PARITY, OBESITY AND BREAST CANCER SURVIVAL: DOES INTRINSIC SUBTYPE MODIFY OUTCOMES?

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ABSTRACT

Xuezheng Sun: Parity, Obesity and Breast Cancer Survival: Does Intrinsic Subtype Modify Outcomes?
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Purpose: Parity and obesity have shown distinct associations with the breast cancer risk by intrinsic subtype. Little is known whether their influence on prognosis also varies by intrinsic subtype, although their general prognostic associations have been reported previously.

Methods: Study subjects were 1,140 invasive breast cancer patients from the phases I and II of the population-based Carolina Breast Cancer Study (CBCS), with tissue blocks available for subtyping using immunohistochemical markers. Parity was measured by number of full-term birth and time since last birth. Obesity was measured by body mass index (BMI) and waist hip ratio (WHR). Vital status was determined using the National Death Index. The association of exposures with breast cancer (BC)-specific and overall survival was assessed using the Cox proportional hazards model. **Results**: During the follow-up (median =13.5 years), 450 patients died, with 61% due to breast cancer (n=276). For obesity, WHR, but not BMI, was associated with an increased risk of all-cause mortality (≥ 0.84 vs. <0.77, adjusted hazard ratio (HR) = 1.50, 95% confidence interval (CI) =1.11-2.05), independent of age, race, adjusted lifestyle and socioeconomic factors. According to intrinsic subtypes, high BMI (≥ 30 kg/m^2) was an independent factor for all-cause mortality (adjusted HR=2.25, 95% CI=1.14-4.46, <0.25 kg/m^2 as reference) among patients with basal-like tumors, while high WHR (≥ 0.84) was associated with poor overall survival (adjusted HR=1.75, 95% CI=1.20-2.56, <0.77 as reference) among patients with luminal tumors. For parity, both high parity (3+ births) and recent birth (< 5 years before

diagnosis) were associated with BC-specific mortality (parity: adjusted HR=1.76, 95% CI=1.13-2.73; birth recency: adjusted HR=1.90, 95% CI=1.10-3.34), with stronger effect observed in luminal tumors than basal-like tumors. The subtype-specific prognostic associations of parity and obesity were suggested to vary by follow-up period (greater HRs detected in patients surviving ≥5 years), but not by race or menopause. C**onclusions**: Our study suggests the influence of obesity and parity on breast cancer prognosis may vary by intrinsic subtype. These results may contribute to a better understanding of how pregnancy and obesity influence the natural history of different breast cancer subtype, and help tailor treatment and optimize intervention strategies.

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AA	African American
ASCO	American Society Of Clinical Oncology
BC	Breast Cancer
BCSM	Breast Cancer Specific Mortality
BLBC	Basal-Like Breast Cancer
BMI	Body Mass Index
CBCS	Carolina Breast Cancer Study
CI	Confidence Interval
CK5/6	Cytokeratin 5/6
DAG	Diagram/Directed Acyclic Graph
DCIS	Ductal Carcinoma In Situ
ER	Estrogen Receptor
EGFR	Epidermal Growth Factor Receptor
HC	Immunohistochemistry
HER2	Human Epidermal Growth Factor Receptor 2
HR	Hazard Ratio
HRT	Hormone Replacement Therapy
HT	Hormone Therapy
ICD	International Classification Of Diseases
IGF-1	Insulin-Like Growth Factor 1
IL	Interleukin
IRB	Institutional Review Board

kg	Kilograms
m	Meters
mg	Milligram
NDI	National Death Index
OC	Oral Contraceptive
OR	Odds Ratio
OS	Overall Survival
PR	Progesterone Receptor
RFS	Relapse Free Survival
RR	Risk Ratio
SES	Socioeconomic Status
SHBG	Sex Hormone Binding Globulins
TNBC	Triple-Negative Breast Cancer
UNC	University Of North Carolina
WHO	World Health Organization
WHR	Waist-To-Hip Ratio

CHAPTER 1: BACKGROUND

Despite many advances in screening, prevention and novel treatment, breast cancer remains the most frequent cancer and the second leading cause of cancer mortality in women (1, 2). Approximately 232,340 new cases of invasive breast cancer and 39,620 breast cancer deaths are expected to occur among US women in 2013 (3, 4). The burden of the disease is likely to rise over the next 20 years due to the increasing population of older adults and minorities, with invasive breast cancer expected to reach 294,000 in 2030 (5). The increasing public health burden underpins the importance of breast cancer research. The establishment of breast cancer heterogeneity, namely intrinsic subtype using global gene expression techniques, suggests the need to reweigh epidemiological findings in this new context. Specifically, evaluating the influence of breast cancer heterogeneity on the association of breast cancer risk factors with breast cancer prognosis would provide novel insights into the previously mixed observations on these associations.

1.1. Heterogeneity of breast cancer

1.1.1. Biological heterogeneity

Breast cancer has long been recognized as a heterogeneous disease. Previously, this complexity was mainly characterized by hormone receptors, including estrogen receptor (ER) and progesterone receptor (PR). This classification reflects etiological heterogeneity, which also has been supported by epidemiologic data. Distinct associations with risk factors, such as age (6, 7), race/ethnicity (8, 9), reproductive factors (7, 10, 11), anthropometric factors (12, 13), and

lifestyle factors (14), across subtypes defined by hormone receptor status have been observed in many studies across different populations. The differences by hormone receptor status are also observed in screening mammography detection (15), clinicopathological characteristics (16, 17), treatment availability (18, 19), and prognosis (19, 20). Although hormone receptors provide a rough picture for breast cancer heterogeneity, the intra-category heterogeneity has been recognized as tumors with the same hormone receptor status can respond differently to therapy, resulting in different outcomes. In response to this recognition, several additional biomarkers (such as P53, Ki67, and Her2) have been developed to capture heterogeneity from proliferation, apoptosis, migration, and other aspects of breast cancer biology not captured by hormone receptor status (21-23). Previous analyses usually describe breast cancer heterogeneity using individual biomarkers. The mechanism of breast cancer heterogeneity is complex, involving distinct cellular origins, genetic and epigenetic alterations, and paracrine signals from surrounding cells (24). Considering the complex etiology of tumor subtypes, an individual biomarker is obviously insufficient to portray distinct tumor phenotypes. Recent advances in new techniques (such as cDNA microarray) have allowed a more comprehensive profiling of breast cancers across thousands of genomic biomarkers.

Discovery of intrinsic breast cancer subtype

In 2000, using cDNA microarray and hierarchical clustering, Perou and colleagues categorized breast cancer into five intrinsic subtypes based on differences in molecular patterns: luminal A, luminal B, Her2-enriched, basal-like, and normal-like (25). Later, the robustness and universality of this new taxonomy was confirmed in larger, multi-ethnic populations using different microarray platforms and across different breast cancer histological types (26-32).

Luminal A and luminal B tumors are predominately ER positive and express genes similar to luminal mammary epithelial cells. In contrast, basal-like tumors are predominately ER negative and express genes associated with normal myoepithelial cells of the outer layer of the breast duct (24, 26, 33). Her2-enriched tumors are characterized by high expression of several genes in the ERBB2 amplicon (neu/HER2) (24). Normal-like tumors show high expression levels of many genes known to be expressed by adipose tissue and other non-epithelial genes. Some researchers suggested that normal-like tumors may represent tumor samples contaminated by normal tissue (34). Besides differences in expression of biomarkers of cellular origin, intrinsic subtypes demonstrate differences in proliferation. Compared to basal-like and luminal B tumors, proliferation rates and expression of proliferation-associated genes were lower for luminal A and lowest among Her2-enriched tumors (24, 26, 33, 35). In addition, the rate of TP53 mutation was lower in luminal A tumors, and BRCA1 mutation is more frequent in basal-like tumors than other subtypes (33). In recent studies, more genetic and genomic differences (such as androgen receptor, GATA3, FOXA1, keratin 18, and PI3KCA) (36) have been characterized for each breast cancer subtype, further underscoring their distinction in their biological characteristics.

Pathogenesis by intrinsic subtype

As a reflection of the underlying biology and genomics, intrinsic types vary considerably in their natural disease history. Compared to luminal A tumors, basal-like tumors are more likely to be invasive ductal cancers with high-grade/poor differentiation (25, 37-46). They also have high proliferative index, high nuclear/cytoplasmic ratio, pushing margins of invasion, central necrosis, and lymphocyte-rich stroma (39, 47, 48). In contrast, luminal tumors (both luminal A and B) tend to be well-differentiated with lower nuclear grade. In studies comparing intrinsic

subtypes of invasive breast tumor and ductal carcinoma in situ (DCIS), luminal A tumors are more prevalent than DCIS (49-52). Regarding tumor size and axillary lymph node involvement, findings are inconsistent, with some studies showing larger tumors and higher rates of lymph node positivity in basal-like tumors (27, 42, 53), but others finding no differences (38, 41). Similar to basal-like tumors, Her2-enriched tumors also demonstrate higher grade and larger size than luminal tumors (43, 44, 46), although their progression is thought to be slow (54).

Classification by IHC biomarker

Originally, subtype classification was based on cDNA microarray techniques, which require frozen tissue for RNA extraction and analysis. Since frozen tissues are not routinely available in clinical practice and epidemiological studies, methods are needed for subtyping tumors using formalin-fixed paraffin embedded tissues. To this end, immunohistochemistry (IHC) classification protocols have been developed and used in the vast majority of published reports. The most frequently used IHC classification criteria were developed by Nielsen and colleagues (55). According to this schema, luminal A tumors are immunohistochemically identified by the expression of ER, the presence or absence of PR, and the absence of Her2 expression. Luminal B tumors are hormone receptor positive (ER positive and/or PR positive), but differ from luminal A tumors in that they are positive for expression of Her2. Her2-positive tumors are defined by the lack of expression of hormone receptors and the presence of HER2 tyrosine kinase receptor. Basal-like tumors are defined by the lack of ER, PR and Her2, but the expression of either epidermal growth factor receptor (EGFR) or cytokeratin (CK)5/6+. This categorization approach was revised recently by the St. Galen International Expert Consensus panel, with emphasis on distinguishing between luminal A and luminal B tumors (56, 57). By the newly proposed IHC-based definition, luminal A tumors are ER-positive, Her2-negative, are

Ki-67-positive in less than 14% of cells, and PR-positive in more than 20% of cells. Luminal B tumors are either ER-positive and PR-positive and Her2-positive, or ER-positive and Her2-negative and either Ki-67≥14% or PR≥20% (35, 57). Of note, although representing a convenient approximation, subtypes defined by IHC biomarkers are similar, but not identical, to intrinsic subtypes defined by cDNA microarray.

Due to similarities in these definitions and the availability of ER, PR, and Her2 status in clinical records, triple negative breast cancer (TNBC) and basal-like breast cancer (BLBC) have been used interchangeably in some papers. However, they do not completely overlap, with discordance between 20-30% (58, 59). TNBC is more biologically heterogeneous, including all the intrinsic subtypes, with BLBC accounting for about 70% (60). As the intrinsic subtype definition had superior prognostic value than the three-biomarker classification, intrinsic subtype is considered more precise at describing the intrinsic biological differences in breast cancer (60, 61, 61-65). In this proposal, the literature review focused on studies using intrinsic subtype, but still included reports on TNBC considering the large amount of literature.

1.1.2. Distinct epidemiological risk factor profile by intrinsic subtype

The heterogeneity in biological and histopathological tumor features suggests distinct etiologies, which has been confirmed in epidemiologic studies of risk factors. The risk factors that have been evaluated for associations with intrinsic subtype include age, anthropometric factors, reproductive factors, and lifestyle factors. Among these factors, obesity and parity are reviewed in the later parts of this section. In general, luminal A and TNBC/BLBC have been intensively studied with clear differences observed. Relatively little is known regarding the risk factors for Her2-positive tumors and luminal B tumors, which partially contributes to intra-subtype heterogeneity, potential misclassification, and the rarity of the two subtypes.

Age at diagnosis has been widely studied in association with subtype. Patients with TNBC/BLBC tend to be younger than patients with luminal A and Her2-positive tumors (66-72). This difference has been remarkably consistent across different-sized studies and in different multi-racial/ethnic populations (44, 46, 53, 66, 71, 73-75).

