younger age (<50 years old, OR=1.44; 95% C.I.: 0.95, 2.20), postmenopausal status (OR= 1.14; 95% C.I.: 0.94, 1.75), and high mammographic density (OR=2.02; 95% C.I.: 1.29, 3.16) were associated with interval detection (Table 4.2).

Table 4.3 shows associations between interval-detected vs. screen-detected cancers and clinical characteristics. Interval cancers were associated with aggressiveness as measured by tumor size, stage, and nodal status. Interval cancers were also more commonly hormone receptor negative, but these results were not significant, nor was an association with p53 status. However, interval cancers were statistically significantly associated with triple negative status (OR= 2.45; 95% C.I: 1.10, 5.47) and with basal-like cancer (OR=2.06; 95% C.I: 1.07, 3.95). Associations between mode of detection and molecular variables (ER, PR, HER2, triple negative, basal-subtype) were unchanged after adjusting for tumor size, stage, and nodal status.

Interval cancers were strongly associated with genomic markers (Table 4.4), including PAM50 non-Luminal A subtype (OR=2.94; 95% C.I.: 1.52, 5.71) and PAM50 basal-like subtype (OR=2.68; 95% C.I.: 1.21, 5.94). Mean ROR-PT score was significantly higher in interval than screen-detected cancers (mean =41.0 vs. 26.0; p <0.001). As shown in Figure 4.3, the kernel density distribution is shifted toward higher risk tumors among interval cancers and a higher proportion of ROR-PT high risk tumors, (24/105, 23%) were detected among interval-detected cancers (vs. 3/71, 4% among screen-detected). Associations between interval detection and tumor characteristics were not markedly changed when stratified by density (Table 4.3), race (Table 4.5), or by screening interval (Table 4.6).

4.5 Discussion

Identification of the predictors and characteristics of interval cancers contributes to our knowledge of the risks and benefits of mammography. We found that standard clinical prognosis

features are associated with interval cancers, and that genomic tests indicative of poor prognosis are more common among interval cancers. Previous literature has shown that interval cancers tend to have negative prognostic characteristics [8,12,13,16,43,44], however we found associations to be weaker than reported previously for ER- or PR-[12,44], triple negative[8,43,44], and p53 mutant[44]. With the exception of triple negative subtype, none of these were significantly associated with interval detection.

While multi-gene classification methods have become more prominent clinically, genomic characteristics of interval cancers are not well studied. The only study that has reported associations between PAM50 results and mode of detection was based within a clinical cancer sequencing study in Sweden with 173 patients. That study had similar findings, showing that interval cancer was associated with basal-like subtype[19]. Higher ROR-PT among interval cancers has not been assessed previously. It is striking that only 4% of screen-detected cancers had high ROR-PT, in parallel with high frequency of Luminal A subtype (71%).

While our findings strongly support biologic determinants of interval cancers, masking bias may nonetheless contribute to interval cancer rates. Multiple studies have shown high mammographic density to be associated with interval cancers[45-47], including our own findings herein. However, it is difficult to disentangle tumor biology and mammographic density because younger women have both higher density and more aggressive tumor characteristics[48,49]. We were unable to consider the independent contributions of age, race, and mammographic density due to sample size.

Some limitations of the study should be noted. CMR does not include all breast imaging facilities in North Carolina, so only ~30% of women enrolled in CBCS were linked to CMR. Furthermore, CBCS oversampled younger and African American women, and therefore the

proportion of screen and interval detected cases may vary as a function of the demographic and selection characteristics of CBCS[48]. Therefore our study is not designed to estimate the proportion of screen and interval-detected cases in the general population. Notably, among screened women, we classified 45% of invasive cases as screen-detected. Previous studies based on CMR have reported higher proportions of screen detected cases (e.g. Henderson et al. reported 80% of cases were screen detected using a 1 year-interval[14]; Hofvind et al. reported 60% of cases were screen detected given the 24-month definitions used herein[11]). We were unable to retrospectively review mammographic images to confirm which interval cases arose from false negatives, but we minimized misclassification within screen and interval-detected groups by classifying women with missing screening data as 'unknown'. We note that the unknown category likely includes true screen- and interval-detected cases along with true clinically detected cases. Despite these limitations, this study does provide novel data on genomic characteristics in a racially diverse population.

The goal of mammography is to find aggressive cancers at an earlier stage to increase survivorship and reduce mortality. Our research shows that a high proportion of interval cancers are associated with aggressive biology. Our work also suggests that genomic tests may be useful in distinguishing indolent vs. aggressive screen-detected cancers, given the high prevalence of low-risk tumors among screen-detected cases. If confirmed, these findings indicate that continued evaluation of genomic tools in combination with mammography could help to increase the benefit and reduce negative consequences of screening.

Figure 4.1 Flowchart of eligibility criteria.

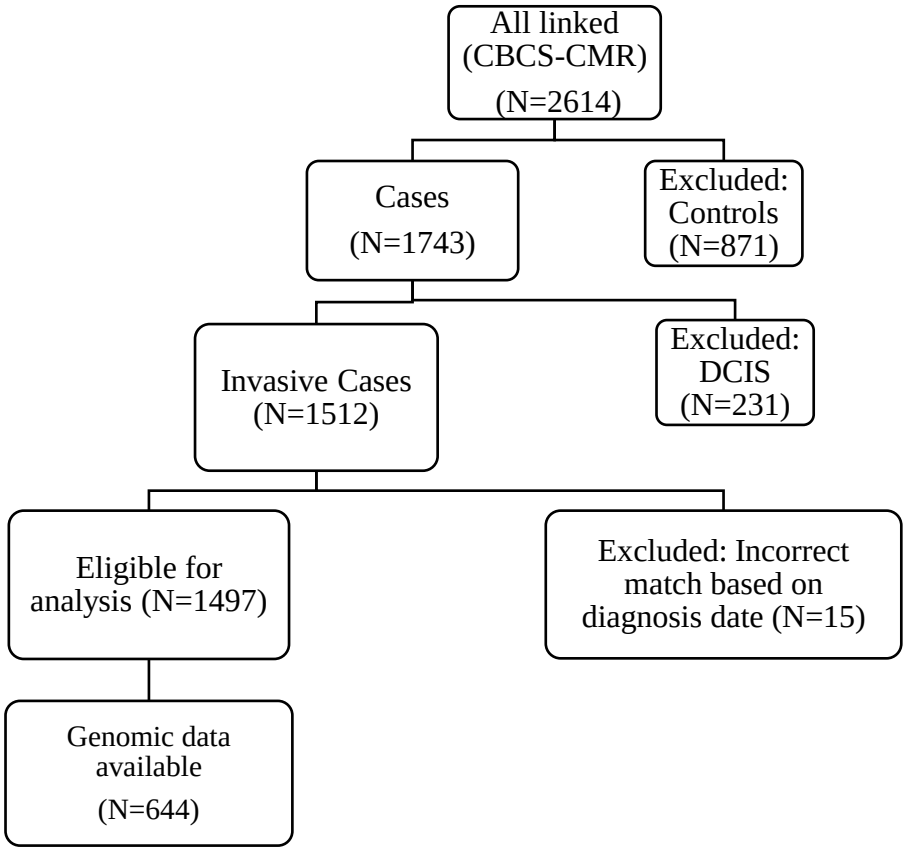
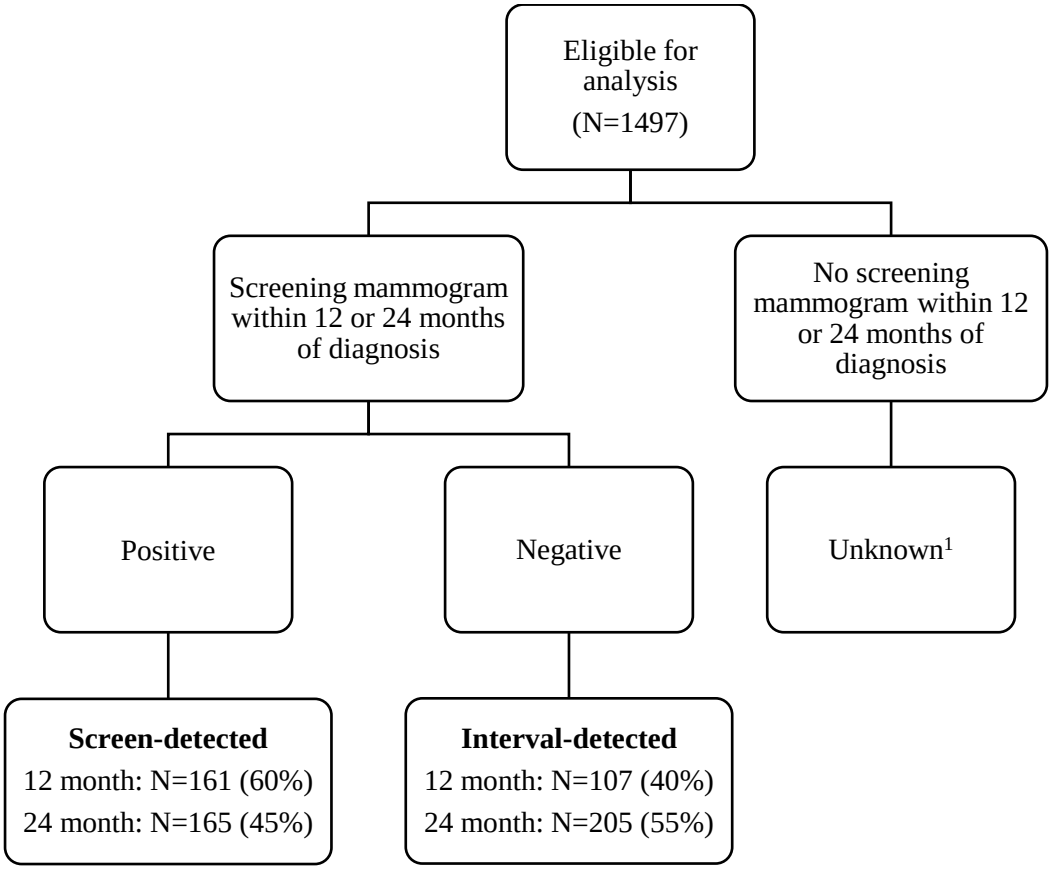


Figure 4.2 Mode of detection categorization, using 12 or 24 month screening interval.