Racial differences in subtype prevalence have also been found consistently. African American and Hispanic women are more likely to have TNBC/BLBC compared to white women (46, 70-72, 76, 77). Her2-positive tumors were more likely to affect Hispanic or Asian women (43, 46, 77). The mechanism underpinning the association of race and subtype is not well understood and remains under investigation. Race is a complex construct consisting of both environmental and genetic differences, each of which may contribute to differences in subtype-specific risk. However, some have argued that racial differences may be independent of socioeconomic status (SES) (43, 78).

Aside from parity, other reproductive factors have been reported to be associated with subtypes. Breastfeeding has been associated with lower risk across all subtypes (53, 67, 69, 71, 76, 79, 80). This protective effect was stronger in, or limited to, BLBC/TNBC in some studies (70, 76, 80), while other studies reported the largest decrease in risk among luminal A or luminal tumors (66, 71, 79). In addition, younger age (<30 years) at first full-term birth was associated with TNBC (70), while older age (≥ 30 years) was associated with luminal tumors (66).

Other breast cancer factors have been less studied and some of them showed heterogeneous associations with breast cancer subtypes. Hormone replacement therapy (HRT) has been associated with luminal and Her2-positive tumors (66, 73, 77). Women with family history of breast cancer tend to have higher risk for luminal tumors (66, 67) and for TNBC before 45 years (81, 82). The association of early age at menarche with breast cancer subtype is controversial.

Some studies found it to be associated with luminal and Her2-positive tumors (67, 76, 83, 84), but other studies found it to be associated with TNBC (70, 71, 82). Lower SES demonstrates a positive relation with TNBC and Her2-positive tumors (71, 72). Current smoking also showed a positive association with Her2-positive tumors in one study (71). This study also found that higher levels of physical activity were associated with decreased risk of all tumor types, except for luminal A (71).

Inconsistent associations between breast cancer subtypes and these risk factors across studies could be explained by differences in study populations, adjusted confounders, or study sample size. The measurement of exposures, such as lactation, smoking, and physical activities, is challenging and varies across studies, which may contribute to the observed inconsistency. Similarly, approaches in subtype categorization might impact the findings across studies. For example, besides ER, PR, Her2, and CK5/6, some studies used Her1, Ki-67, or proliferation grade to define BLBC and luminal tumors (73, 85, 86). This misclassification will be improved after the release of standard (56, 57) and after further progress in our understanding of intrinsic subtype (35, 36, 60). Finally, inconsistent associations of rare subtypes with risk factors may result from small sample sizes.

1.1.3. Distinct prognosis by intrinsic subtype

Histologically similar tumors have variable prognoses and response to therapy, and these differences in clinical behavior are believed to be due to molecular differences (85). Indeed, intrinsic subtypes have shown significant prognostic and predictive value in breast cancer outcomes.

Pattern of relapse and metastasis

One measure of heterogeneous outcomes in breast cancer is the rate of relapse. TNBC and Her2-positive tumors have higher incidence of locoregional recurrence than other subtypes. A recent meta-analysis of 12,592 patients reported an approximately three-fold higher risk of locoregional recurrence among TNBC and Her2-positive tumors compared to luminal tumors after breast-conserving therapy (87). Similar higher risk for TNBC and Her2-positive tumors was also observed in patients with mastectomy (87). These results are consistent with studies of BLBC (88-90). Early blood-borne dissemination has been hypothesized to occur more commonly in BLBC (91). Compared to BLBC/TNBC, Her2-positive tumors more frequently exhibited lymphovascular invasion (41, 44-46, 73, 80) and have more frequent locoregional recurrence following breast-conserving therapy (87).

Similarly, TNBC and Her2-positive tumors have higher risk for distant metastasis than luminal tumors (44, 92). Of note, the location of metastasis also varies by intrinsic subtype. In general, BLBC and Her2-positive tumors are more likely to develop brain and lung metastases, while luminal tumors are associated with bone metastases (46, 62, 93-98). Some studies also suggest that Her2-positive tumors more frequently metastasize to liver (46, 62, 97). These findings, in turn, support subtypes as distinct biological variants of breast cancer that predispose patients to different outcomes.

Overall and breast cancer-specific survival

Significant differences in prognosis exist among intrinsic subtypes, with BLBC and Her2-positive tumors showing the shortest overall and disease -free survivals (26, 33, 88, 90, 99, 100). TNBC has the worst overall and disease-free survival of any subtype (43, 72, 83). Five-year overall survival rate of TNBC has been found to range from 47.7%-85.5% (43-45, 72) and five-

year disease-free survival was 76.0% (43). BLBC has been found to be similar, with five-year overall survival/metastasis-free survival reported between 58% and 66% (26, 33, 88, 90, 99, 100). The estimated five-year overall/disease-free survival percentages of Her2-positive tumor ranged from 63.0%-83.2% and 77.5%-79.1% before and after Trastuzumab treatment, respectively (43-45). In contrast, luminal tumors carry a better prognosis, with five-year overall survival and five-year metastasis-free survival up to 93.3% and 86% respectively (37, 45). Luminal B tumors demonstrate noticeably worse outcomes than luminal A tumors, which may be partially due to variation in response to treatment (26, 33, 99, 101).

Effect modification of the association between intrinsic subtype and breast cancer prognosis by race/ethnicity has been evaluated (43, 72, 83). In one analysis based on a subset of the women's CARE (contraceptive and reproductive experiences) study, the black-white difference was only observed in all-cause mortality among old women (50-64 years) with luminal A tumors (HR=1.88, 95% CI=1.30-3.79), while no black-white differences in mortalities (all-cause and breast cancer-specific mortalities) were observed for women with TNBC (83).

Therapy response

Different biological characteristics also result in different therapy responses. Luminal tumors have more treatment options than other subtypes, including endocrine therapy. Therefore, they usually have better prognosis. The prognosis of Her2-positive tumors is expected to be improved with the use of anti- ERBB2 monoclonal antibody agents, which is generally supported by recent data (102). Since TNBC/BLBC tumors do not express the target for hormone therapy and anti-ERBB2 therapies, cytotoxic chemotherapy (such as DNA-damaging agents) is the only available systemic treatment. Although novel treatments are under

study (103, 104), fewer treatment options available for BLBC are considered the main reason for the poor prognosis (105).

1.2. Obesity, breast cancer risk and prognosis

1.2.1. Obesity, intrinsic subtype, and breast cancer risk

The association between obesity and breast cancer risk has been intensively studied. In a recently published meta-analysis of 2,175,419 subjects, obesity showed a protective effect in premenopausal women (OR=0.93, 95% CI=0.86-1.02) and a detrimental effect in postmenopausal women (OR=1.15, 95% CI=1.07-1.24) (106). For premenopausal women, the proposed explanation for these associations was that obesity is associated with a greater number of anovulatory cycles and thus lower levels of estradiol. For postmenopausal women, it was proposed that obesity is associated with aromatization of steroid precursors to estrogens and therefore higher levels of estradiol (107). Other complimentary mechanisms may also apply, such as insulin-insulin-like growth factor axis, and systemic/chronic inflammation (108).

Considering tumor subtype provides novel insights into the association between obesity and breast cancer risk. Based on a meta-analysis of studies of breast cancer subtype defined by ER and PR, the menopause-modified effect of obesity was stronger when the analysis was limited to ER+/PR+ tumors (premenopausal: RE (risk estimate)=0.80, 95% CI=0.70-0.92. Postmenopausal: RE=1.82, 95% CI=1.55-2.14), while no associations were observed for ER-/PR- (premenopausal: RE =1.04, 95% CI=0.92-1.17. Postmenopausal: RE =1.09, 95% CI=0.96-1.23) (13). The magnitudes of the associations among hormone-positive tumors were similar to the results in women with luminal tumors. Turkoz and colleagues found that being overweight or obese significantly decreased the risk of luminal tumors (overweight: OR=0.63, 95% CI=0.43-0.95. Obesity: OR=0.50, 95% CI=0.32-0.76) among premenopausal women (66).

Regarding intrinsic subtype, most of the studies observed a positive association between obesity and TNBC/BLBC, with a possible difference by menopause status. Kwan and colleagues found that, compared to luminal A tumor patients, premenopausal women with TNBC were more likely to be overweight (OR=1.82, 95% CI=1.03-3.24) or obese (OR =1.97, 95% CI=1.03-3.77) (77). Similar associations among premenopausal women were also detected in a large population (n=6175) from the National Comprehensive Cancer Network (46). These findings based on case-only studies are also confirmed in a case-control study, where compared to healthy premenopausal controls, premenopausal women with higher weight level (categorized as underweight, normal weight, overweight, or obese) had higher risk for TNBC (OR=1.67, 95% CI=1.22-2.28) (81). These results from U.S. studies are consistent with studies from other countries. Yang and colleagues found that BMI was associated with higher risk for BLBC (per five BMI units, OR=1.18, p=0.003) among premenopausal Polish women (73). A study in Turkey found that overweight or obesity significantly increased the risk of TNBC (overweight: OR=1.89, 95% CI=1.06-3.37. Obesity: OR=1.90, 95% CI=1.00-3.61) among premenopausal women (66). Similar findings were also observed in premenopausal Chinese women, who experienced a nearly four-fold increased risk of TNBC among obese patients compared to under-weighted patients (OR=3.7, 95% CI=1.2-12.1) (109). In contrast to the observations in premenopausal women, the subtype-specific effects of obesity disappeared after menopause, and obesity shows a homogeneously positive association with breast cancer (46, 66, 73, 76, 77, 81, 109). The association between obesity and luminal B or Her2-positive breast cancer has been less reported. Gaudet et al. found an increased risk for luminal B tumors among obese patients (81). Weight gain since 18 years and BMI at age 18 was also reported to be associated with higher risk for luminal B and Her2-positive tumors (80).

Besides menopause status, race/ethnicity has been evaluated as a potential effect modifier in some studies. According to the most recently published meta-analysis among premenopausal women, a weak inverse association with BMI was observed for both African American and white women (AA: RR per 5kg/m2=0.95, 95% CI=0.91-0.98. White: RR=0.93, 95% CI=0.91-0.95), while a positive association was observed in Asian women (RR=1.08, 95% CI=1.01-1.16) (110). Interestingly, WHR showed different results from BMI, with WHR associated with increased risk for breast cancer across all racial groups (African American: RR for 0.1 unit increment=1.06, 95% CI=1.01-1.12. White: RR=1.09, 95% CI=1.04-1.14. Asian: RR=1.19, 95% CI=1.15-1.24) (110). These differences may reflect the different prevalence of subtypes by racial/ethnic groups. However, so far few studies assessed the association by both race and subtype.

Compared to BMI, few studies have used other types of anthropometric indices to evaluate obesity. Different indices capture different aspects of obesity. For instance, BMI is considered to better reflect overall body obesity, while WHR measures central/abdominal obesity. It has been suggested that the association of obesity with breast cancer risk varies depending on the specific measurements used (110). However, little is known regarding the difference between measures of obesity by intrinsic subtype. In CBCS, Millikan and colleagues detected an association with WHR, but not BMI (76). However, the association with WHR was not confirmed in other study (71).

1.2.2. Obesity, intrinsic subtype, and breast cancer prognosis

The prognostic effect of obesity has been investigated extensively. Past reviews and meta-analyses have consistently found an association between obesity and poor breast cancer survival (111-116). Based on a recent meta-analysis of 43 studies, compared with non-obese breast

cancer patients, obese breast cancer patients had a 33% increased risk for poor outcomes (HR for all cause death= 1.33, 95% CI=1.21-1.47; HR for breast cancer-specific death=1.33, 95% CI=1.19-1.50) (113). In addition, obesity is associated with increased risk of contralateral breast (RR=1.37, 95% CI=1.20-1.57) and a second primary breast cancer (RR=1.40, 95% CI=1.24-1.58) (116). The relationship between obesity and prognosis varies by menopausal status, with a stronger effect observed in pre-menopausal than post-menopausal women (113, 117). Although the understanding of obesity's role in prognosis is mostly based on studies where BMI was measured at or after diagnosis of breast cancer, a meta-analysis suggested that obesity before breast cancer diagnosis had a stronger association with prognosis (114). Two studies examining the impact of WHR on breast cancer mortality suggested that high WHR had an unfavorable effect on breast cancer prognosis (118, 119), although WHR's association with mortality was weaker than that of BMI (113).

Variation has been observed in the association between obesity and prognosis by breast cancer subtype. In a meta-analysis of 14 studies, overall mortality in obese women was worse compared with non-obese women (pooled HR=1.31, 95% CI=1.17–1.46) for ER+/PR+ tumors, as well as for ER-/PR- tumors (HR=1.18, 95% CI=1.06–1.31). The pooled HRs for breast cancer-specific mortality were 1.36 (95% CI=1.20–1.54) for ER+/PR+ tumors and 1.46 (95% CI=0.98–2.19) for ER-/PR- tumors, respectively (120).

Studies addressing whether the effect of obesity on breast cancer outcomes varies by intrinsic subtype are few, and the results are mixed (**Table 1.1**). One study in premenopausal women reported an increased risk for breast cancer-specific mortality associated with obesity (obese vs normal weight: HR=1.4, 95%CI=1.0-2.1) among TNBC, but no association was observed among luminal tumors (66). This finding is inconsistent with other studies, where no

association was observed between obesity and prognosis among TNBC (121-124). Sparano and colleagues used data of three trials (N=4770 breast cancer patients overall) and found that, among TNBC patients, obesity was not associated with overall mortality (obese vs normal weight: HR=1.11, 95% CI=0.85-1.46), disease-free mortality (HR=1.02, 95% CI=0.80-1.30), or breast cancer-specific mortality (HR=1.00, 95% CI=0.74-1.36) (121). However, among luminal A tumors, obesity was positively associated with all of the three prognostic measures (HR for overall death=1.37, 95% CI=1.13-1.67; HR for disease-free death=1.24, 95%CI=1.06-1.46; HR for breast cancer-specific death=1.40, 95% CI=1.11-1.76) (121). The investigators found similar results when subtype was defined by ER/PR status (121). These findings are consistent with a recently published hospital-based study in Japan, where among ER+/PR+ patients, but not ER-/PR- patients, women with higher BMI tended to have poorer overall and breast cancer-specific survivals compared with women with lower BMI (117). The null association was also observed in similar TNBC-only studies (122-124). While low statistical power due to small sample size may contribute to the null association, the magnitude of association was notably close to the null value of 1 in these studies on TNBC (121-123). Few studies have evaluated obesity in association with survival following Her2-positive tumor diagnosis. One large study based on data from an adjuvant treatment trial did not detect any prognostic value of obesity among Her2-positive tumors (121). Other studies found that obesity was associated with higher risk for mortality and distant metastasis in this subtype (66, 121, 125).

Whether menopausal status modifies the association between obesity and breast cancer prognosis is not clear. A meta-analysis reported increased hazard for overall mortality in obese versus non-obese women (HR=1.23, 95% CI=1.07–1.42) among premenopausal patients and also among postmenopausal women (HR=1.15, 95% CI=1.06–1.26). For breast cancer-specific

mortality, increased risk for obese versus non-obese women was also found for premenopausal women (HR=1.18, 95% CI=0.82–1.70) and postmenopausal women (HR=1.38, 95% CI=1.11–1.71), respectively (120). The relationships between obesity, prognosis, breast cancer subtype, and menopausal status have been less studied, partially due to sparse sample size when stratifying on multiple variables. While some studies did not detect different prognoses by menopausal status (122, 126), Dignam and colleagues limited analysis to ER- breast cancer and found a positive association between obesity and the risk for contralateral breast cancer among postmenopausal patients, but not among premenopausal patients (127). Another study in premenopausal patients detected an association with breast cancer-specific death among TNBC and Her2-positive tumors (both ER-negative subtypes), but not among luminal tumors (66).

While race/ethnicity appears to modify subtype-specific survival (41, 83, 100, 128) and obesity-associated survival (129-132), so far no data have been published evaluating obesity, race/ethnicity, subtype, and breast cancer outcome simultaneously.

1.3. Parity, breast cancer risk, and prognosis

1.3.1. Parity, intrinsic subtype, and breast cancer risk

The association between parity and breast cancer risk has been extensively studied. Parity, narrowly defined as the number of live births a woman has given, has been linked to breast cancer as a protective factor for a long time (133, 134). Later studies show that for each individual birth, the effect of parity on breast cancer risk varies temporally (usually defined as year since birth), with a transiently increased risk in the first 5-7 years after last child birth (135, 136). This transient increase in risk is believed to be caused by stimulation of the malignant cell transformation during breast involution (137-139), but several years after birth there is a long-

term reduction in risk. Reduced lifetime risk in later years after childbirth is believed to be induced by the differentiation of normal mammary stem cells (135, 140).

The heterogeneity of breast cancer is reflected in different relationships between parity and various subtypes. In a recent meta-analysis on the association of parity (measured by number of births) and breast cancer by ER/PR status, parity reduced the risk for ER+/PR+ tumors (relative risk (RR) per birth=0.89, 95% CI=0.84-0.94), but not for ER-/PR- tumors (RR per birth=0.99, 95% CI=0.94-1.05) (11). These results are consistent with the findings of studies on intrinsic subtype. Compared to luminal tumors, high parity and recency of last birth/pregnancy are associated with an increased risk for BLBC/TNBC (69-71, 73, 76, 81, 141). In addition, a positive association between parity and risk of Her2-positive tumors was also observed (76, 85).

Racial/ethnic groups have different distributions of reproductive factors and tumor subtypes, respectively. Results to date suggest quantitative differences by race/ethnicity (142-144), with a stronger protective effect of parity on overall breast cancer risk observed in African American women than white women (144). However, little is known as to whether race/ethnicity adds another layer modifying the relationship between parity and breast cancer subtype.

1.3.2. Parity, intrinsic subtype, and breast cancer prognosis

While epidemiological research has established associations between parity and breast cancer risk, it remains unsettled whether parity has prognostic value in breast cancer. While some studies reported no association between parity and prognosis (88, 145-156), some studies reported that parity, particularly high parity ($n \geq 4$), was an adverse prognostic factor (155, 157-164), and still others showed a better prognosis among parous women (149, 165, 166). Temporal factors such as time since last birth are thought to play a particularly critical role in

breast cancer prognosis. Based on a recent meta-analysis of 30 studies, breast cancer diagnosed within 2 years after birth/pregnancy (usually defined as pregnancy-associated breast cancer) had a higher risk for death (HR=1.44, 95% CI=1.27-1.63) and relapse (HR=1.60, 95% CI=1.19-2.16) (167). The effects of parity on prognosis are likely to be attenuated with time since pregnancy/birth (150, 153, 162, 163, 168-171).

The association between parity and survival seems to vary with the age of the patients. In young patients (definition varies across studies, at most younger than 50 years), nulliparous women appeared to have a better prognosis than parous women (157, 161-163), while an inverse relationship was observed in older patients (>50 years) (157, 172). Meanwhile, some studies have found that the prognostic effect of recent birth was limited to young women, but not older women (152, 154, 157, 162, 163, 171, 173).

ER/PR status has been considered in the studies on the association of parity and breast cancer survival, but usually as a confounder rather than an effect modifier (145, 151, 162, 163, 174-176). So far there are only two studies examining parity associations with survival by breast cancer subtype (160, 171). In the study by Trivers and colleagues among women aged 20-54, the associations of parity and years since last birth with all-cause mortality did not depend upon ER status (effect estimates were not reported) (160). The effect modification of intrinsic subtype was also examined among young Japanese women (age range=20-44), and the adverse effect of recent birth on all-cause mortality was only observed in women with luminal A tumors (0-2 years vs nulliparous, HR=3.07, 95%CI=1.30-7.27) (171). Little is known regarding the role race/ethnicity or menopausal status plays in the association of parity, breast cancer subtype, and prognosis.

1.4. Linkage between risk factors and prognosis

Whether the influence of risk factors on breast cancer etiology is persistent after disease diagnosis is critical to understanding mechanisms and to improve prognosis. The rationale underlying the linkage between risk factors and prognosis in previous studies was based on biological mechanisms, which are similar to those relating risk factors to cancer incidence. Few previous studies characterize these relationships considering the potentially differences in disease development and progression, or assess the differences in breast cancer outcomes by risk factors and by prognosis measures (BC-specific mortality vs overall mortality). In this project, we evaluated the association of obesity and parity with breast cancer prognosis, with an emphasis to address these issues.

Obesity status is dynamically changing and, over the lifespan, cumulative. Most of the evidence of its relationship with breast cancer risk comes from studies in which obesity status was measured close to breast cancer diagnosis. Using this data to evaluate the obesity/breast cancer association assumes that obesity status measured around diagnosis ($obesity_{t1}$) is highly correlated with obesity status during the phase of causal action ($obesity_{t0}$) (Figure 1.1 A). This assumption is reasonable given the previous literature (177, 178). The same assumption is employed in studies of the association between obesity and prognosis. In the discussion below, obesity is conceived as the status at t0 that is measured at t1. As shown in Figure 1.1 A, obesity could influence breast cancer prognosis by altering susceptibility to more aggressive subtypes, or through a pathway other than breast cancer subtype (e.g. through treatment tolerance). Based on this model, after accounting for obesity's influence on subtype susceptibility in analysis stratified by intrinsic subtype, there will be a "residual" association of obesity with breast cancer-specific mortality observed within each stratum due to its direct effect on breast cancer prognosis. The

magnitude of the "residual" association will vary by subtype. For example, since BLBC is the most aggressive subtype with the fewest treatment options, the subtype itself is likely to explain the majority of poor prognosis. Therefore, we would expect that obesity is not associated with breast cancer-specific mortality, which has been indicated in several recently published studies (121-124). These differences in the association among intrinsic subtypes will provide information on the different role of obesity in development and progression of each subtype. Since obesity has been shown to impact numerous diseases, particularly chronic diseases, its association with overall mortality is likely to be greater than BC-specific mortality, particularly in long-term survival (83, 179).

Different risk factors influence breast cancer prognosis by different pathways. In previous studies, the association of number of births/pregnancies with overall mortality/breast cancer-specific mortality disappeared after adjusting for tumor characteristics (147, 151, 157). Therefore, we hypothesize that its association with breast cancer prognosis is only through breast cancer, without a direct pathway (Figure 1.1 B). We will not observe its effect in the analysis stratified by intrinsic subtype, if subtype counts for tumor differences mediating the prognostics effect of parity. Other data suggested that breast cancer-specific mortality yielded similar estimates as all-cause mortality (155, 166), and even stronger estimates in some analyses (158, 160, 166). These data support our hypothesis that parity's effect on breast cancer prognosis is mainly due to its influence on breast cancer, but not deaths due to other diseases.

Unlike the number of births/pregnancies, the association of birth recency with breast cancer outcome remained after adjusting for tumor characteristics and treatment (167). This association with survival suggests that these traditional parameters of tumor characteristics may not appropriately capture the unique properties of pregnancy-related breast cancer. Recent studies

showed TNBC was overrepresented among pregnancy-related breast cancer (141, 176, 180), which suggests intrinsic subtype may work as a more accurate phenotype to describe its underlying properties. In turn, considering intrinsic subtype in the analysis on birth recency and breast cancer prognosis may illuminate the etiology of pregnancy-related breast cancer and better characterize the prognostic effect of parity.

1.5. Limitations

1.5.1. Potential selection bias

Our study examined the association of obesity and parity with breast cancer prognosis by intrinsic subtype among breast cancer patients. Since obesity and parity are risk factors for breast cancer, evaluating their prognostic effect among breast cancer patients may induce selection bias, specifically collider stratification bias, due to conditioning on breast cancer, which is a collider in this context (Figure 1.2) (181). In the study limited to breast cancer patients, unmeasured/unknown confounders (U) of breast cancer and breast cancer prognosis may work as a bypass linking obesity/parity to mortality, resulting in a spurious/distorted association between obesity/parity and mortality (182-186). The magnitude of this bias depends on several factors: (a) the presence of unmeasured/unadjusted factors (U); (b) the prevalence of U in the study population; (c) the effect of U on breast cancer risk in exposed individuals; (d) the effect of U on breast cancer risk in unexposed individuals; and (e) the effect of U on prognosis among breast cancer patients (183, 187).