[1] Women who had unknown mode of detection were excluded from this study.

Table 4.1 Characteristics of full analytic set and pre-diagnosis mammography use for women with mammography recorded in CMR >2 years before diagnosis of invasive breast cancer (N=209).

	Full analysis data set (N=370) N (%)		Women with mammography recorded > 2 years before diagnosis (N=209) N (%)	Mean number of visits (SD)	p	Mean number of exams (SD)	p	Mean number of screening mammograms (SD)	p
Age									
<50	148 (40)		70 (33)	4.7 (4.0)		3.6 (3.1)		2.9 (2.7)	
≥50	222 (60)		139 (66)	7.1 (5.2)	0.02	5.8 (4.0)	<0.0001	5.3 (3.8)	<0.0001
Race									
White	197 (53)		115 (55)	6.5 (5.3)		5.2 (4.0)		4.5 (3.8)	
African American	173 (47)		94 (45)	6.1 (4.6)	0.5	4.8 (3.7)	0.5	4.4 (3.4)	0.8
Menopausal status									
Pre	134 (36)		62 (30)	4.7 (3.7)		3.7 (3.0)		3.1 (2.8)	
Post	236 (64)		147 (70)	7.0 (5.3)	0.003	5.6 (4.1)	0.001	5.1 (3.8)	<0.0001
Education									
≤ High school	194 (52)		112 (54)	6.7 (5.4)		5.4 (4.1)		4.9 (3.9)	
> High school	176 (48)		97 (46)	5.8 (4.5)	0.2	4.6 (3.6)	0.1	4.0 (3.3)	0.1
Income									
< 30,000	137 (40)		81 (42)	6.9 (5.4)		5.6 (4.3)		5.0 (3.7)	
≥ 30,000	211 (61)		114 (58)	5.9 (4.6)	0.2	4.6 (3.6)	0.1	4.1 (3.6)	0.1
Missing	22		14						
Family history[a]									
No	282 (79)		156 (77)	6.3 (4.9)		5.0 (3.8)		4.3 (3.5)	
Yes	76 (21)		47 (23)	6.6 (5.5)	0.7	5.0 (4.2)	1.0	4.9 (4.0)	0.4

Missing	**12**		**6**						
Marital status									
Married	217 (59)		131 (63)	6.2 (5.2)		4.9 (3.9)		4.3 (3.5)	
Single	42 (11)		25 (12)	6.4 (5.0)	0.8	4.9 (3.7)	1.0	5.1 (4.6)	0.4
Widowed/ divorced	111 (30)		53 (25)	6.7 (4.4)	0.5	5.5 (3.9)	1.0	4.7 (3.4)	0.4
HRT use									
Never	244 (68)		126 (63)	5.8 (4.4)		4.7 (3.6)		3.9 (3.4)	
Current/ former	117 (32)		75 (37)	6.6 (5.1)	0.2	5.2 (3.8)	0.3	5.2 (3.9)	0.03
Missing	9		8						
BI-RADS mammographic breast density[b]									
Non-dense	178 (55)		109 (64)	6.2 (5.4)		5.1 (4.2)		4.5 (3.9)	
Dense	145 (45)		61 (36)	5.7 (4.4)	0.5	4.5 (3.4)	0.4	3.8 (2.9)	0.2
Missing	47		39						
Mode of detection									
Screen	165 (45)		85 (41)	6.0 (4.8)		4.9 (3.7)		4.0 (3.3)	
Interval	205 (55)		124 (59)	6.5 (5.1)	0.5	5.1 (4.0)	0.7	4.8 (3.9)	0.1

[a]First degree family history of breast cancer.

[b]Non-dense= BI-RADS categories 1&2; Dense= BI-RADS categories 3&4.

Table 4.2 Univariate odds ratios (OR) and 95% confidence intervals (95% CI) for demographic/personal characteristics comparing interval-detected vs. screen-detected cancers.

	Screen-detected (N=165)	Interval (N=205)	OR (95% CI)
	N (%)	N (%)	
Age			
≥50	107 (65)	115 (56)	1.00
<50	58 (35)	90 (44)	1.44 (0.95, 2.20)
Race			
White	85 (52)	112 (55)	1.00
Black	80 (48)	93 (45)	0.88 (0.59, 1.33)
BMI			
Underweight	0	3 (1)	
Normal	37 (23)	53 (26)	1.00
Overweight	127 (77)	147 (72)	0.81 (0.50, 1.31)
Missing	1	2	
CBCS Phase			
Phase 1&2	73 (44)	72 (35)	1.00
Phase 3	92 (56)	133 (65)	1.01 (0.62, 1.65)
Menopausal status			
Post	108 (65)	128 (62)	1.00
Pre	57 (35)	77 (38)	1.14 (0.94, 1.75)
Education			
> High school	75 (45)	101 (49)	1.00
High school or less	90 (55)	104 (51)	0.86 (0.57, 1.29)
Marital status			
Married	94 (57)	123 (8)	1.00
Single	18 (11)	24 (12)	1.02 (0.52, 1.99)
Divorced	53(32)	58 (29)	0.84 (0.53, 1.32)
Family History [a]			
No	124 (79)	158 (79)	1.00
Yes	33 (21)	43 (21)	1.02 (0.66, 1.73)
Missing	8	4	
HRT use			
Never	107 (67)	137 (68)	1.00
Current/ Former	53 (33)	64 (32)	0.94 (0.61, 1.47)
Missing	5	4	

BI-RADS mammographic breast density [b]			
Non-dense	85 (54)	77 (38)	1.00
Dense	73 (46)	124 (62)	2.02 (1.29, 3.16)
Missing	7	4	

[a] First degree female family history.

[b] Low density= BI-RADS categories 1&2; High density= BI-RADS categories 3&4.

Table 4.3. Interval vs. Screen-detected cancers: Associations with clinical characteristics stratified by mammographic density.

	Overall			Non-dense			Dense		
	SDC (N=165)	Interval (N=205)	Adjusted OR (95% CI)[a]	SDC (N=93)	Interval (N=85)	Adjusted OR (95% CI) [a]	SDC (N=51)	Interval (N=94)	Adjusted OR (95% CI) [a]
	N (%)	N (%)		N (%)	N (%)		N (%)	N (%)	
Age									
≥50	107 (65)	115 (56)	1.0	65 (70)	52 (61)	1.00	30 (59)	46 (49)	1.00
<50	58 (35)	90 (44)	1.44 (0.95, 2.20)	28 (30)	33 (39)	1.47 (0.79, 2.74)	21 (41)	48 (51)	1.49 (0.75, 2.97)
Tumor size									
≤ 2 cm	115 (70)	103 (52)	1.0	69 (78)	42 (51)	1.00	33 (66)	44 (49)	1.00
> 2 cm	44 (27)	94 (46)	2.33 (1.48, 3.65)	20 (22)	41 (49)	3.22 (1.66, 6.26)	17 (34)	45 (51)	2.00 (0.97, 4.12)
Missing	6	8		4	2		1	5	
Nodal status									
Negative	123 (75)	127 (62)		68 (74)	51 (60)	1.00	38 (75)	61 (66)	1.00
Positive	41 (25)	77 (38)	1.78 (1.13, 2.81)	24 (26)	34 (40)	1.78 (0.94, 3.39)	13 (25)	32 (34)	1.58 (0.73, 3.42)
Missing	1	1		1	0		0	1	
Stage									
I/ II	151 (94)	172 (86)		85 (94)	69 (83)	1.00	46 (92)	81 (89)	1.00
III/ IV	9 (6)	28 (14)	3.22 (1.43, 7.25)	5 (6)	14 (17)	3.21 (1.09, 9.44)	4 (8)	10 (11)	1.38 (0.40, 4.74)
Missing	5	5		3	2		1	3	
ER									
Positive	112 (71)	124 (65)	1.0	62 (70)		1.00	35 (70)	58 (67)	1.00
Negative	46 (29)	66 (35)	1.25 (0.79, 1.98)	26 (30)	31 (39)	1.44 (0.75, 2.77)	15 (30)	29 (33)	1.15 (0.54, 2.45)

Missing	**7**	**17**		**5**	**6**		**1**	**7**	
PR									
Positive	94 (61)	96 (50)	1.0	48 (57)	35 (45)	1.00	33 (66)	50 (56)	1.00
Negative	60 (39)	96 (50)	1.53 (0.99, 2.37)	36 (43)	43 (55)	1.57 (0.84, 2.95)	17 (34)	39 (44)	1.58 (0.76, 3.28)
Missing	11	13		9	7		1	5	
HER2									
Positive	20 (14)	23 (12)	1.0	11 (13)	6 (8)	1.00	6 (13)	11 (13)	1.00
Negative	127 (86)	162 (88)	1.24 (0.64, 2.38)	72 (87)	69 (92)	1.84 (0.63, 5.33)	39 (87)	75 (87)	1.32 (0.43, 4.00)
Missing	18	20		10	10		6	8	
p53 IHC									
Wild type	75 (71)	78 (67)	1.0	43 (72)	31 (66)	1.00	23 (70)	38 (68)	1.00
Mutant	30 (29)	39 (33)	1.23 (0.69, 2.18)	17 (28)	16 (34)	1.28 (0.55, 2.95)	10 (30)	18 (32)	1.08 (0.41, 2.81)
Missing	60	88		33	38		18	38	

[a] All odds ratios, except those for age, are adjusted for age and menopausal status

Table 4.4 Odds ratios for molecular characteristics for linked invasive cases.