Since breast cancer-specific mortality is already conditional on breast cancer, collider stratification bias does not apply to the analysis of breast cancer-specific mortality. Here we mainly discuss the influence of this bias on overall survival. Based on the analysis by Glymour and colleagues, selection bias will distort the association only if "U" has a large effect on breast

cancer risk or mortality (in their simulation: RR>2.5) (183). Although a large proportion of breast cancer incidence and mortality cannot be explained by known factors, it is unlikely that unidentified variables with large effects on risk and progression, or highly-prevalent unidentified variables with small effects, exist because breast cancer is such an intensively-studied disease. Most likely, the effects of unknown factors are minor, functioning as white noise without biasing results considerably. Additionally, since the CBCS has minimized unmeasured factors by collecting information on almost all identified predictors of breast cancer incidence and survival, the magnitude of this bias is less likely to be large enough to change our results qualitatively.

Moreover, Figure 1.2 shows that the "U" has to be a risk factor for breast cancer and also influences overall survival through diseases other than breast cancer. Therefore, this factor would have to be a common cause of two diseases, one of which is breast cancer. Concurrence with a second lethal disease is not common for young women with breast cancer, but may exist for older patients. In addition, long follow-up may provide enough time to develop a second lethal disease with common causes. Therefore, in our proposed project, the results of older women and long-term survival are more likely to be influenced by collider stratification bias, although the bias likely will not be large.

1.5.2. Exposure misclassification

In the proposed study, we assume that obesity and parity status do not change after the diagnosis of breast cancer. This assumption may not hold since many studies have observed weight change due to treatment, age, and lifestyle factors (188, 189). It was estimated that 50-96% of breast cancer patients experienced weight gain (188). Since we only have information on BMI around the time of diagnosis, our results may be biased by weight changes during follow-up. An analysis of 12,915 breast cancer patients from four prospective cohorts found that the

mean weight change was 1.6 kg during a follow-up averaging 8.1 years (189). This weight change is not likely to cause a considerable proportion of patients to change their obesity category. Moreover, we adjusted for key factors associated with weight gain in our analysis, such as age, menopausal status, tumor stage, and SES, to minimize this information bias.

Compared to obesity, parity is less likely to change after breast cancer diagnosis. Hartmen and colleagues reported that the birth rate among young breast cancer survivors (≤45 years) was about half of the general population (standardized birth ratio=0.52, 95% CI=0.47-0.57) (190). Therefore, the static assumption is reasonable for parity.

1.5.3. Subtype misclassification

Although intrinsic subtype has largely improved the categorization of breast cancer heterogeneity, the subtypes defined by the IHC approach in CBCS are similar, but not identical, to intrinsic subtypes by cDNA microarray. Therefore, misclassification of subtype is unavoidable, particularly for luminal A and B tumors. In CBCS, the definition by Nielsen and colleagues (55) for luminal B did not identify all luminal B tumors as only 30-50% of this subtype expressed Her2 receptor, which leads to some luminal B tumors being classified as luminal A. This misclassification may result in the observed prognosis of luminal A tumors being worse than it should be (41). The IHC-based definition of luminal B has been updated recently as tumors that are (a) ER-positive and Her2-negative and (Ki-67 ≥ 14% or PR ≤ 20%), or (b) ER-positive and PR-positive and Her2-positive (35, 57). In the proposed project, to avoid the bias due to this misclassification, we combined luminal A and B tumors as a single luminal subtype.

In 2010 the American Society of Clinical Oncology (ASCO) lowered the IHC cutoff for determining ER positivity from the previous value of 10% to 1% (191). The information of ER

positivity in the CBCS Phases I and II was from clinical records, where cutoffs ranged from more than 0% to more than 20%. Therefore our intrinsic subtyping may classify some luminal tumors, defined by the updated cutoffs, as Her2-positive or basal-like tumors. This misclassification will dilute the differences among subtype, and consequently may cover the modification of subtype on the associations of obesity and parity with breast cancer prognosis.

1.5.4. Others

CBCS Phases I and II do not have treatment information. Treatment of breast cancer is determined by both physician recommendation and patient preferences. Physician recommendation is mainly based on comprehensive consideration of disease-related factors (e.g. tumor stage and hormone receptor status) and patient-related factors (e.g. age, co-morbidities) (192, 193). Patient preference is mainly influenced by education, culture, and income (194, 195). CBCS includes information on most of these determinants. In the proposed project, adjusting for tumor and patient characteristics could minimize this potential bias. The development of trastuzamab and other Her2-targeted agents in recent years has improved prognosis of Her2-positive tumors (102). The CBCS was conducted prior to the introduction of trastuzumab and other Her2-targeted agents, which decreases the bias due to temporal changes in therapy.

In this study, we considered race and menopausal status as potential effect modifiers. Too many stratification variables may result in sparse cell counts and low statistical power to detect associations. In CBCS, the sample size of Her2-positive tumor is small (n=116, 8% of CBCS subjects). Therefore, we may have low power to get precise estimation for this subtype.

1.6. Summary

Breast cancer is a heterogeneous disease with multiple intrinsic subtypes having different risk factors and prognoses. Obesity and parity, two factors that have consistently different associations with different intrinsic subtypes, have been linked to breast cancer prognosis in previous studies. However, little is known whether these relationships are modified by intrinsic subtype. This proposed project is the first to investigate the associations of parity and obesity with breast cancer prognosis by intrinsic subtype. Our results will contribute to characterization of different pathways linking risk factors to prognosis, and provide important insights to optimize treatment and management strategies in breast cancer patients and ultimately improve breast cancer survival.

Table 1.1: Obesity and breast cancer survival by intrinsic subtype

Author (year)	Study design	Population	Obesity	Subtype	Results
Mazzarella (2013) [a]	Hospital-based study in Italy	1250 non-metastatic Her2+ BC before Trastuzumab use. 43% 35-50 years; 41% 51-65 years. Median FU=8.2 years.	BMI at diagnosis		Obese vs under/normal
				Her2+/ER+	Recurrence risk: HR=0.75, 95% CI=0.43-1.31 Overall mortality: HR=1.05, 95% CI=0.53-2.09
				Her2+/ER-	Overall mortality: HR=1.79, 95% CI=1.03-3.10 Recurrence risk: HR=1.34, 95% CI=0.84-2.13
Turkoz (2013) [b]	Hospital-based study in Turkey	733 non-metastatic premenopausal BC. Mean age=40.13 years. Median FU=2.4 years	BMI at diagnosis		Obese vs normal
				TNBC	BCSM: HR=1.4, 95%CI=1.0-2.1, p=0.04
				Her2+	BCSM: p=0.037. HR and 95% CI not provided
				Luminal	BCSM: p=0.40. HR and 95% CI not provided
Mowad (2013) [c]	Medical center-based study in US	183 stage 1-3 TNBC. Mean age=49.8 years. Median FU=3.54 years. 69% AA	BMI at diagnosis		Not clearly described the reference level. Likely obesity level used as ordinal variable
				TNBC	Overall mortality: HR=1.36, 95% CI=0.77-2.42 Recurrence risk: HR=1.01, 95% CI=0.67-1.52
Dawood S (2012) [d]	Hospital-based study in US	2311 stage 1-3 TNBC. ~50% <50 years. Median FU=3.25y. ~20% AA.	BMI at diagnosis		Obese vs over-weighted:
				TNBC	Overall mortality: 1.00, 95% CI=0.83-1.20 Recurrence risk: 1.09, 95% CI=0.92-1.29

Table 1.1: Obesity and breast cancer survival by intrinsic subtype

Author (year)	Study design	Population	Obesity	Subtype	Results
Sparano JA (2012) [e]	Data from adjuvant treatment trials in US	4770 stage 1-3 BC. Age ranged 22-84. Median FU= 7.9 years. ~85% white.	BMI at diagnosis		Obese vs normal
				Luminal	Overall mortality: HR=1.37, 95% CI=1.13-1.67 Recurrence risk: HR=1.24, 95% CI=1.06-1.46
				Her2+	Overall mortality: HR=0.99, 95% CI=0.73-1.34 Recurrence risk: HR=1.06, 95% CI=0.82-1.38
				TNBC	Overall mortality: HR=1.11, 95% CI=0.85-1.46 Recurrence risk: HR=1.02, 95% CI=0.80-1.30
Ademuyiwa FO (2011) [f]	Hospital-based study in US	418 stage 1-3 TNBC. Mean age=55 years. Median FU=3.1 year. 80% white.	BMI at diagnosis		Obese vs normal:
				TNBC only	Overall mortality: HR= 0.94, 95% CI=0.54-1.64 Recurrence: HR= 0.81, 95% CI=0.49-1.34

[a] Adjusted for age at diagnosis, menopausal status, number of positive lymph nodes, tumor size, grade, percent of estrogen receptor-positive cells (as a continuous variable), perivascular invasion and type of surgery.
[b] Adjusted for age, tumor size, nodal involvement, grade, lymphvascular and perineural invasion, extracapsular extension and hormonal status.
[c] Not explicitly described in the text. Likely the HRs were adjusted for age, race, tumor grade, T stage, N stage and PMRT (postmastectomy radiotherapy).
[d] Adjusted for age, race, stage of disease, lymphovascular invasion, systemic adjuvant treatment, and radiation therapy.
[e] The HRs were adjusted for age, race, premenopausal vs other, tumor size, axillary nodal status, surgery, use of radiation therapy, and use of systemic therapy.
[f] Adjusted for age at diagnosis, race, receipt of chemotherapy, year of diagnosis, grade, histology, stage, and presence of lymphovascular invasion

Figure 1.1: Hypothesized associations between obesity, parity, intrinsic subtype, and breast cancer prognosis

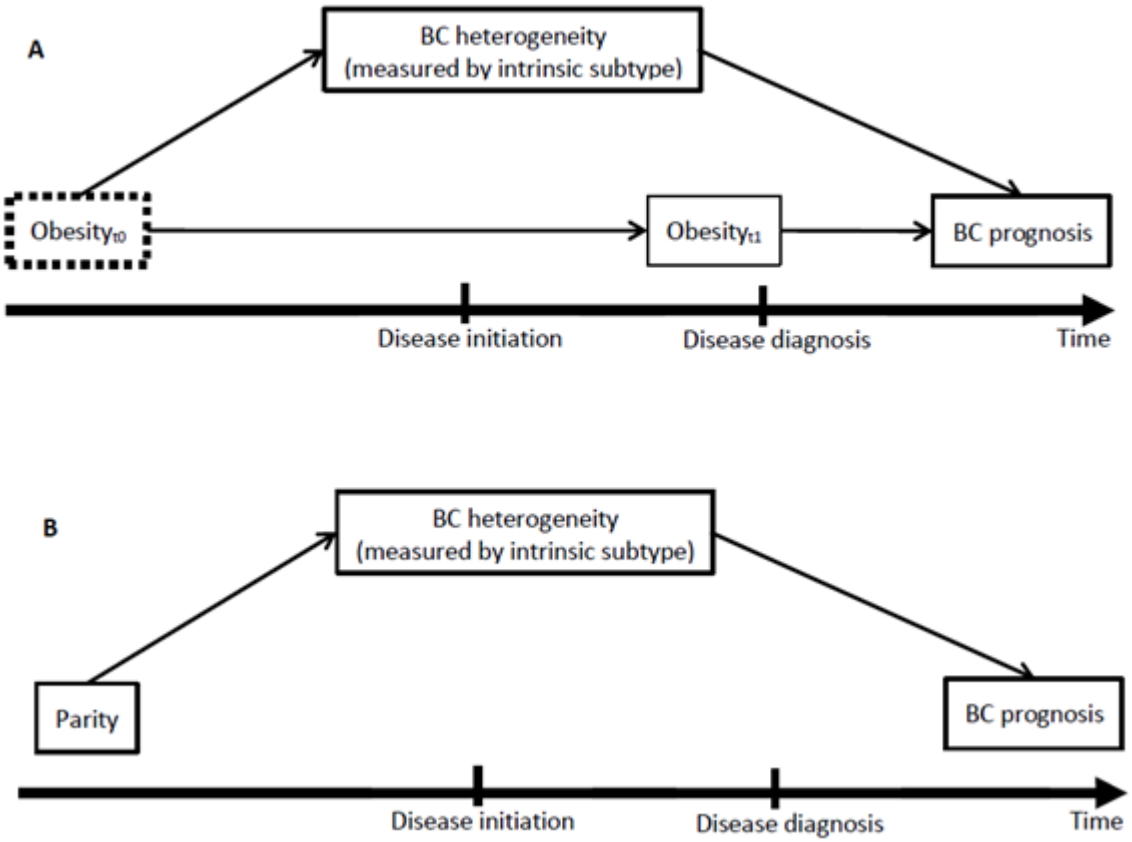
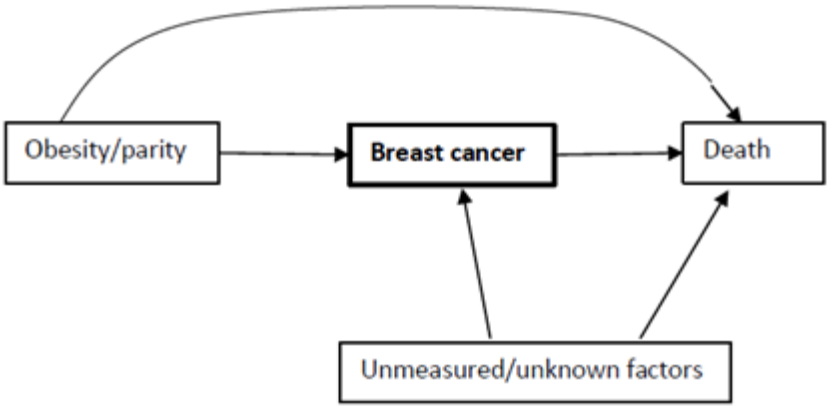


Figure 1.2: Collider stratification bias



CHAPTER 2: SPECIFIC AIMS

During the past ten years, the understanding of heterogeneity of breast cancer has been improved by global gene expression analyses. Several intrinsic subtypes have been identified, including luminal A, luminal B, Her2-enriched, and basal-like (25, 26, 196). These breast cancer subtypes show significant differences in risk factor profiles (41, 73, 76, 77, 141), as well as prognosis (41, 88, 97, 100, 197, 198). The recognition and improved classification of intrinsic subtype have been adding new insights to breast cancer research.

Whether breast cancer risk factors influence prognosis has great public health significance. However, findings have been mixed (88, 114, 115, 157, 158). Moreover, few studies have considered the role of breast cancer heterogeneity in the analysis. In this project, we studied obesity and parity, two risk factors that have consistently shown distinct association with breast cancer subtype and also have been suggested to affect breast cancer outcomes.

Aim1: To evaluate whether obesity is associated with overall mortality and breast cancer-specific mortality, considering intrinsic subtype as an effect modifier. We hypothesized that obesity was associated with higher overall and breast cancer-specific mortality among all breast cancers, but the magnitude of the association varied by subtype. Obesity was evaluated by body mass index (BMI, based on anthropometric information self-reported prior to diagnosis and measured after the diagnosis during interview), and waist-to-hip ratio (WHR, based on anthropometric information measured after the diagnosis during interview).

Aim2: To evaluate whether parity is associated with overall mortality and breast-cancer specific survival, considering intrinsic subtype as an effect modifier. We

hypothesized that parity was associated with higher overall and breast cancer-specific mortality among all breast cancers, but the magnitude of this association varied by subtype. Parity was described by number of full-term births and time since last birth.

The two parallel aims were addressed using the cases from the Carolina Breast Cancer Study (CBCS) (Phases I and II). Vital status of included cases was ascertained through the National Death Index. Cox regression methods was used to estimate adjusted hazard ratios (HR) and 95% confidence interval (CI) in all cases and stratified by breast cancer subtype. Potential effect modification by race and menopausal status was also evaluated.

CHAPTER 3: RESEARCH METHODS

3.1. Population and participants

3.1.1. Carolina breast cancer study

The CBCS is a population-based case-control study aimed at identification of genetic and environmental causes of breast cancer among African American and white women from North Carolina (41, 76, 199, 200). The CBCS study area included 24 counties (including suburban, small town, and rural areas) in eastern and central parts of the state, with over-sampling of younger and African American women. In this project, we included CBCS patients with invasive breast cancer who were recruited in the first two phases, Phase I (1993-1996) and Phase II (1996-2001).

Breast cancer patients were identified from the North Carolina Central Cancer Registry using rapid case ascertainment. Eligible patients were those who were newly diagnosed for a first primary breast cancer between May 1, 1993 and December 31, 2001, were aged 20-74 years at the time of diagnosis, and resided in the 24 counties. Among eligible patients, cases were selected using randomized recruitment with predetermined probabilities, with the aim to balance representation by age (<50 y vs. ≥ 50 y) and race (African Americans vs. whites) to further improve the statistical validity of comparisons among these subgroups. Under this strategy, the following sampling fractions were used: 100% of younger (defined as 20-49 years) African American women, 75% of older (defined as 50-74 years) African American women, 67% of younger white women, and 20% of older white women (200). Other than the oversampling of younger and African American women by design, the CBCS population is approximately

representative of cases reported to the North Carolina Central Cancer Registry in the study region during the study period (200).

For recruitment prior to patient contact, a letter was sent to the physician providing cancer care requesting permission to invite the woman to participate in the study. Potential participants with physician permission were contacted first by letter and then by a telephone call. If a woman agreed to participate, an appointment was scheduled for an in-person interview at the woman's home or other agreed-upon location. Home visits and interviews for cases and controls were conducted by registered nurses and interviews lasted about 1-1.5 hours. The interviewers were matched with patients on race for those aged 50 years or older. Interviewers administered a structured questionnaire that included detailed information about family history of cancer and reproductive history, including age at menarche, age at first full-term pregnancy, number of children, breastfeeding, age at menopause, oral contraceptive use, and use of hormone replacement therapy. Body measurements including waist and hip circumferences and weight were obtained at the time of the interview. For cases, consent for retrieving tumor tissue, pathology reports, and medical documentation was obtained at the time of interview.

The CBCS Phases I &II included 1,803 invasive breast cancer cases (787 African American and 1,016 white women). The overall contact rate (contacted/eligible) and the cooperation rates (enrolled/contacted) of invasive cases were 97.6% and 78.0% respectively. The overall response rate (product of contact and cooperation rates) was 76.0%, with subgroups of patients ranging from 69.9% for African Americans aged 50 years or older to 81.2% for whites less than 50 years old (201, 202). Compared with women who participated in the CBCS, nonparticipants were more likely to be of lower socioeconomic status, to have a lower educational level, and to have a recent history of unemployment (202).

All tumor blocks were processed at the University of North Carolina (UNC) SPORE Core Tissue Procurement Analysis Facility in Chapel Hill, NC. Approval for release of formalin-fixed, paraffin-embedded tumor tissue blocks was obtained for 94% of cases. Patients with smaller or early-stage tumors were less likely to provide blocks because they were either unavailable or had insufficient tissue for subtype analysis. The Hematoxylin and Eosin (H&E)-stained slides were produced from each of the paraffin-embedded blocks and reviewed in a standardized fashion by the study pathologist who was blinded to the demographic characteristics of participants. The pathologist confirmed the diagnosis of breast cancer, assigned a histologic classification, and described tumor features in more detail (200). All tumors were graded according to the Nottingham modification of the Scarff–Bloom–Richardson system (203), taking into account tubule formation (architectural grade), pleomorphism (nuclear grade), and mitotic activity (mitotic grade). In 6 % of the cases, the grades could not be properly determined, usually due to an insufficient amount of diagnostic tissue or poor histology.

The current project was limited to 1,140 invasive breast cancer patients with available information on intrinsic subtype. In obesity analysis, the sample size was 1,109 after additionally excluding patients without information on BMI or WHR.

3.1.2. Data acquisition

Permission to use the data was obtained from the former principal investigator of CBCS, Dr. Robert Millikan and current principal investigator Dr. Andrew Olshan has subsequently confirmed permission to use the data. Additionally, IRB approval was obtained for analyzing the data.

3.2. Data analysis

3.2.1. Exposure assessment and categorization

Obesity

Obesity was measured by BMI and WHR to reflect different types of obesity. The information on height and body weight was self-reported prior to diagnosis and measured after the diagnosis during interview. BMI was computed by dividing the weight in kilograms by the square of the height in meters. The World Health Organization definition is used to classify patients as underweight (BMI <18.5 kg/m^2), normal (BMI 18.5-24.9 kg/m^2), overweight (BMI 25-29.9 kg/m^2), and obese (BMI ≥30 kg/m^2). In our analysis, the "underweight'' and ''normal" BMI classes was combined as one group. Waist and hip circumferences were only measured at the time of interview. WHR was calculated as the ratio of waist to hip circumference (cm) and used as a measure of abdominal adiposity. Cut points for WHR were tertiles (two cutoff points: 0.77 and 0.83) based upon the distribution in controls (76). The lowest obesity level was used as the reference in the analysis.

Parity

Parity was evaluated as both number of full-term live births and recency of last birth. Information on both variables was collected during interview. Number of full-term live births was grouped into three categories: nulliparous (reference), 1-2 children, and ≥3 children. Recency of last birth was calculated as the year of diagnosis minus the year of the last birth, and was grouped into four categories: 0 (reference), <5 years, 5-<10 years, and ≥10 years.

3.2.2. Breast cancer subtype assessment and definition

The CBCS used the most frequently-used IHC classification criteria developed by Nielsen and colleagues (55), where luminal A tumors are defined as ER+; either PR+ or PR-; and Her2-. Luminal B tumors are defined the same as luminal A for ER and PR but differ in positive Her2 expression. Her2-positive tumors are defined by the lack of expression of ER and PR and

the presence of HER2 receptor (ER-/PR-/Her2+). BLBCs are defined by the lack of ER, PR and Her2, and the expression of CK5/6+.

To evaluate ER/PR status, tumor blocks were sectioned and stained for a panel of IHC markers at the Immunohistochemistry Core Laboratory at UNC-Chapel Hill. ER and PR status were obtained from medical records for 80% of invasive cases. For ER/PR information from medical records, the status was determined in various clinical laboratories, the vast majority using an IHC method with cutoffs for receptor positivity ranging from more than 0 to more than 20 percent for assays performed on paraffin-embedded tissues (about half) and of 10 or 15 fmol/mg for assays performed on frozen tissues (about half). For 11% of the cases with missing status for ER/PR on medical records, ER/PR status was determined at the UNC laboratory. ER/PR status was missing for the remaining 9 percent of the cases (41, 200, 204). The staining of Her2 was categorized using a 0 to 3 scoring system, and assignment of Her2 positivity was defined as any Her2 staining (41, 204). CK 5/6 was scored positive if any cytoplasmic and/or membranous staining was seen (200, 205). Compared to cases excluded from subtype analyses, cases included in subtype analyses were less likely to be stage I (39% vs. 48%) and more likely to be stage II (51% vs. 39%), with little differences observed in stage III (8% vs. 10%) or stage IV (3% vs. 4%). There were no differences between the included and excluded cases in age, race, menopausal status, lymph node status, nuclear grade, histologic grade, or survival (41, 76, 201).

3.2.3. Breast cancer-specific survival and overall survival assessment

The National Death Index provided vital status and dates of deaths on the CBCS cases through December 31, 2011. Deaths among cases were determined using weighted probabilistic scores and predetermined matching cutoffs to establish a maximum of 1 match per individual. These data were derived from death certificates and included all causes of death for overall survival and breast cancer-specific survival with high sensitivity (98%) and specificity (100%) (206). International Classification of Diseases (ICD) breast cancer codes 174.9 (ICD-9) or C50.9 (ICD-10) were used to identify deaths due to breast cancer on the death certificate. The main outcome of interest in our survival analysis is time to death, which is defined as the number of years between breast cancer diagnosis and death from breast cancer or any cause. Women alive at the end of follow-up or the last known follow-up date were considered censored in overall mortality analyses. In the cause-specific mortality analyses, women who died from causes other than breast cancer were additionally counted as censored.

3.2.4. Effect modification

Besides intrinsic subtype, race and menopausal status were considered as potential effect modifiers. Race was based on self-report at the time of in-person interviews. The two races included in this project were White and African American. Menopausal status was determined using information from the interview. Women younger than 50 years who had undergone natural menopause, bilateral oophorectomy, or irradiation to the ovaries were classified as postmenopausal; otherwise they were classified as premenopausal. For women aged 50 or older, menopausal status was assigned based upon cessation of menstruation. Considering that there is no standard definition for menopausal status and the small sample size of perimenopausal women (n=95), perimenopausal women were excluded to avoid misclassification (n=95).