	Screen-detected (N=165)	Interval (N=205)	Adjusted OR (95% CI)[a]
	N (%)	N (%)	
3-marker subtype			
Luminal A	99 (68)	102 (55)	1.00
Luminal B	14 (10)	16 (9)	0.95 (0.43, 2.08)
HER2	6 (4)	7 (4)	1.12 (0.36, 3.45)
Triple negative	26 (18)	60 (32)	2.45 (1.10, 5.47)
Missing	20	20	
5-marker subtype			
Luminal A	67 (64)	64 (47)	1.00
Luminal B	12 (12)	30 (22)	2.45 (1.14, 5.25)
HER2	6 (6)	4 (3)	NR[b]
Basal	19 (18)	38 (28)	2.06 (1.07, 3.95)
Missing	61	69	
PAM50			
Luminal A	51 (71)	46 (46)	1.00
Luminal B	4 (6)	18 (18)	5.29 (1.63, 17.10)
HER2	5 (7)	8 (8)	1.82 (0.54, 6.15)
Basal	12 (17)	29 (29)	2.68 (1.21, 5.94)
Missing	93	104	
PAM50			
Luminal A	51 (71)	46 (46)	1.00
Non-Luminal A	21 (29)	55 (54)	2.94 (1.52, 5.71)
Missing	93	104	
p53			
Wild type	42 (55)	55 (52)	1.00
Mutant	34 (45)	51 (48)	1.13 (0.63, 2.05)
Missing	89	99	
ROR-PT			
Low/ Medium	68 (96)	81 (77)	NR[b]
High	3 (4)	24 (23)	NR[b]
Missing	94	100	

[a] All odds ratios are adjusted for age and menopausal status.

[b] Odd ratios are not reported where cell size < 5 observations.

Table 4.5 Interval vs. Screen-detected cancers: Associations with clinical characteristics stratified by race.

	Black			White		
	SDC (N=80)	Interval (N=93)	Adjusted OR (95% CI) [a]	SDC (N=85)	Interval (N=112)	Adjusted OR (95% CI) [a]
	N (%)	N (%)		N (%)	N (%)	
Age						
≥50	57 (71)	52 (56)	1.00	50 (59)	63 (56)	1.00
<50	23 (29)	41 (44)	1.95 (1.04, 3.68)	35 (41)	49 (44)	1.11 (0.63, 1.97)
Tumor size						
≤ 2 cm	53 (71)	40 (44)	1.00	62 (74)	63 (59)	1.00
> 2 cm	22 (29)	50 (56)	2.79 (1.44, 5.40)	22 (26)	44 (41)	1.95 (1.04, 3.65)
Missing	5	3		1	5	
Nodal status						
Negative	20 (25)	35 (38)	1.00	21 (25)	42 (38)	1.00
Positive	59 (75)	58 (62)	1.83 (0.93, 3.59)	64 (75)	69 (62)	1.79 (0.95, 3.36)
Missing	1	0		0 (0)	1	
Stage						
I/ II	73 (96)	78 (85)	1.00	78 (93)	94 (87)	1.00
III/ IV	3 (4)	14 (15)	4.39 (1.2, 16.07)	6 (7)	14 (13)	1.84 (0.67, 5.11)
Missing	4	1		1	4	
ER						
Positive	49 (65)	50 (57)	1.00	63 (76)	74 (73)	1.00
Negative	26 (35)	38 (43)	1.35 (0.71, 2.57)	20 (24)	28 (27)	1.13 (0.57, 2.21)
Missing	4	3		2 (2)	10	
PR						
Positive	44 (59)	33 (38)	1.00	50 (63)	63 (59)	1.00
Negative	30 (41)	53 (62)	2.29 (1.21, 4.36)	30 (38)	43 (41)	1.06 (0.58, 1.94)

Missing	**6**	**7**		**5 (6)**	**6**	
HER2						
Positive	9 (13)	15 (18)	1.00	11 (14)	8 (8)	1.00
Negative	61 (87)	70 (82)	0.77 (0.31, 1.92)	66 (86)	92 (92)	2.26 (0.83, 6.13)
Missing	10	8		8	12	
3-marker subtype						
Luminal A	45 (65)	38 (45)	1.00	54 (71)	64 (64)	1.00
Luminal B	5 (7)	11 (13)	2.14 (0.66, 6.92)	9 (12)	5 (5)	0.35 (0.10, 1.18)
HER2	4 (6)	4 (5)	NR[b]	2 (3)	3 (3)	NR[b]
Triple negative	15 (22)	32 (24)	2.28 (1.06, 4.91)	11 (14)	28 (28)	1.89 (0.84, 4.25)
Missing	11	8		9	12	
5-marker Subtype						
Luminal A	34 (63)	19 (30)	1.00	33 (66)	45 (62)	1.00
Luminal B	5 (7)	18 (29)	8.10 (2.25, 27.93)	8 (16)	12 (16)	0.95 (0.34, 2.70)
HER2	4 (9)	3 (5)	NR[b]	1 (2)	1 (1)	NR[b]
Basal	11 (20)	23 (37)	3.70 (1.45, 9.47)	8 (16)	15 (21)	1.34 (0.50, 3.60)
Missing	26	30		35	39	
p53 IHC						
Wild type	39 (70)	35 (67)	1.00	36 (73)	43 (66)	1.00
Mutant	17 (30)	17 (33)	1.08 (0.47, 2.46)	13 (27)	22 (34)	1.31 (0.57, 3.03)
Missing	24	41		36	47	

[a]All odds ratios, except those for age, are adjusted for age and menopausal status.

[b] Odd ratios are not reported where cell size < 5 observations.

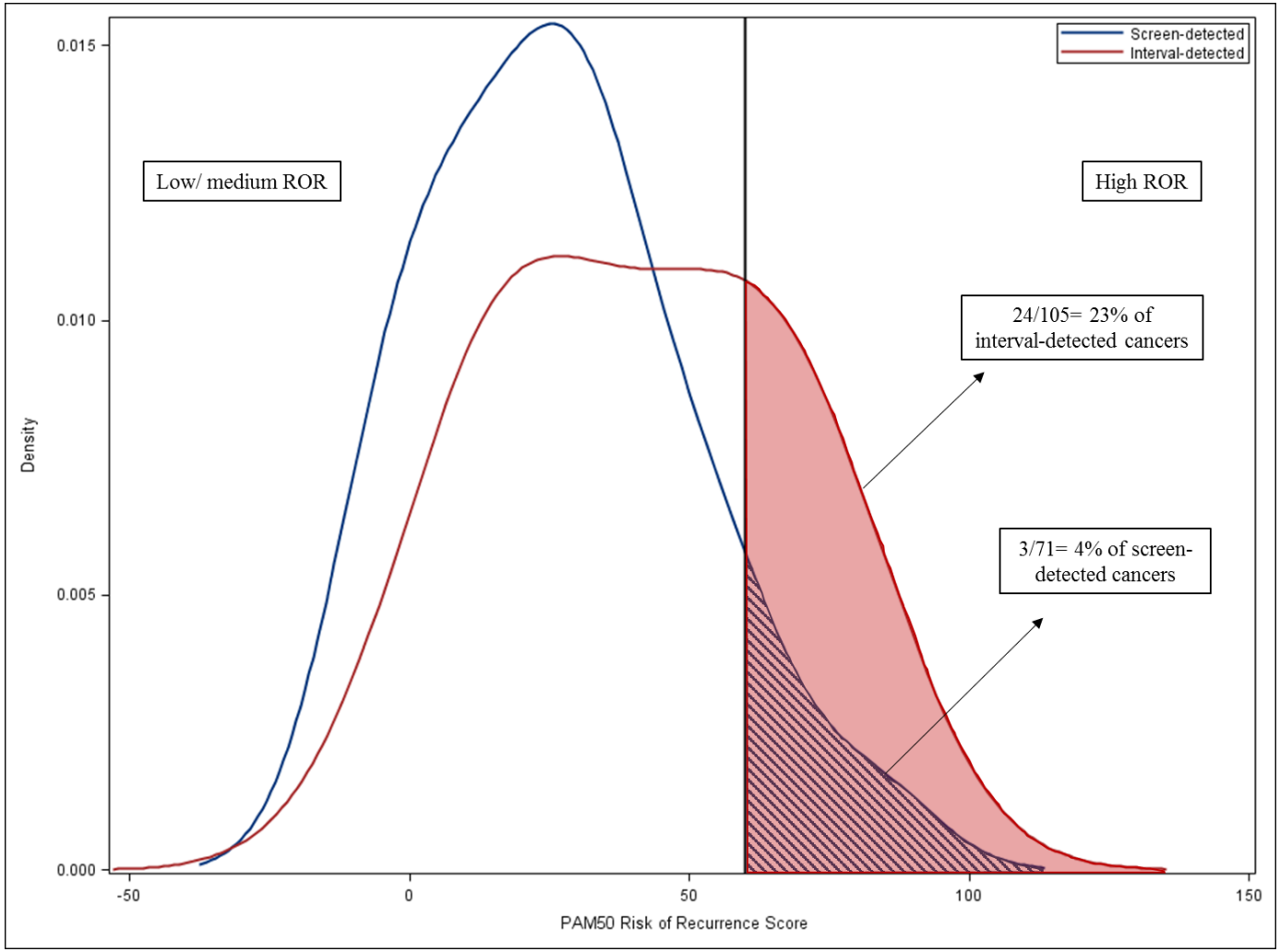
Table 4.6 Summary table comparing odds ratios of interest (Interval vs. screen-detected cancers).

	1 year interval[a] OR (95% CI)	**2 year interval[b] OR (95% CI)**
<50 vs. ≥50	1.84 (1.12, 3.03)	1.44 (0.95, 2.20)
Black vs. white	0.78 (0.48, 1.27)	0.88 (0.59, 1.33)
High vs. low density	2.34 (1.38, 4.00)	2.02 (1.29, 3.16)
≤ 2 cm vs. < 2 cm	3.40 (2.00, 5.79)	2.33 (1.48, 3.65)
ER- vs. ER+	1.44 (0.83, 2.50)	1.25 (0.79, 1.98)
PR- vs. PR+	1.53 (0.91, 2.61)	1.53 (0.99, 2.37)
Triple negative vs. Luminal A	2.50 (1.33, 4.71)	2.45 (1.10, 5.47)
Basal vs. Luminal A	2.24 (1.05, 4.76)	2.06 (1.07, 3.95)

[a] Mode of detection constructed using 1 year interval: interval-detected (N=107), screen-detected (N=161).

[b] Mode of detection constructed using 2 year interval: interval-detected (N=205), screen-detected (N=165).

Figure 4.3 Kernel density distribution of PAM50 risk of recurrence (ROR) score for interval- and screen-detected cancers. ROR distributions of screen-detected and interval-detected cancers are blue and red, respectively. The area shaded under the curve represents the proportion of cancers that have high risk of recurrence score. Of 105 interval-detected cancers that had genomic data available, 24 cancers (23%) had high ROR score. Of 71 screen-detected cancers that had genomic data available, 3 cancers (4%) had high ROR score.



CHAPTER 5: MAMMOGRAPHIC IMAGING FEATURES AND MOLECULAR AND GENOMIC BREAST CANCER SUBTYPE

5.1 Overview

Introduction: Breast cancers detected by mammography may appear as masses, with calcifications, or with other imaging features. Patterns of imaging features by breast cancer subtype are not well-characterized. We examined the association between age, race, and molecular and genomic subtypes of breast cancer and distinct mammographic features.

Methods: We identified 412 women diagnosed with a primary invasive breast cancer from 1993-2013 and who had imaging features recorded on a mammogram within two years of diagnosis by linking the Carolina Breast Cancer Study and the Carolina Mammography Registry. Linear regression was used to estimate prevalence differences (PD) as measures of associations between imaging features (masses and calcifications) and patient, immunohistochemical, and genomic characteristics.

Results: Overall, masses and calcifications were reported in 49% and 20% of cases, respectively. Both young (<50 years) and African-American women showed higher relative frequency of masses and lower relative frequency of calcifications compared to older (≥ 50) and White women. Masses were less frequent among interval-detected vs. screen-detected women (33% vs. 46%, p=0.04). Relative to Luminal A breast cancers (42% presenting as masses), PAM50 Basal-like and HER2-enriched subtypes were more likely to present as masses (59% and 72%, respectively). High risk of recurrence (ROR-PT) score was also associated with presenting as only a mass (50% vs. 28% among low ROR-PT tumors, p=0.03). Conversely, few Basal-like

and ROR-PT high cancers presented with calcifications (n=4/49 basal-like and n=3/30 ROR-PT high).

Conclusions: Distinct molecular and genomic subtypes of breast cancer present with distinct mammographic features. Improving detection of aggressive subtypes may depend upon ability to accurately and sensitively detect masses.

5.2 Introduction

Mammography is the most common breast cancer screening method, and consists of a low dose x-ray image of the breast. The presence of mammographic imaging features is used in the detection and diagnosis of breast cancer, with masses being most common, followed by calcifications, architectural distortion, and asymmetry[140,141]. The likelihood of detecting a tumor using screening mammography may vary as a function of the imaging characteristics of the breast cancer[155,156]. If breast cancer subtype is associated with specific imaging features, screening efficacy may vary by subtype accordingly[91,92,222].

In this study, we describe associations between imaging features and molecular and genomic breast cancer subtypes as a step towards understanding the relationship between subtype, imaging features, and mammographic detection. Specifically, we evaluated genomic subtypes (Basal-like, Luminal A, Luminal B, HER2-enriched) using RNA expression patterns[92,104,111-113] along with the research version of the clinically-utilized PAM50 risk of recurrence (ROR-PT)[127], a genomic risk score that incorporates tumor subtype, expression-based measures of proliferation, and clinical tumor size. There have been a small number of published studies that have examined the association between breast cancer subtype and imaging features[141,142,153] but no studies have examined genomic tests in association with mammographic features.

5.3 Methods

5.3.1 Data Sources

Carolina Breast Cancer Study

The Carolina Breast Cancer Study (CBCS) is a population-based study designed to identify both genetic and environmental risk factors for breast cancer among North Carolina women[190]. The current analysis uses data from all three study phases of CBCS (Phase 1, 1993-1996; Phase 2, 1996-2001; and Phase 3, 2008-2013). Randomized recruitment was used to oversample both African American and younger cases (under age 50)[114,192] in all phases. The first two phases of CBCS recruited both cases and controls from 24 counties of eastern and central NC[190]. Cases were women aged 20 to 74 diagnosed with a primary invasive breast cancer between May 1, 1993 and December 31, 2000 and identified through rapid case ascertainment from the North Carolina Central Cancer Registry. Cases of *in situ* cancer were also enrolled in Phase 2. A total of 1803 invasive breast cancer cases were enrolled in Phases 1&2. Phase 3 of CBCS recruited invasive cases only (N=3000) from 44 counties in NC.

CBCS Variables

Women in the CBCS were interviewed at baseline by a nurse, at which point they also provided written informed consent for medical record requests. All demographic (age at diagnosis and race), clinical (tumor size and stage), and molecular data used in this study came from CBCS.

The following IHC markers were used to distinguish intrinsic subtype: ER, PR HER2, human epidermal growth factor-1 (EGFR), and cytokeratin 5/6 (CK5/6. For Phases 1&2 of the CBCS, previously described assays were used to stain and quantify these IHC markers[111,204,205]. ER and PR status were determined from medical records for the 80% of women who had these data available from medical records[204]; for the remaining cases with paraffin-embedded tissue

available, IHC analysis was performed at the University of North Carolina Translational Pathology Laboratory (TPL). Positivity for ER and PR status was defined as having more than 5% of cells showing nuclei-specific staining[111]. Tumors with HER2 staining in more than 10% of cells were considered HER2 positive[205]. Positivity of EGFR was defined as any staining and positivity for CK 5/6 was defined as any cytoplasmic and/or membranous staining. Previously identified IHC definitions for intrinsic subtypes were used[111,206]. Methods to distinguish intrinsic subtypes in CBCS Phase 3 were described in detail by Allot et al.[128]. Briefly, tissue microarrays (TMAs) were constructed and stained by the TPL and were digitally imaged using the Aperio ScanScope XT (Aperio Technologies, Vista CA). Automated digital image analysis was performed to quantify IHC staining using a Genie classifier and the Nuclear V9 algorithm (Aperio Technologies, Vista CA), for ER and PR and a Genie classifier and Membrane V9 algorithm for HER2.

PAM50 gene expression subtyping was performed on a subset (n=2007) of samples with available formalin-fixed paraffin embedded cores or unstained slides from CBCS Phases 1-3 as described previously[128]. RNA was isolated using the RNeasy FFPE Kit (Qiagen) and Nanostring analyses were performed in the Rapid Adoption Molecular laboratory and the Translational Genomics laboratory at UNC. Tumors were classified as Luminal A, Luminal B, HER2-enriched, Basal-like, and normal-like using the PAM50 predictor[127]. A subset of the PAM50 genes were also used to construct the risk of recurrence score, taking into account proliferation and tumor size (ROR-PT)[208]. ROR-PT is the research correlate to the clinically used Prosigna assay (NanoString Technologies Inc., Seattle, WA, USA), which has been clinically validated[209]. ROR-PT is a continuous score, but can be categorized (Low/Medium/High) using published protocols[127].

Carolina Mammography Registry

The Carolina Mammography Registry (CMR)[193] is a large community-based mammography registry that has studied the performance and outcomes of mammography in North Carolina since 1994 and participates in the Breast Cancer Surveillance Consortium (BCSC)[216]. CMR collects data from breast imaging facilities across North Carolina. Data from patients and radiologists include patient demographics, prior screening history, breast cancer risk factors including family history of breast cancer, radiologist-reported breast density using Breast Imaging Reporting and Data System (BI-RADS) classifications, reason for the visit, screening and diagnostic procedures performed, and radiologists' interpretation of the examination using the American College of Radiology BI-RADS assessment categories and the recommended follow-up.

CMR Variables

All mammography data used in this analysis, including mammographic density, type of examination, screening dates, and screening outcomes came from the CMR. In the CMR, mammographic density is recorded at each mammogram by the interpreting radiologist using BI-RADS classifications. For all analyses, mammographic density was categorized as non-dense (BI-RADS 1 and 2) or dense (BI-RADS 3 and 4)[217].

Mammogram findings were reported by the radiologists using BI-RADS assessment categories[202]. Screening mammograms and results were defined using BCSC definitions[218]. A mammogram was considered to be screening if: the woman was 18 or older, had no breast implants or prior mastectomy, no history of breast cancer, the indication for the examination was routine screening, it was the first examination sequence of the day, bilateral screening views were done, there was no imaging in the previous 9 months, and the overall assessment code was

not BI-RADS 6. A positive screening mammogram is defined as a screening mammogram with a BI-RADS assessment code of 4 (suspicious abnormality) or 5 (highly suggestive of malignancy). Screening mammograms with a BI-RADS assessment code of 0 (incomplete) or 3 (probably benign finding) with a recommendation for biopsy, fine needle aspiration (FNA), or surgery were also considered positive. A negative screening mammogram was defined as a screening mammogram with a BI-RADS assessment category of 1, 2, or 3 with no recommendation for biopsy, FNA, or surgery.