3.2.5. Confounding

Potential confounders were selected based on the available literature and conceptual diagrams/directed acyclic graphs (DAG) as shown in Figure 3.1 and Figure 3.2**.** Reproductive factors other than parity (breastfeeding, age at first menarche, age at first birth, and age at last birth) were not be considered as confounders because they are not up-stream factors and literature review suggested no association between these reproductive factors and breast cancer prognosis. Although tumor characteristics were not up-stream factors of obesity/parity, they were adjusted in the model building process to account for fundamental differences in tumors not captured by intrinsic subtype, and also to minimize bias due to the missing information on treatment. More details on measurement and definition of potential confounders are listed in **Table 3.1**.

3.2.6. Statistical methods

The associations of obesity/parity with prognosis were evaluated separately. Survival curves by obesity/parity categories were be generated using the Kaplan-Meier method. The log-rank test was used to test whether there was a difference between categories in the probability of death at any time. To estimate the size and precision of differences in survival between obesity/parity categories, we performed univariate Cox regression to estimate hazard ratios and corresponding confidence intervals (obesity: BMI<25kg/m^2 and the first tertile of WHR as reference categories; number of live birth and birth recency: nulliparous as references). The interpretation of a HR was the relative risk of death comparing those who were exposed to some characteristic to those who were not, over the entire study period.

Kaplan-Meier survival curves, log-rank tests, and Cox regression are based on the assumption that censoring is non-informative and unrelated to prognosis. In other words, the

people who are censored at some point in time are representative of those subjects who survive up to that point in time, conditional on explanatory variables (207, 208). This assumption of censoring was unlikely to be violated in the CBCS, as patients entered the study based on their date of diagnosis, and date and cause of death were obtained from the National Death Index without knowledge of obesity/parity. Therefore, in this proposed project, it was unlikely that informative censoring was a large source of bias. Another important assumption for log-rank tests and Cox regression is that the ratio of survival probabilities between compared groups does not depend on time. This assumption was evaluated using Kaplan-Meier survival curves and log-log plots of survival. In addition, a separate model was used with the inclusion of a time-dependent cross-product term for the natural log of survival time (days) and the covariate of interest. The assumption was considered to hold if the interaction term is not significant at $p \geq 0.05$.

Effect modification by intrinsic subtype was initially assessed by examining stratum-specific estimates. It was also evaluated by including the product interaction terms. If the *p* value for the likelihood ratio test comparing models with and without the interaction term(s) was <0.10, then effect modification was considered significant on a multiplicative scale. The results of stratified analyses were presented no matter how intrinsic subtype tests as a significant effect modifier. The effect modifications of race and menopausal status were also evaluated only within luminal and basal-like tumors using similar approaches.

Variables listed in Table 3.1 were examined as potential confounders. Models were built based on types of potential confounders: modeling started with exposure, age, race, and study phase; proceeded to adjustment for identified potential confounders; and finally included adjustment for tumor characteristics.

Table 3.1: Measurement and definition of potential confounders[a]

Variables	Measurement	Code in statistical analysis
Age at diagnosis	Collected during interview	Continuous and categorical (<40, 40-49, 50-59, or ≥60)
Parity[b]	Collected during interview	0, 1-2, 3+
Family history of breast cancer (first degree)	Collected during interview	Categorized as yes or no
Education	Collected during interview	Categorized as high school and post high school, college and above, or lower than high school
Family income	Collected during interview	Categorized as 15-30K, 30-50K, >50K, or <15K.
Smoking[b]	Collected during interview	Categorized as never, former, or current
Physical activity[b]	Collected during interview	Categorized as yes or no
HRT	Collected during interview	Defined as any hormone replacement therapy and categorized as never user, current user, or past user
OC	Collected during interview	Categorized as never or ever, where ever user was defined as 3+months of OC use before BC diagnosis.
Lymph node status	Extracted from medical record	Categorized as positive or negative
Tumor stage (Nottingham)	Extracted from medical record	Based on the American Joint Committee on Cancer (AJCC, 5th edition), categorized as I, II, or III+IV
Nuclear grade	Extracted from medical record	Categorized as marked pleomorphism or slight/moderate
Histologic grade	Evaluated in participating hospitals based on the H&E slides prepared at UNC	Categorized as poorly differentiated, or well/moderately differentiated
Histology group	Evaluated in participating hospitals based on the H&E slides prepared at UNC	Categorized as ductal or others
Mitotic index	Extracted from medical record	Categorized as high if index is greater than 10 mitotic figures per 10 high-power fields; otherwise as low

[a] The definition of race and menopause was described in 4.2.4.
[b] Considered as potential confounders in Aim 1 (obesity association).

Figure 3.1: Diagram illustrating associations of obesity, intrinsic subtype, breast cancer outcome, and other related factors

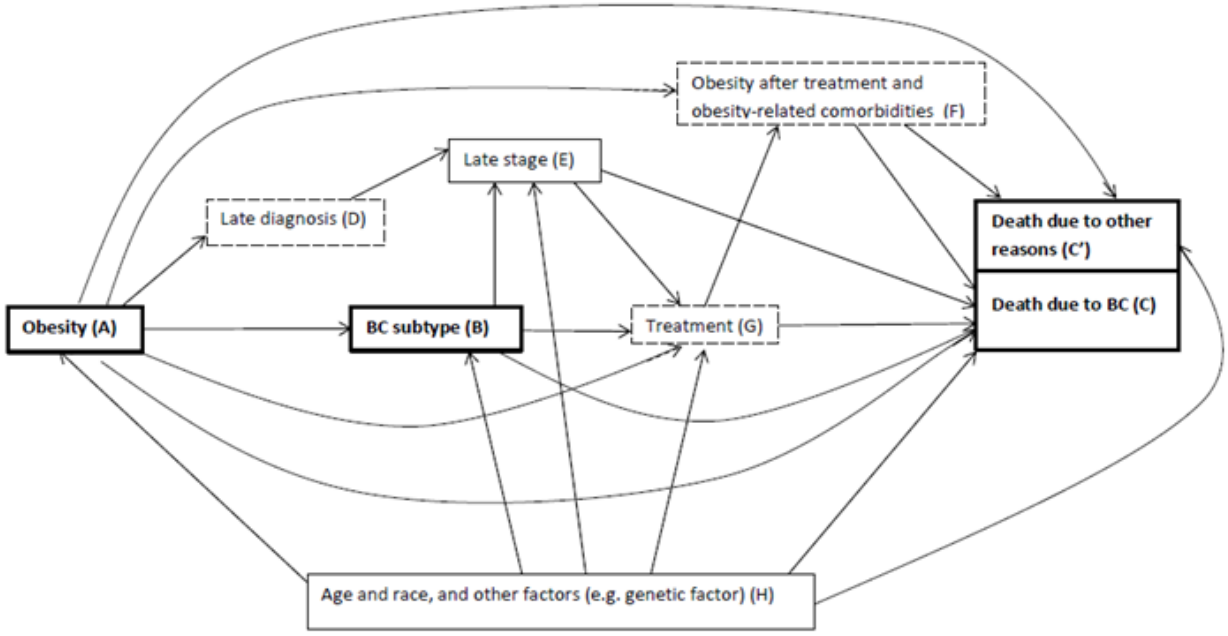
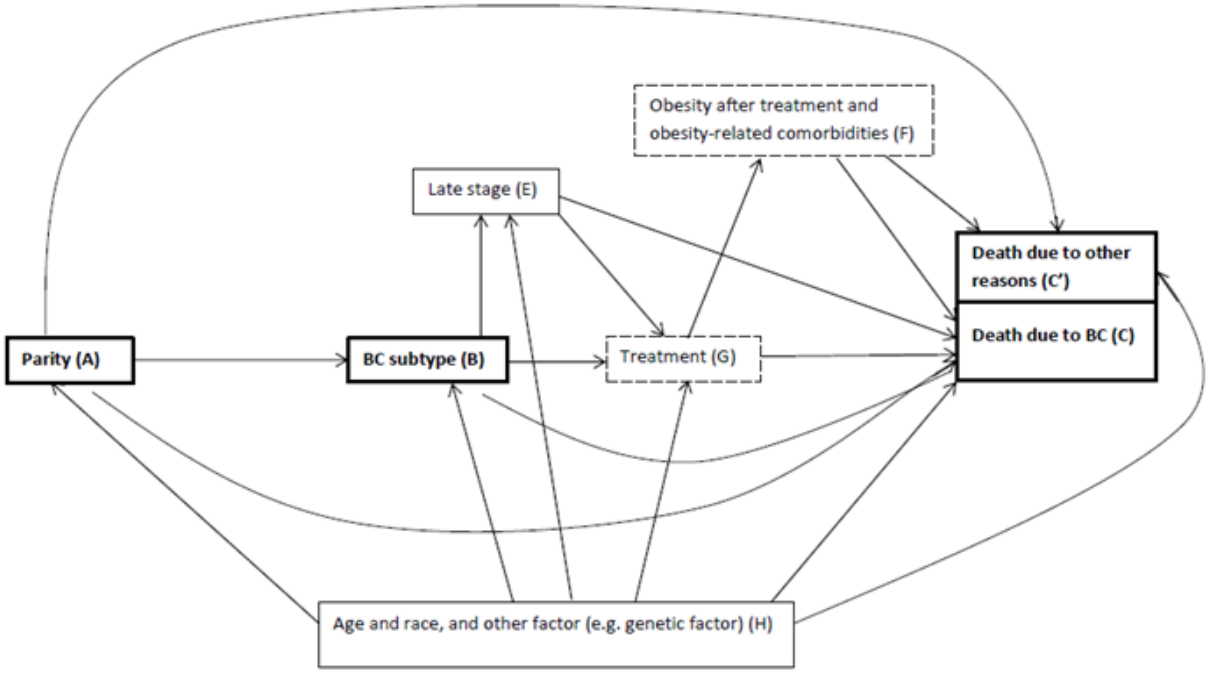


Figure 3.2: Diagram illustrating associations of parity, intrinsic subtype, breast cancer outcome, and other related factors



CHAPTER 4: PARITY AND BREAST CANCER SURVIVAL

4.1. Background

Reproductive history is an important established determinant of breast cancer risk. Increased appreciation of etiologic heterogeneity in breast cancer has added complexity to our current understanding of the associations. Risk of basal-like breast cancer and triple-negative breast cancer is suggested to increase with multiple births and recency of last birth/pregnancy (69-71, 73, 76, 81, 141), while risk of luminal tumors follows patterns established for breast cancer overall. These results suggest that pregnancy and associated events may have contrasting mechanistic effects, including increasing short term risk for some intrinsic molecular tumor subtypes, while providing long-term protection against others.

The proposed biological mechanisms linking parity and increased basal-like/ triple-negative breast cancer risk include increased hormonal stimulation, expansion of stem/progenitor cells, growth stimuli, and pro-inflammatory and wound-healing changes in microenvironment during breast involution (137-139, 209, 210). These mechanisms could influence both risk and prognosis, although the latter issue has not been well studied.

It remains unsettled whether parity has prognostic value in breast cancer. While some studies reported no association between number of births and prognosis (88, 145-156), other studies reported that multiple births was associated with a poorer prognosis (155, 157-164), and still others showed improved prognosis among multiparous women (149, 165, 166). These discrepancies may be attributed to different distributions of potential effect measure modifiers

such as race and menopausal status, and a different profile of the intrinsic subtype across study populations. Results regarding time since last birth are relatively consistent. Recent birth appears to be associated with poor outcome among breast cancers overall (157, 167, 170, 171). Little is known whether the influence of recent birth on prognosis varies by subtype.

Using data from the Carolina Breast Cancer Study (CBCS), a large population-based case-control study, we assessed the impact of multiparity and recent birth, on overall and breast cancer (BC)-specific survival. These associations were evaluated among breast cancers as a whole and in strata defined by specific breast cancer subtypes (basal-like and luminal).

4.2. Methods

4.2.1. Study population

The CBCS is a population-based case-control study, the details of which have been described previously (76, 200). Briefly, a total of 1,808 patients aged 20-74 years diagnosed with primary invasive breast cancer during 1993-1996 (Phase I) and 1996-2001 (Phase II) were identified using rapid case ascertainment from NC Central Cancer Registry, with African American and young cases (aged 20-49 years) oversampled using randomized recruitment (200, 211). Participants were interviewed in person within 1 year of the diagnosis by trained nurses who collected anthropometric measurements and questionnaire responses. Clinicopathological information was abstracted from clinical records and pathological reports. The study procedures for recruitment and enrollment into the CBCS were approved by the Institutional Review Board of the University of North Carolina (UNC). All study participants gave written informed consent.