CBCS-CMR Linkage

All cases and controls from Phases 1, 2 and 3 of CBCS (N=7331) were matched to all women in CMR from 1994-2014 inclusive (N=657,060) using probabilistic linkage. The following identifiers were used to match records: last four digits of social security number (SSN), first name, last name, middle initial, date of birth, and address. Because some women in CBCS Phase 3 did not consent to use of SSNs, Phase 3 of CBCS was linked separately for those with and those without SSN.

Matches (women that were in both CBCS and CMR) were determined using thresholds set on linking probabilities of the identifiers chosen. The final linked dataset included 2,614 women (871 controls and 1,743 cases of DCIS or invasive breast cancer). The sensitivity of linkage (100%) was the same for women linked using SSN information and those linked without, but linkage specificity was higher (97.1% vs. 95.2%) for those with SSN. Linkage was performed by the Cancer Information and Population Health Resource (CIPHR) at the University of North Carolina[219]. Consistent with screening patterns in the general population, CBCS women with records in the CMR were more likely to be cancer cases, older, post-menopausal, and have used hormone replacement therapy.

Eligibility Criteria

The eligibility criteria applied in this study are shown in Figure 5.1. As a secondary quality control measure for the linkage, information from one commonly collected variable between the two data sets, date of diagnosis, was compared. Both CBCS and CMR collected data for this variable from the NC Central Cancer Registry; date of diagnosis should therefore be the same if the match from the linkage was correct. There were 15 of 1512 (0.1%) women where dates of diagnosis did not match. After manual review, it was determined that these women represented false matches and these women were excluded from analysis. The linked dataset contained 1497 women. Of these women, 412 had imaging feature data available and were included in this study.

5.3.2 *Defining Interval vs. Screen-detected Cases*

Invasive breast cancer cases were classified as interval- or screen-detected based on the result of the most recent pre-diagnostic screening mammogram and the date of breast cancer diagnosis. Screening interval recommendations varied from 1-2 years[28-31] during the study period (1993-2013). Mode of detection was defined using a 24month screening interval (Figure 1). For example, using the 24-month screening interval, if a positive screening mammogram was recorded in the 24 months before the diagnosis date, the cancer was classified as screen-detected. If a negative screening mammogram was recorded in the 24 months before diagnosis, the cancer was classified as interval-detected. Women who met neither screen-detected nor interval-detected definitions, including those who did not have a screening mammogram recorded in the 24 months prior to diagnosis, were classified as "missing".

5.3.2 Defining absence/ presence of imaging features

The indication for the study (screening/diagnostic/ follow-up), breast composition, important findings (imaging features), and final assessment (negative, benign, etc.) are recorded for each imaging exam. BI-RADS classifications, which can be used to predict malignancy[211-213], for each imaging feature are noted by the radiologist for each imaging exam performed. Imaging features used in this analysis were mass and calcifications. Architectural distortion and asymmetry were not used due to low prevalence in our study sample. All data on these features were extracted from the most recent diagnostic exam (recorded within two years before to 30 days after diagnosis) when possible. Data from the most recent (within two years before diagnosis) screening mammogram was used for women who did not have diagnostic exam data available. Due to power considerations, imaging features were categorized dichotomously. A feature was considered "absent" when BI-RADS=1; a feature was categorized as "present" when BI-RADS=2, 3, 4, or 5. The imaging feature variables used in analysis were any mass (mass ± calcifications), any calcifications (calcification ± mass), and mass only (mass without calcifications). Presence of any mass was the most common (49%), followed by mass only (42%), and any calcifications (20%).

5.3.3 Statistical analysis

Chi-square tests were used to study differences in mammographic feature presentation by patient, clinical, molecular, and genomic factors. Prevalence differences and their associated 95% confidence intervals were calculated using generalized linear models. Differences in frequencies of imaging features were observed by CBCS phase of study; therefore, all prevalence difference analyses were controlled for CBCS phase. A sensitivity analysis using only data from

diagnostic exams was also performed; results were similar to what is presented here. All analyses were done in SAS version 9.3 (SAS Institute, Cary, NC).

5.4 Results

Table 5.1 describes prevalence differences between imaging features and demographic characteristics. Masses in the absence of calcifications were more common among women <50 (50% vs. 35%, p=0.004). We also observed racial differences in prevalence of mammographic features. Calcifications were 9% less frequent among African-American vs. White women (25% vs. 16%, p=0.02). Conversely, masses were 18% (58% vs. 40%, p <0.001) more frequent and masses in the absence of calcifications were 20% more frequent (52% vs. 32%, p <0.001) in African-American compared to White women. We also found that tumor size was associated with imaging features; larger tumors (>2 cm) had higher frequency of masses (56% vs. 45%) and masses without calcifications (47% vs. 39%) relative to tumors ≤ 2 cm. Both masses (33% vs. 46%, p=0.04) and masses without calcifications (28% vs. 40%, p=0.05) were less frequent among interval-detected vs. screen-detected cancers. No differences in frequency of mammographic features were seen by stage or mammographic density.

Table 5.2 shows associations between molecular and genomic characteristics and imaging features. We did not find statistically significant associations for any of the individual hormone receptors or for IHC intrinsic subtype, although we observed a trend of a higher prevalence of masses among ER- vs. ER+, PR- vs. PR+, and all non-Luminal A subtypes vs. Luminal A cancers. We found stronger associations when using PAM50 genomic subtype, most notably with respect to cancers that presented as mass only. Basal-like (53% vs. 33%, p=0.02) and HER2-enriched (66% vs. 33%, p=0.01) cancers more frequently presented with masses only compared to Luminal A cancers. There was a higher

prevalence masses without calcifications among women with high vs. low (50% vs. 28%, p=0.03) or medium vs. low (44% vs. 28%, p=0.04) ROR-PT score. In addition, only 3 women with calcifications had high ROR-PT score. In an additional set of models, adjusted for age and race, associations between mammographic features and tumor molecular characteristics were no longer observed (Table 5.3).

5.5 Discussion

In the current study, we evaluated associations between molecular phenotypes and how cancers present mammographically. By identifying if specific subtypes present with features that may be difficult to detect, examining subtype-specific differences in mammographic features may contribute to our understanding of mammography efficacy. We observed a consistent pattern when studying IHC and genomic subtypes, with triple negative, HER2, and Basal-like cancers more commonly presenting as masses relative to Luminal cancer. Younger (<50) and African-American women were also more likely to have their tumors detected as masses, in line with higher rates of aggressive cancers in these groups[114]. We extended previous insights on molecular associations to include genomic data for the first time and found that subtype associations with imaging features were slightly stronger when using PAM50 vs. IHC subtype. When considering the risk of recurrence score, both masses and masses without calcifications were associated with a high ROR-PT score. Calcifications were rare in high risk genomic subtypes.

The prevalence of imaging features in our study corresponds with what has been reported in other populations, with the majority of cancers detected with masses and a smaller proportion presenting with calcifications (20-27%)[141,155]. Likewise, our findings that aggressive features such as ER and PR negativity and HER2 positivity are associated with masses rather than

calcifications and are in accordance with previous studies[141,153,154,225,226]. Associations between imaging features and race are not well-studied. One other study has reported racial differences, also finding that prevalence of masses were higher among Black women[141].

Our data are consistent with previous reports implying that calcifications are associated with smaller size. We observed that smaller tumors (<2 cm) were more frequent in cases with calcifications. Previous literature suggests that the majority of breast cancers detected via calcifications are DCIS rather than invasive[179,227]. Sensitivity of mammography to calcification detection has also led to concerns of overdetection. Our results are consistent with this, as we found that a small minority of tumors (10%) presenting with calcifications had high ROR-PT score, while a larger proportion of those presenting with masses (19%) had high ROR-PT. It is possible that combining information on imaging features with genomic testing, as a companion diagnostic, could help distinguish indolent and aggressive screen-detected cancers. Furthermore, use of imaging technology that is more sensitive to mass detection, such as tomosynthesis[228,229], may lead to improved detection of aggressive cancers.

Biological mechanisms explaining associations between mammographic features and breast cancer subtype have not yet been characterized. If the prevalence of specific mammographic features reflects the product of their incidence and duration, lower prevalence of calcifications may be expected for cancers with rapidly growing tumors, such as those that are the Basal-like subtype. However, it is unknown whether all tumor subtypes have a state that is detectable via calcifications, or whether the unique biological characteristics of Basal-like breast cancers, for example, preclude a calcification state entirely. The presence of a small number of Basal-like cases with calcifications in our study suggest that this subtype can present with calcifications. Identifying the mechanism of how imaging features such as calcifications and

masses develop can provide further insight into how aggressive cancers are able to avoid detection.

Limitations of this study include that data on the clinical relevance of our imaging features was limited for our study. That is, calcifications that were noted on a mammogram may not have been central to detection for a given case. We also lacked detailed information of mammographic features such as mass shape (irregular/ lobulated/ oval/ round), mass margins (noncircumscribed/ circumscribed), and calcification type (round/ amorphous/ fine linear), some of which have been shown to be associated with breast cancer subtype[187,230,231]. Heterogeneity within imaging feature groups may have attenuated some of our associations with subtype. For example, round masses are associated with triple negative cancer and irregular masses are associated with Luminal cancer[186,187]; we could not distinguish the two in our data. In addition, we observed that after adjustment for age and race, there was no longer an association observed between molecular characteristics and mammographic features; although the precision of the estimate decreased, the direction of associations remained similar, suggesting that we were underpowered to detect these associations. Strengths of this study include an assessment of imaging features by race, which is not commonly reported. In addition, associations between imaging features and PAM50-derived variables have not been reported previously.

In summary, considering imaging features, mode of detection, and breast cancer subtype together provides a more complete picture of how specific groups of cancers can escape detection through mammography. As it appears that the majority of Luminal cancers are detected in the presence of calcifications, this work also raises interesting biological questions, such as whether aggressive tumors possess a detectable calcification state, or whether they pass through this state too quickly to be detected in this state given current screening intervals. More studies

are needed to assess how age and race are related to mammographic feature presentation. In addition, future work linking genomics to image features will continue to develop our understanding of the limits of mammography and to identify clinical testing or screening technologies that could lead to improved screening and diagnosis.

Figure 5.1 Flowchart of eligibility criteria.

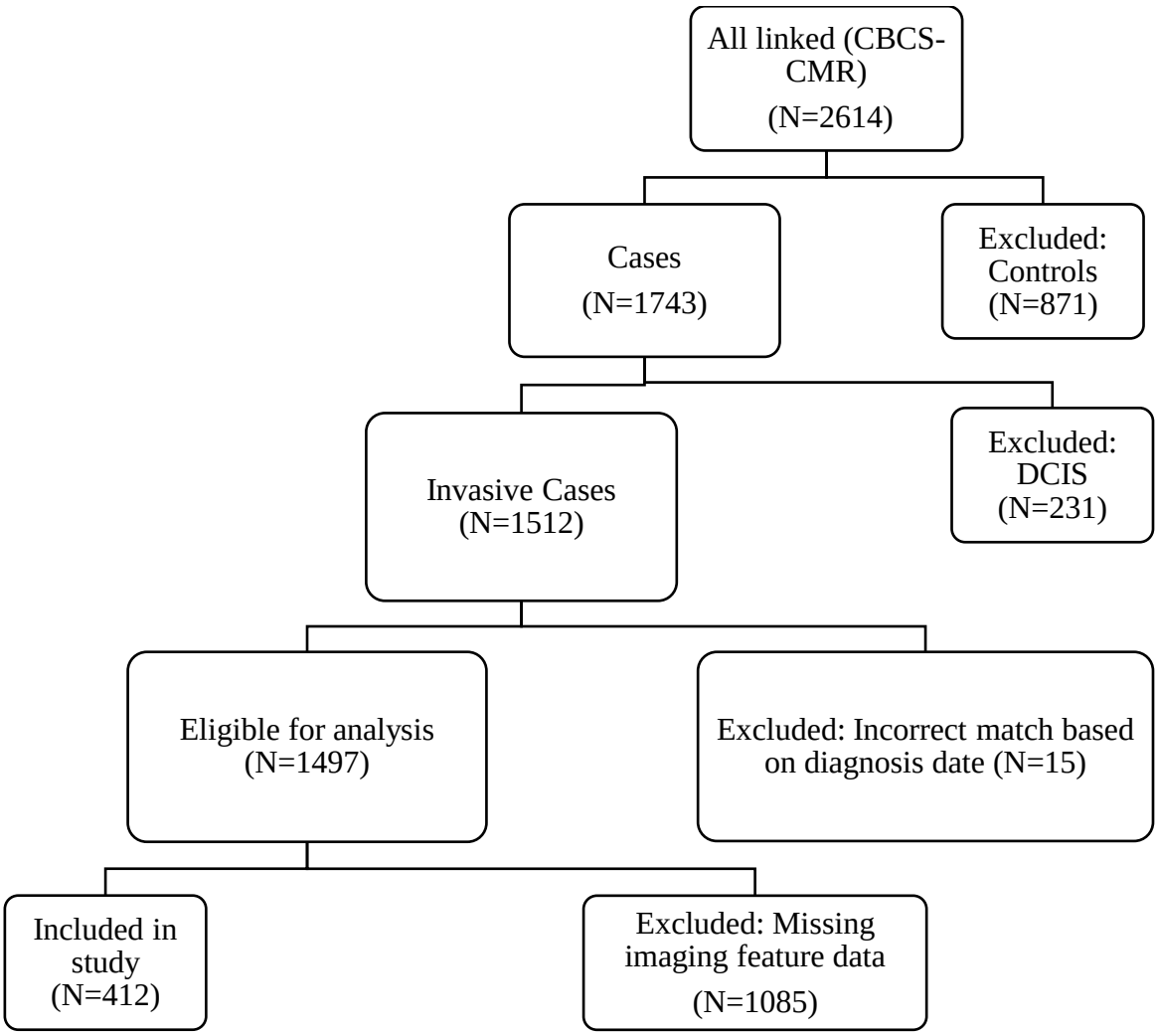


Table 5.1 Prevalence and prevalence differences (PD) of mammographic features by demographic characteristics among CBCS-CMR linked invasive cases (N=412).

	Total	Any mass			Any calcification			Mass only		
Age	N (%)	N (%)	PD[a] (95% C.I.)	p	N (%)	PD[a] (95% C.I.)	p	N (%)	PD[a] (95% C.I.)	p
≥ 50	222	98 (44)			56 (25)			77 (35)		
< 50	190	103 (54)	8.49 (-1.05, 18.04)	0.08	28 (15)	-10.88 (-18.57, -3.20)	0.006	95 (50)	13.89 (4.52, 23.26)	0.004
Race										
White	201	81 (40)			51 (25)			64 (32)		
African-American	208	120 (58)	18.27 (8.94, 27.60)	<0.001	33 (16)	-9.18 (-163.98, 1.38)	0.02	108 (52)	20.43 (11.29, 29.56)	<0.001
BI-RADS mammographic breast density[b]										
Non-dense	191	99 (52)			34 (18)			87 (46)		
Dense	196	93 (47)	-5.84 (-15.65, 3.97)	0.2	42 (21)	3.30 (-4.65, 11.26)	0.4	79 (40)	-6.75 (-16.47, 2.97)	0.2
Missing	25	9			8			6		
Stage										
I/II	347	172 (50)			69 (20)			149 (43)		
III/ IV	52	26 (50)	-3.16 (-17.02, 10.71)	0.7	11 (21)	1.26 (-10.65, 13.17)	0.8	21 (40)	-0.81 (-0.15, 0.12)	0.9
Missing	13	3			4			2		
Tumor size										
≤ 2 cm	217	97 (45)			50 (23)			84 (39)		

> 2 cm	**180**	**100 (56)**	**10.69 (1.04, 20.33)**	**0.03**	**30 (17)**	**-6.43 (-0.14, 1.43)**	**0.1**	**85 (47)**	**8.61 (-0.90, 18.12)**	**0.08**
Missing	15	4			4			3		
Mode of detection										
Screen	125	58 (46)			31 (25)			50 (40)		
Interval	132	44 (33)	-12.45 (-24.11, 0.78)	0.04	25 (19)	-5.13 (-15.35, 5.10)	0.3	37 (28)	-11.45 (-2.69, 0.21)	0.05
Missing	155	99			28			85		

[a] All prevalence differences adjusted for CBCS Phase.

[b]Non-dense= BI-RADS categories 1&2; Dense= BI-RADS categories 3&4.

Table 5.2 Prevalence and prevalence difference (PD) of mammographic features by molecular/ genomic characteristics among CBCS-CMR linked invasive cases (N=412).

	Total	Any mass			Any calcification			Mass only		
ER	N (%)	N (%)	PD[a] (95% C.I.)	p	N (%)	PD[a] (95% C.I.)	p	N (%)	PD[a] (95% C.I.)	p
Positive	221	100 (45)			45 (20)			84 (38)		
Negative	170	91 (54)	5.28 (-4.69, 15.25)	0.3	37 (22)	1.41 (-6.79, 9.60)	0.7	78 (46)	4.69 (-5.13, 14.51)	0.3
Missing	21	10			2			10		
PR										
Positive	199	87 (44)			39 (20)			74 (37)		
Negative	192	106 (55)	9.97 (0.19, 19.75)	0.05	42 (22)	2.28 (5.76, 10.31)	0.6	90 (47)	7.94 (-1.70, 17.58)	0.1
Missing	21	8			3			8		
HER2										
Positive	46	28 (61)			12 (26)			22 (48)		
Negative	308	147 (48)	-13.77 (-28.91, 1.37)	0.1	56 (18)	-7.87 (-21.29, 5.56)	0.3	128 (42)	-6.05 (-21.49, 9.38)	0.4
Missing	58	26			11			22		
IHC subtype										
Luminal A	196	86 (44)			37 (19)			74 (38)		
Luminal B	22	13 (59)	17.43 (-4.22, 39.07)	0.1	4 (18)	-0.35 (-16.87, 17.57)	1	11 (50)	14.01 (-8.66, 36.69)	0.2
Triple negative	111	60 (54)	3.27 (-0.54, 7.09)	0.1	19 (17)	-0.56 (-3.54, 2.43)	0.7	53 (48)	3.11 (-0.68, 6.91)	0.1
HER2+	24	15 (63)	8.72 (-1.84, 19.28)	0.1	8 (33)	7.17 (-2.58, 16.91)	0.1	11 (46)	3.12 (-7.44, 13.68)	0.6
Missing	59	27			16			14		
PAM50										
Luminal A	85	36 (42)			15 (18)			28 (33)		
Luminal B	17	7 (41)	-2.40 (-28.85, 24.05)	0.9	7 (41)	22.94 (-2.36, 48.24)	0.1	4 (23)	-9.23 (-12.31, 30.77)	0.4

Basal-like	**49**	**29 (59)**	**16.50 (-0.74, 33.73)**	**0.06**	**4 (8)**	**-9.60 (-20.70, 1.50)**	**0.1**	**26 (53)**	**19.88 (2.88, 36.87)**	**0.02**
HER2-enriched	18	13 (72)	31.41 (7.31, 55.51)	0.01	2 (11)	-6.66 (-23.27, 9.95)	0.4	12 (66)	32.67 (7.60, 57.74)	0.01
Missing	235	113			53			100		
ROR-PT										
Low	43	16 (37)			8 (19)			12 (28)		
Medium	101	54 (53)	18.67 (-3.48, 40.83)	0.1	18 (18)	-1.09 (-15.02, 12.84)	0.9	44 (44)	17.51 (0.46, 34.56)	0.04
High	30	16 (53)	18.67 (-3.48, 40.83)	0.1	3 (10)	-7.87 (-23.77, 8.03)	0.3	15 (50)	24.27 (2.68, 45.85)	0.03
Missing	235	113			53			100		

[a] All prevalence differences adjusted for CBCS Phase.