4.2.2. Breast cancer subtype classification

The details of breast cancer subtyping have been published previously (41, 76). Briefly, whole, formalin-fixed paraffin-embedded tumor tissues were sectioned and stained for a panel of immunohistochemical (IHC) markers in the IHC Core Laboratory at UNC. The following markers were used to determine breast cancer intrinsic subtypes: luminal A (ER+ and/or PR+, HER2-), luminal B (ER+ and/or PR+, HER 2+), basal-like (ER-, PR-. HER2-, HER1+ and/or cytokeratin 5/6+), HER2-enriched (ER-, PR-, HER2+), and unclassified (negative for all five markers). We combined luminal A and luminal B as luminal tumors due to the small number of luminal B tumors (n=111) and, more importantly, recent revisions to the IHC definition of luminal B (35, 57). Luminal A and B tumors cannot be reliably distinguished without additional markers (such as Ki-67) or nanostring data (212). In the CBCS, the demographic and tumor characteristics in patients with luminal A and B tumors were comparable except luminal B tumors more likely to be lymph node positive (p=0.01).

4.2.3. Exposure and outcome assessment

Parity status was evaluated as number of full-term births. Recency of last birth was calculated as the year of diagnosis minus the year of the last full-term birth. Their values were 0 for nulliparous women.

Linkage with the National Death Index provided vital status, dates of deaths, and cause of death on the CBCS cases through December 31, 2011. Deaths among cases were determined using weighted probabilistic scores and predetermined matching cutoffs to establish a maximum of 1 match per individual (206). International Classification of Diseases (ICD) breast cancer codes 174.9 (ICD-9) or C50.9 (ICD-10) were used to identify deaths due to breast cancer on the death certificate.

4.2.4. Statistical analysis

The current analysis was limited to 1,140 African American or White patients (9 other race cases excluded) with available information on intrinsic subtype (659 cases without subtype information excluded), parity, and birth recency. The demographic and tumor characteristics of the excluded cases were compared with those of the included cases; no significant differences were detected, except that excluded cases were less aggressive (more likely to have negative lymph node status, tumor size ≤ 2cm, and stage I). After referring categorization of previous studies (76, 160, 171) and the distribution in this study population, number of full-term live births was grouped into three categories: nulliparous, 1-2 births, and ≥3 births. Birth recency was grouped into four categories: nulliparous, <5 years, 5-<10 years, and ≥10 years. To describe the characteristics of the study population, the distribution of age at diagnosis, menopausal status, race, BMI, WHR, family history of breast cancer, education, family income, smoking, alcohol intake, physical activity, the usage of hormone replacement therapy (HRT) and oral conceptive (OC), lymph node status, intrinsic subtype, tumor size, tumor stage, histology type, nuclear grade, histologic grade, and mitotic index, were evaluated by multiparity and birth recency categories by Chi-square test or Student's t-test (Table A.1 and Table A.2). The assessment and definition of these variables have been described previously (76). Patients living as of December 31, 2011 were censored, and those who died of causes other than breast cancer were censored for BC-specific analysis. Kaplan-Meier survival curves and log-rank tests were used to compare the difference in overall and BC-specific survivals by multiparity and birth recency.

Cox regression analysis was used to estimate hazard ratio (HR) and 95% confidence interval (CI) for overall death and BC-specific death, with nulliparous as the reference. Model

adjusted for study design factors (including age, race, and study phase) was considered as the primary model. Then education and family income were adjusted as potential confounders based on selection with the aid of a directed acyclic graphs using on *a priori* knowledge. Lastly, tumor characteristics, including tumor stage, tumor size, lymph node status, and histological type, were additionally adjusted to evaluate the influence of other prognostic factors on the associations of interest.

Stratified analyses were performed to evaluate the effect modification by intrinsic subtype. Only basal-like and luminal strata are presented because unclassified tumors are of biologically uncertain subtype and because too few patients (n=73) were HER2-enriched for stable estimation. The difference in the hazard ratios by race and menopausal status within luminal and basal-like tumors was also assessed. In the analysis by menopausal status, perimenopausal women were excluded to avoid misclassification (n=95). In addition, because studies have suggested that factors predicting survival in early years after diagnosis may differ from those in later years (e.g. with tumor biological and pathologic characteristics dominant in early years and lifestyles dominant in later years (213)), analyses were conducted conditional on follow-up length: data were truncated at five years to evaluate five-year mortality and then survival was assessed conditional upon surviving the first five years. Exploratory analyses were conducted to characterize the dose-response relationship of multiparity and birth recency with mortality. In addition, to assess the potential interactions of breastfeeding-multiparity or multiparity-birth recency, compound variables were created and their corresponding HRs were calculated (breastfeeding-multiparity: nulliparous, 1-2 births and ever breastfed, 1-2 births and never breastfed, 3+ births and ever breastfed, and 3+ births and never breastfed; multiparity-birth

recency: nulliparous, 1-2 births and last birth <10 years, 1-2 births and last birth ≥ 10 years, 3+ births and last birth <10 years, and 3+ births and last birth ≥ 10 years).

The proportional hazards assumption in each Cox model was assessed using log-log plots of survival and time-dependent cross-product terms of the survival time (years) and the variables of interest, and showed no violation of the assumptions. All statistical tests were two sided with α=0.05, all analyses were performed using SAS version 9.2 (SAS Institute), and all figures were generated using R 3.0.0.

4.3. Results

4.3.1. Patient and tumor characteristics

Among 1,140 breast cancer patients in this study, the average age at diagnosis was 51 years (SD=11.5 years, range=23-74 years). Approximately half of patients were African American (45%) and premenopausal (49%) per the sampling strategy of CBCS. A total of 967 (85%) women were parous, among which 416 (43%) had 3 or more births. 165 (17% of parous patients) had last full term birth within 10 years of breast cancer diagnosis. The patient demographics by multiparity and by birth recency were detailed in Table A.1 and Table A.2, respectively. Compared with nulliparous patients, patients with high parity (3+ births) were significantly older, and were more likely to be African American, obese (BMI≥30 kg/m^2), lower socioeconomic status (SES) (measured by education and family income), alcohol abstainers, and non-OC users. Patients with high parity (3+ births) also tended to have last birth more than 10 years previous to diagnosis. Consequently, birth recency was associated with similar characteristics as multiparity. Additionally, patients who had given birth 10 years more before breast cancer diagnosis were more likely to be smokers and HRT users than patients with last birth within 5 years.

Luminal tumors comprised the majority of breast cancers (n=731, 64%), followed by basal-like tumors (n=205, 18%), unclassified (n=131, 11%), and HER2-enriched tumors (n=73, 6%). Compared with nulliparous patients, parous patients were more likely to have basal-like (frequency was highest in women with birth within 5-<10 years) and lymph node positive tumors (Table A.1 and Table A.2). Among parous patients, lymph node positive and poorly differentiated tumors were more frequent in women with recent birth (<5 years).

4.3.2. Associations of multiparity and birth recency with prognosis

The median follow-up time was 13.5 years, ranging from 0.2 years to 18.7 years. By the end of follow-up (December 31, 2011), there were 450 deaths, with 61% due to breast cancer (n=276). Among breast cancer deaths, 159 (58%) had occurred within 5 years of diagnosis, and 78 (28%) deaths occurred between 5 and 10 years. Patients with higher parity tended to have poorer overall and BC-specific survival (overall, Figure B.1; BC-specific, Figure B.2). In patients with three or more births, compared with nulliparous patients, the HR was 1.77 (95% CI=1.18-2.66) for BC-specific mortality after adjusting for age, race, and study phase (Table A.3), while the difference in overall mortality disappeared (HR=1.09, 95% CI=0.82-1.45, Table A.4). Birth recency was only showed association with BC-survival, with HR of 1.83 (95% CI=1.07-3.13, reference=nulliparous) for women who gave birth within <5 years before diagnosis (HR adjusted for age, race, and study phase, Table A.3; survival curves, Figure B.2). When modeling parity as continuous variables, the risk for BC-specific mortality increased by 10% (HR=1.10, 95% CI=1.03-1.18, p trend <0.01) for each additional birth, while no significant linear association detected for birth recency. The magnitude of associations of multibirth and birth recency with BC-specific survival remained similar after adjusting for education and family income (parity3+, adjusted HR=1.76, 95% CI=1.13-2.73; recency <5 years, adjusted HR=1.90,

95% CI=1.10-3.34), but were attenuated after further adjustment for tumor characteristics (parity3+, adjusted HR=1.42, 95% CI=0.91-2.23; recency <5 years, adjusted HR=1.37, 95% CI=0.77-2.45).

In stratified analyses, multiparity and birth recency showed distinct associations by intrinsic subtype (Figure B.2). Consistent with results among all cases, no association of multiparity and birth recency with overall survival was detected in either luminal or basal-like tumors, except that birth within 10 years suggested a poor outcome in patients with luminal tumor (Table A.4). Higher parity and more recent birth predicted poorer BC-specific survival, with a stronger association observed in luminal tumors than in basal-like tumors, although HR estimates in basal-like tumors were imprecise (Table A.3). These associations found in both subtypes were independent of age, race, and SES factors, but were attenuated after adjustment for tumor characteristics. Only among luminal tumors did birth recency remain significantly associated with BC-specific survival independent of tumor characteristics (adjusted HR=2.35 for last birth < 5 years, 95% CI=1.05-5.27, reference=nulliparous).

We further evaluated stratified HRs according to follow-up period, menopausal status, and race. Compared with effect estimates for the first five years, HRs for BC-specific survival were suggested to be greater after conditioning on survival to 5 years, particularly among patients with luminal tumors (Table A.5). No significant differences were detected by menopausal status or race, although a stronger effect of birth recency was suggested in White women with luminal tumors.

We explored the combined effect of multiparity and birth recency on breast cancer prognosis. As presented in Figure B.3, after adjustment for age, race, study phase, and SES factors, parous patients with parity ≥ 3 births and recency < 10 years had the worst prognosis

(adjusted HR=2.02, 95% CI=1.09-3.73; reference=nulliparous), followed by patients with parity 1-2 births and recency < 10 years (adjusted HR=1.69, 95% CI=1.06-2.67) and parous patients with recency ≥ 10 years (parity ≥ 3 birth and recency ≥ 10 years: adjusted HR=1.47, 95% CI=0.87-2.50; parity 1-2 birth and recency ≥ 10 years, adjusted HR=1.42, 95% CI=0.92-2.21), and nulliparous patients had the best prognosis. The influence of breastfeeding was also evaluated (Figure B.4), and no significant modification of hazard ratios by breastfeeding status was observed (Figure B.5).

4.4. Discussion

In this study, patients with high parity or recent birth had worse BC-specific survival compared to nulliparous patients. This association was independent of age, race, and SES factors, and was attenuated, but not fully explained by tumor characteristics. No effect measure modification by race or menopausal status was detected among luminal tumor patients or basal-like tumor patients. The influence of parity and birth recency varied by intrinsic subtype and follow-up period, with stronger effects observed in long-term survivors (i.e. among those with survival ≥ 5 years) and in patients with luminal tumors.

Birth recency, defined as time interval from last birth until diagnosis, has consistently been related to deleterious tumor characteristics (e.g. advanced stage, high histological grade, and high proportion of hormone receptor-negative tumors) (141, 162, 170, 174), and consequently poor prognosis (150, 153, 162, 163, 168-171). Multiparity has also been associated with higher mortality, particularly BC-specific mortality (158, 165). The current findings are in line with previous studies, and confirm that this association is not strongly modified by race, with HRs that are similar between White and African American women (153).

While effects of multiparity and recency of birth are most often considered as separate dimensions of exposure, we considered the joint effects of these two variables. Women with high parity and short time since last birth had the highest BC-specific mortality. The combined effect of multiparity and recency was not equal to the productive effect of parity and recency (Figure B.3), which suggests an interaction between the two factors (214, 215). These findings indicate that as quantitative and temporal measures of pregnancy respectively, multiparity and recency may influence the natural history of breast cancer through distinct pathways, yet likely also share some mechanisms. Pregnancy is a complex factor. More factors (e.g. breastfeeding, age at first birth, and interval between births) than number and recency of births, are likely to modify its role in breast cancer development and progression. Our study provided a rough picture of the associations of parity and prognosis in the setting of intrinsic subtype. To better characterize the complicated effect of parity, studies with large data using more subtle analysis approaches is definitely needed (216, 217).