Table 5.3 Prevalence and adjusted prevalence difference (PD) of mammographic features by molecular/ genomic characteristics among CBCS-CMR linked invasive cases (N=412).

	Total	Any mass			Any calcification			Mass only		
ER	N (%)	N (%)	PD[a] (95% C.I.)	p	N (%)	PD[a] (95% C.I.)	p	N (%)	PD[a] (95% C.I.)	p
Positive	221	100 (45)			45 (20)			84 (38)		
Negative	170	91 (54)	0.15 (-9.70, 10.00)	1.0	37 (22)	3.03 (-4.66, 10.72)	0.4	78 (46)	0.73 (-8.40, 9.85)	0.9
Missing	21	10			2			10		
PR										
Positive	199	87 (44)			39 (20)			74 (37)		
Negative	192	106 (55)	6.43 (-3.38, 16.24)	0.2	42 (22)	2.75 (-5.04, 10.55)	0.5	90 (47)	5.29 (-3.93, 14.50)	0.3
Missing	21	8			3			8		
HER2										
Positive	46	28 (61)			12 (26)			22 (48)		
Negative	308	147 (48)	9.51 (-5.27, 24.28)	0.2	56 (18)	-8.57 (-21.60, 4.46)	0.2	128 (42)	0.39 (-15.20, 15.98)	0.9
Missing	58	26			11			22		
IHC subtype										
Luminal A	196	86 (44)			37 (19)			74 (38)		
Luminal B	22	13 (59)	12.88 (-8.34, 34.09)	0.2	4 (18)	-3.86 (-22.82, 15.10)	0.7	11 (50)	6.71 (-16.72, 30.14)	0.6
Triple negative	111	60 (54)	1.93 (-1.81, 5.67)	0.3	19 (17)	-1.15 (-4.23, 1.93)	0.5	53 (48)	1.78 (-1.80, 5.36)	0.3
HER2+	24	15 (63)	11.81 (-1.10, 24.63)	0.5	8 (33)	7.96 (-1.91, 17.84)	0.1	11 (46)	2.00 (-12.64, 8.63)	0.7
Missing	59	27			16			14		
PAM50										
Luminal A	85	36 (42)			15 (18)			28 (33)		
Luminal B	17	7 (41)	-1.90 (-28.39, 24.59)	0.9	7 (41)	18.90 (-8.28, 46.08)	0.2	4 (23)	-11.64 (-37.07, 13.79)	0.4

Basal-like	**49**	**29 (59)**	**13.97 (-4.33, 32.28)**	**0.1**	**4 (8)**	**-0.49 (-16.51, 15.52)**	**1**	**26 (53)**	**14.53 (-2.87, 31.94)**	**0.1**
HER2-enriched	18	13 (72)	27.09 (1.10, 53.09)	0.04	2 (11)	-7.89 (-29.89, 14.11)	0.5	12 (66)	22.08 (-4.78, 48.93)	0.1
Missing	235	113			53			100		
ROR-PT										
Low	43	16 (37)			8 (19)			12 (28)		
Medium	101	54 (53)	14.10 (-3.26, 31.47)	0.1	18 (18)	-0.66 (-14.28, 12.97)	0.9	44 (44)	11.50 (-4.12, 27.11)	0.1
High	30	16 (53)	11.51 (-14.64, 37.67)	0.4	3 (10)	3.93 (-20.98, 28.84)	0.8	15 (50)	17.50 (-7.70, 42.69)	0.2
Missing	235	113			53			100		

[a] All prevalence differences adjusted for age, race, and CBCS Phase.

CHAPTER 6: DISCUSSION

6.1 Summary of Findings

It has been argued that mammography preferentially detects indolent cancers. The purpose of this dissertation research was to better characterize cancers missed by mammography as a means of assessing whether this statement is true. To do this, in the first aim, screen vs. interval-detected invasive breast cancer cases were compared. We found that aggressive cancer characteristics such as large tumor size, high stage, and triple negative subtype were more likely to occur ass interval cancers, suggesting that mammography is in fact missing some aggressive cancers. In the second aim we then evaluated one explanation for why aggressive cancers may be missed, namely that they present with different imaging features. We found that ER-, PR-, triple negative, basal-like cancers more commonly presented with masses and seldom presented as calcifications. Results from both aims were strengthened using genomic methods, with high risk of recurrence (ROR-PT) score being associated with both interval cancers and cancers that present as a mass.

Joining these results together can provide a more complete picture of how specific groups of cancers can escape detection through screening. The underlying biology of the tumor may affect mammographic detection rates in two ways. First, aggressive cancers may evade detection by possessing biologic characteristics that lead to rapid progression, resulting in detection between screenings. Secondly, they can present with features that are difficult to detect mammographically. Overall, these results aligned with our study hypotheses, that cancers detected outside of screening would possess negative prognostic characteristics compared to

those detected by screening and that subtype-specific patterns of mammographic features would exist.

6.2 Significance

Understanding characteristics of cancers that evade mammographic detection helps to pinpoint areas of improvement for breast cancer screening. Although mammography is currently the most common breast cancer screening method, it is surrounded by significant controversy. Missed cancers, or false negatives of mammography, which occur in approximately 1 out of 1000 women when using digital mammography[232], is one major source of debate. Missed cancers, which are often attributed to masking caused dense breasts, represent a challenge not only on the individual level but also with respect to public perceptions of mammography, as evidenced by the rapid passing of density notification laws in the majority of the United States. There is strong evidence that the sensitivity of mammography is reduced among women with dense breasts[71,167]; however as found in this study and others[72-74], associations between aggressive tumor characteristics and interval vs. screen detection were stronger among women with fatty breasts, suggesting that cancer biology also plays a role in missed cancers. This is important to highlight as it means that intrinsic technological limitations of mammography in dense breasts may not be the sole reason for missed cancers. Another aspect of mammography that causes debate is overdetection; though mammography increases the proportion of early stage cancer detected, lower impact of mammography in reducing incidence of advanced stage cancer has been observed, suggesting overdiagnosis of indolent cancers[15-17].

Newer breast cancer screening methods such as tomosynthesis address some of these limitations of mammography. First, tomosynthesis is hypothesized to be more sensitive among women with dense breasts, and to be more sensitive cancers, reducing false negative breast

cancer screening rates[233,234]. In addition, tomosynthesis may also detect smaller masses[228,229]. Put into context of the findings of the second aim of this dissertation, this suggests that tomosynthesis may be better at detecting aggressive cancers than mammography and in the future, could be an alternative or companion screening test to conventional two-dimensional mammography.

In addition to improving imaging methods, technology may drive improvements in genomic testing to reduce the harms of screening. Even if mammography leads to overdetection of indolent cancers, our results suggest, that the risk of recurrence score could be used a companion diagnostic at the time of diagnosis to determine whether a breast cancer is indolent or has a high risk of recurrence. If confirmed, these findings could help guide not only treatment decisions, but also breast-conservation options.

6.3 Limitations

In Aim 1, some limitations arose due to utilization of data linkage methods to combine molecular and mammography data. We expect that we had incomplete registry information on mammographic information for some women. The CMR does not cover all breast imaging facilities in North Carolina, so we may have missed mammography information for linked women who visited a facility that is not part of CMR either before or after utilizing a CMR facility. This type of missing information would lead to misclassification of mode of detection. Because CMR does not include all breast imaging facilities in North Carolina, only ~30% of women enrolled in CBCS were linked to CMR. Furthermore, CBCS oversampled younger and African American women, and therefore the proportion of screen and interval detected cases may vary as a function of the demographic and selection characteristics of CBCS[48]. Therefore, our study was not designed to estimate the proportion of screen and interval-detected cases in the

general population. We were also unable to retrospectively review mammographic images to identify interval cases that arose from false negatives. Studies have shown that the false negative rate among interval cancers is around 20%[72,157,158].

Data on clinical relevance of our imaging features was limited for our study. That is, calcifications that were noted on a mammogram may not have been central to detection for a given cases. We also lacked detailed information of mammographic features such as mass shape (irregular/ lobulated/ oval/ round), mass margins (noncircumscribed/ circumscribed), and calcification type (round/ amorphous/ fine linear), some of which have been shown to be associated with breast cancer subtype[187,230,231]. Heterogeneity within imaging feature groups may have attenuated some of our associations with subtype. For example, round masses are associated with triple negative cancer and irregular masses are associated with Luminal cancer[186,187]; we could not distinguish the two in our data. Despite these limitations, the research presented here provides valuable information on mammography and is one of the first to incorporate genomic data, which is becoming increasingly utilized in the clinical setting.

6.4 Future Directions

There are several areas of uncertainty that should be prioritized in future research on breast cancer screening. First, there are remaining uncertainties about efficacy of mammography by race. In both aims, we observed racial differences in the presentation of cancers, which have not been well-studied. In Aim 1, associations between aggressive cancer characteristics and interval-detected cancers were stronger in Black women compared to White women. In Aim 2, Black women were more likely to present with masses compared to White women, and were less likely to present with calcifications. Mode of detection and mammographic features may reflect underlying subtype-specific associations by race since triple negative cancers, which are fast

growing and are likely to present with a mass, are more prevalent among African-American women. Future directions of this research should leverage the resources of surveillance consortia to study the biological characteristics of interval cancers that occur among Black women. Alternatively, clinical trials designed to assess the efficacy of tomosynthesis vs. digital mammography should evaluate race-specific performance in both arms.

Another important area for future research is the use of health insurance data in studies of mammography and breast cancer screening. United States-based studies often have had less complete mammographic data relative to European studies based on single-payer health care data. Use of insurance claims data could provide a more complete history of mammography use for women, allowing for better classification of mode of detection. In addition, such studies could help to better characterizing screening behavior. Screening behavior is commonly tracked by evaluating both initiation and adherence. Factors that affect screening initiation and adherence may lead to selection bias in studies that evaluate tumor biology of screen-detected vs. non-screen-detected cancers. Understanding these factors is important when interpreting the results of mammography.

Finally, our data suggest that the biologic features of cancer subtypes are often present from the earliest stages, affecting not just clinical outcomes but patterns of detection. However, the specific biological mechanism underlying the association between subtype and imaging features are still poorly understood. Specifically, the natural history of calcifications are not well-understood, and are crucial in understanding why basal-like cancers are often not detected with calcifications. It is unclear whether basal-like cancers have an early, calcification stage that could be detectable by more frequent screenings, or whether some cancers are only detectable as

masses. One approach to addressing this question would be to identify genes or histologic features that are associated with calcifications.

6.5 Conclusions

The linked Carolina Breast Cancer Study and the Carolina Mammography Registry data set provided a unique resource, with both data sets contributing high quality data. Screening and mammographic data from CMR was complemented with epidemiologic, clinical, and molecular data from CBCS. Using a racially diverse data set with well-characterized tumor biology, this dissertation elucidated the relationship between breast cancer subtype, imaging features, and mode of detection in a heterogeneous population of North Carolina women. Understanding the limitations and failures of mammography highlight priority areas of screening improvement and helps prioritize research questions in the context of evolving radiologic practices.

APPENDIX A: MAMMOGRAPHY RECOMMENDATIONS (CBCS 1-3)

CBCS Phase 1 (1993-1996)

	1990	1991	1992	1993
Organization				
ACS	35-39: Baseline Mammogram 40-49: Every 1-2 yrs 50+: yearly		40-49: Every 1-2 yrs 50+: yearly	
ACOG	35-39: Baseline Mammogram 40-49: Every 1-2 yrs 50+: yearly			
USPSTF				

CBCS Phase 2 (1996-2001)

	1993		1994	1995	1996	1997	1998
Organization							
ACS		40-49: Every 1-2 yrs 50+: yearly				40+ yearly	
ACOG		40-49: 1-2 yrs 50+: yearly					
USPSTF					40+: Every 1-2 years		

CBCS Phase 3 (2008-2013)

	2005	2006	2007	2008	2009	2010
Organization						
ACS	40+: yearly					
ACOG	40-49: 1-2 years 50+: annual					
USPSTF	40+: Every 1-2 years					Before 50: up to the woman 50-74: biennial 75+: insufficient evidence

APPENDIX B: STUDIES OF INTEREST

Table B1. Percent of interval cancers in different study populations

Author	Study Population	Screening Interval	Percent of cancers that were interval-detected
Ikeda, 1992[50]	Malmo Mammographic Screening Trial	18-24 months	17%
Klemi, 1997[49]	Population based screening program in Finland	1-3 years, depending on age	Age 40-49: 1 year interval: 27% Age 40-49: 3 year interval: 39% Age 50-74" 2 year interval: 18%
Porter, 1999[48]	Women from HMO (Group Health Cooperative of Puget Sound) also enrolled in Breast Cancer Screening Program	2 years	28%
Hofvind, 2009[54]	Screening program in Norway	2 years	26%
	CMR	1-2 years	38%
Kirsh, 2011[69]	Ontario Breast Screening Program	Biennial screening, but women who were determined to be high risk were screened annually	13.8%, of which 77% were true interval cancers
Nederend, 2014[52]	Breast cancer screening program in the Netherlands	2 years	23.9%
Bento, 2014[53]	Breast cancer screening program in Portugal	2 years	20.2%
Henderson, 2015[51]	Breast Cancer Surveillance Consortium	1 year	14.7%

Table B2. Studies of interest related to Aim 1: Subtype & mode of detection

Author, year	Population	Outcome	Interval used	Predictor	Key Results
Kirsh, 2011[69]	Ontario Breast Screening Program (women>50) January 1, 1994-December 31, 2002	Screen-detected (referent, n=450) vs. Interval (n=375) IC= diagnosed before the next recommended screening visit after a negative screening mammo SDC= Diagnosed after a positive screening mammo	Most women participating in OBSP are screened every two years, women considered at high-risk for BC are examined annually	Lymph node +	OR=1.41, 95%CI (1.01,1.96)
				Tumor size (<10 mm referent)	**10-15 mm**: OR=2.04, 95% CI (1.34, 3.11) **16-20 mm:** OR=3.70, 95% CI (2.28, 5.95) **>20 mm**: OR=4.83, 95% CI (3.09, 5.75)
				Stage at diagnosis (I is referent)	**II:** OR=2.16, 95% CI (1.39, 3.36) **III or IV**: OR=4.46, 95% CI (1.12, 17.70)
				ER -	OR=1.68, 95% CI (1.09, 2.59)
				PR -	OR=2.07, 95% CI (1.43, 2.98)
Domingo, 2014[68]	Population based screening program in Spain (women 50-69) 2000-2009	Screen-detected (referent, n=1297) vs. Interval (n=455) IC: primary BC arising after a negative screening episode, with or without further assessment, and before the next invitation to screening, or within 24 months for women who reached the upper age limit	Women invited to participate by written letter every 2 years	Lymph node +	SDC: 29.8% IC: 49.6%
				ER-	SDC: 17.5% IC: 36.8%
				Luminal A+B	SDC: 83.4% IC: 66.4%
				Triple negative	SDC: 9.9% IC: 19.9%

Rayson, 2011[91]	Nova Scotia Breast Screening Program	Screen-detected (referent, n= 481) Interval (n=241) IC= interval cancers were true interval cancers- negative screening mammos were re-reviewed by 3 independent radiologists	1 year and 2 year	Triple negative	**Women 40-49**: OR= 1.36, 95% CI (0.19, 9.67) **Women 50-69 with 1 year interval**: OR=1.72, 95% CI (0.29, 10.2) **Women 50-69 with 2 year interval**: OR=2.28, 95% CI (1.05, 4.94)
Caldarella, 2013[77]	Population based screening program in Italy	Screen-detected (referent,N=211) Interval (N=66)	2 year	Triple negative (LumA is referent)	OR= 3.52 (1.12, 11.13)
				HER2 (LumA is referent)	OR= 1.57 (0.46, 5.29)
Gilliland, 2000[70]	New Mexico Mammography Project	Screen-detected (referent, n=63) Interval (n=64)	1 year	p53 expression	OR=2.96, 95% CI (1.07, 8.20)
Collett, 2005[138]	Norwegian Breast Cancer Screening Program	Screen (referent, n=95) Interval (n=95)	2 year	p53 high expression	OR=4.0, 95% CI (1.6, 12.0)

*Interval cancer was cancer detected 24 months after negative screening mammogram

Schroen, 1996[102]	Netherlands, hospital based, retrospective analysis of women referred for breast cancer from 1975-1990	SDC (N=173) Interval (N=76) Other [patients who were never invited to screening program, patients who chose not to attend, and patients who developed breast cancer >2 years after attending the screening program] (N=688)	2 year	Tumor size >5 cm Positive lymph node status	SDC: 9% Interval: 10% Other: 12% SDC: 19% Interval: 40% Other: 32%

Table B3. Studies of interest related to Aim 2: Subtype & imaging features

Author, year	Population	Subtype	Key Results
An, 2015[225]	Women <30 who underwent surgery to treat breast cancer in South Korea	Triple negative (n=6) ER+ (n=40) HER2 enriched (n=4)	80% of triple negative breast and 65% of ER+ cancers presented with a mass and no calcifications
Boisserie-Lacroix, 2013[187]	Database from French hospital	Triple negative (n=92) ER+/PR+/HER2+ (n=95)	8.7% of TN present as mass with calcification vs. 5.3 of Luminal B
Ko, 2010[153]	Database from Korean hospital	Triple negative (N=87) ER+/PR-/HER2- (n=93) ER-/PR-/HER2+ (n=65)	TN cancers usually presented with a mass HER2 more likely to present with calcifications
Wang, 2010[226]	Chinese women who underwent breast surgical treatment	Basal like (n=40) Non-basal like (n=227)	Basal-like more likely to present with mass and less likely to present with architectural distortion
Yang, 2008[154]	Premenopausal women <45	TN (n=38) HER2+ (n=67) ER+ (n=93)	TN more likely to be associated with a mass HER2 more likely to present with calcifications
Killelea, 2013[141]	Database from Yale hospital	LumA (n=703) LumB (n=78) HER2 (n=59) TN (n=145)	TN more likely to be associated with a mass HER2 more likely to present with calcifications Luminal cancers more likely to present with architectural distortion

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