Most previous studies of parity and survival considered subtype (usually defined by hormone receptor status) as a confounder (163, 171, 173, 174). However, the heterogeneous association of parity with breast cancer risk across intrinsic subtype indicates that the underlying mechanisms may be different for each subtype (69-71, 73, 76, 81, 141). We found that multiparity and birth recency had weaker effects among patients with basal-like tumors than patients with luminal tumors. The trend of decreased mortality risk with time since last birth has been reported previously (146, 150, 169, 170), and in our study was only observed in luminal tumor patients. To our knowledge, only one previous study has examined the influence of multiparity and birth recency on mortality by breast cancer subtype (171). This study was based on 526 young patients with invasive breast cancer (20-44 years) in Japan. Although no

association between multiparity and mortality was detected, worse prognosis was observed in patients with more recent birth. Similar to our results, this association was stronger in luminal tumor patients than for all breast cancer patients (HR for ≤2 years group=3.07 vs 2.19, reference=nulliparous). Unfortunately, the very small sample size of triple negative tumors (n=79) and lack of subtype-specific markers for basal-like breast cancer hampered this study's ability to make inferences about basal-like breast cancer.

If the mechanism by which parity influences breast cancer risk is to shift tumors toward more aggressive characteristics at diagnosis, then the effect of parity variables on survival should be diminished upon adjustment for tumor characteristics or may vary temporally following diagnosis. Factors influencing early survival may be more related to intrinsic tumor characteristics and subsequent treatment, while later survival may depend upon host factors. Thus, we adjusted for tumor characteristics and conducted survival analyses condition upon surviving the first five years. In this study and others (163, 171, 173, 174), adjustment for tumor characteristics only modestly influences the effect of parity. With regard to conditional survival, parity was most strongly related to the reduced survival among women who survived at least five years, with significant relationships confined to luminal tumors. This suggests different biological mechanisms driving parity-associated survivorship in basal-like vs. luminal cancers.

There are many mechanisms that have been proposed for pregnancy-associated breast cancer progression. High level of pregnancy hormones is a plausible mechanism, given the influence of estrogen in breast cancer progression (210, 218-220). Additionally, considering the relatively long latent period of breast tumors, the hormonal milieu of pregnancy may, as a selection force, change the course of the disease by stimulating growth and promotion of existing tumor cells. This pathway is expected to specifically influence ER-positive tumors. However,

this mechanism is less compelling for ER-negative tumors. One potential explanation is that the frequency of basal-like tumor clones may be higher than luminal tumor clones in young women (221). Alternatively, the post-partum /post-lactation involution is also widely accepted, wherein inflammatory changes that accompany involution may promote tumor progression (210). Our previous research showed that parity-induced changes in microenvironment gene expression differed by ER status (222). Thus, mechanisms may differ by ER status of tumors. Pregnancy may have both hormonal and microenvironmental effects on ER-positive/luminal tumors (223).

Our study should be interpreted in light of some limitations. First, the CBCS oversampled young and African American patients, which resulted in a higher proportion of patients with basal-like tumors in our study population. Even so, stratified analyses by subtype still suffered from small sample size and imprecise estimates, particularly when evaluating the association differences by race and menopausal status in basal-like tumors. The study in HER2-enriched tumors was also underpowered. In our analysis, we adjusted for several key determinants of therapy (e.g. age, lymph node status, and SES) (193, 194), however treatment data was not collected. Treatment heterogeneity within tumor classes has likely increased our variation, however it is unlikely to bias our results substantially. Finally, although the different biological features and prognosis have been establish in gene expression studies(224), classification of luminal A and B in epidemiologic studies remains problematic. Recent data show that stratification of Luminal A vs. B using HER2 status (as has been done previously in the CBCS study) results in misclassification of both tumor types (212). To avoid this misclassification, we combined luminal A and B in this analysis, which hampered investigating differences in prognostic association of parity in these two strata.

In conclusion, our study identified multiparity and birth recency as predictors of breast cancer outcome. Moreover, our results deepen the understanding of parity-associated survival by suggesting that the effect of parity may vary by intrinsic subtype, which will help optimize subtype-specific treatment strategies to improve breast cancer survival. Studies with large sample size of uncommon subtypes and known treatment profiles are needed to validate our findings and to further investigate the potential interaction of parity and treatment.

CHAPTER 5: OBESITY AND BREAST CANCER SURVIVAL

5.1. Background

The association between obesity and poor breast cancer survival has been well-studied. Based on the most recently published meta-analysis, compared to lean patients, obese patients had 41% and 35% higher risk for all-cause deaths and BC-specific deaths, respectively (225). Proposed mechanisms include adverse disease features, hormonal influences, chronic inflammatory microenvironment, adipokines, epithelial-mesenchymal transition, insulin and insulin growth factor axis, and comorbidities that may interfere with treatment (226-228). Considering the obesity epidemic in the United States (229, 230), obesity may become an important facet of cancer management, thus it is important to understand how obesity affects breast cancer survival.

Despite wide acceptance of a plausible association between obesity and progression, inconsistent results are still observed across epidemiologic studies and population subgroups (225). In a study of 4,538 breast cancer patients aged 35-64 years, obesity was associated with mortality in White but not African American women (231). Another study among premenopausal women even observed a protective effect of obesity on BC-specific mortality (232). One contributing factor of these inconsistent results may be failure to fully account for breast cancer subtype. Obesity shows distinct relationships with risk of specific breast cancer subtype (46, 73, 77, 81). The same molecular mechanisms active during etiology may also promote progression in some subtypes. Previous studies investigating this hypothesis have primarily defined subtype by hormone receptor status (estrogen receptor, progesterone receptor,

or both), and these studies have also had inconsistent findings (120). A meta-analysis suggested that the influence of obesity may be stronger in hormone receptor-positive tumors than hormone receptor-negative tumors (120).

In recent years, it has been observed that ER-negative tumors are heterogeneous (25, 60). While ER-positive tumors are predominantly luminal subtype, strata defined by ER-negative status include a mix of tumors including HER2-positive, basal-like, and triple-negative tumors that are unclassifiable (25, 41). However, few studies have examined obesity-associated survival by intrinsic subtype. Among studies that have evaluated adiposity and survival by subtype, most have used only BMI as the primary obesity measure, and studies conflict on whether BMI or WHR may be more strongly linked with breast cancer subtypes (71, 76). Data on central obesity (such as WHR) is rare in epidemiologic studies (113), despite the importance of this adiposity measure (233, 234).

Using data from the Carolina Breast Cancer Study (CBCS), a large population-based case-control study, we assessed the impact of BMI and WHR on overall and breast cancer (BC)-specific survival. These associations were evaluated among breast cancers as a whole and in strata defined by specific breast cancer subtypes (basal-like and luminal).

5.2. Methods

5.2.1. Study population

The CBCS is a population-based case-control study, the details of which have been described previously (76, 200). Briefly, a total of 1,808 patients aged 20-74 years diagnosed with primary invasive breast cancer during 1993-1996 (Phase I) and 1996-2001 (Phase II) were identified using rapid case ascertainment from NC Central Cancer Registry, with African American and young cases (aged 20-49 years) oversampled using randomized recruitment (200,

211). Participants were interviewed in person within 1 year of the diagnosis by trained nurses who collected anthropometric measurements and questionnaire responses. Clinicopathological information was abstracted from clinical records and pathological reports. The study procedures for recruitment and enrollment into the CBCS were approved by the Institutional Review Board of the University of North Carolina (UNC). All study participants gave written informed consent.

5.2.2. Breast cancer subtype classification

The details of breast cancer subtyping have been published previously (41, 76). Briefly, whole, formalin-fixed paraffin-embedded tumor tissues were sectioned and stained for a panel of immunohistochemical (IHC) markers in the IHC Core Laboratory at UNC. The following markers were used to determine breast cancer intrinsic subtypes: luminal A (ER+ and/or PR+, HER2-), luminal B (ER+ and/or PR+, HER 2+), basal-like (ER-, PR-. HER2-, HER1+ and/or cytokeratin 5/6+), HER2-enriched (ER-, PR-, HER2+), and unclassified (negative for all five markers). We combined luminal A and luminal B as luminal tumors due to the small number of luminal B tumors (n=111) and, more importantly, recent revisions to the IHC definition of luminal B (35, 57). Luminal A and B tumors cannot be reliably distinguished without additional markers (such as Ki-67) or nanostring data (212). In the CBCS, the demographic and tumor characteristics in patients with luminal A and B tumors were comparable except luminal B tumors more likely to be lymph node positive (p=0.01).

5.2.3. Exposure and outcome assessment

Waist circumference, hip circumference, height, and body weight were measured by trained nurses at the time of interview. BMI was computed by dividing the weight in kilograms by the square of the height in meters. The World Health Organization (WHO) definition was

used to classify patients as underweight (BMI <18.5 kg/m^2), normal (BMI 18.5-24.9 kg/m^2), overweight (BMI 25-29.9 kg/m^2), and obese (BMI ≥30 kg/m^2). Underweight patients were combined with normal-weight patients due to small number (n=23, 2%). WHR was calculated as the ratio of waist to hip circumference (cm). Since the criteria for central obesity is not standardized (235), tertiles of the WHR distribution in CBCS controls (with cutoff points of 0.77 and 0.83) were used (76).

Linkage with the National Death Index provided vital status, dates of deaths, and cause of death on the CBCS cases through December 31, 2011. Deaths among cases were determined using weighted probabilistic scores and predetermined matching cutoffs to establish a maximum of 1 match per individual (206). International Classification of Diseases (ICD) breast cancer codes 174.9 (ICD-9) or C50.9 (ICD-10) were used to identify deaths due to breast cancer on the death certificate.

5.2.4. Statistical analysis

Our analysis included 1,109 patients, after excluding 9 cases with race other than White or African American, 659 cases without immunohistochemical subtype information, and 31 cases with missing data on anthropometric measures. The demographic and tumor characteristics of the excluded cases were compared with those of the included cases; no significant differences were detected, except that excluded cases were less aggressive (more likely to have negative lymph node status, tumor size ≤ 2cm, and stage I). The demographic, lifestyle, clinical and other characteristics of the study population were evaluated by BMI and WHR using Chi-square test or Student's t-test (Table A.7 and Table A.8). The assessment and definition of these variables have been described previously (76). Patients living as of December 31, 2011 were censored, and those who died of causes other than breast cancer were censored for

breast cancer (BC)–specific analysis. Kaplan–Meier survival curves and log-rank tests were used to compare the difference in overall and BC-specific survivals by BMI and WHR.

Cox regression analysis was used to estimate the hazard ratio (HR) and 95% confidence interval (CI) for overall death and BC-specific death, with BMI <25 kg/m^2 or bottom WHR tertile as the reference category. Confounders were selected based on *a priori* knowledge and with the aid of a directed acyclic graph. To understand the influence of confounders on the study associations, we added these covariates sequentially. Multivariable models included a minimal model (age, race, and study phase; Model 1), then a model additionally adjusted for selected socioeconomic factors (education and income) and lifestyle factors (smoking, alcohol intake, physical activity, and parity; Model 2), and finally, a model adjusted for tumor characteristics (tumor stage, tumor size, lymph node status, and histological type; Model 3).

Stratified analyses were performed to evaluate effect modification by intrinsic subtype. Only basal-like and luminal strata are presented because unclassified tumors are of biologically uncertain subtype and because too few patients (n=73) were HER2-enriched for stable estimation. The difference in HRs by race and menopausal status within luminal and basal-like tumors was also assessed. In the analysis by menopausal status, perimenopausal women were excluded to avoid misclassification (n=95). In addition, because studies have suggested that factors predicting survival in early years after diagnosis may differ from those in later years (e.g. with tumor biological and pathologic characteristics dominant in early years and lifestyles dominant in later years (213)), analyses were conducted conditioned on follow-up length: data were truncated at five years to evaluate five-year mortality and then survival was assessed conditional upon surviving the first five years.

The proportional hazards assumption in each Cox model was assessed using log-log plots of survival and time-dependent cross-product terms of the survival time (years) and the variables of interest, and showed no violation of the assumptions. All statistical tests were two sided with α=0.05, all analyses were performed using SAS version 9.2 (SAS Institute), and all figures were generated using R 3.0.0.

5.3. Results

5.3.1. Patient and tumor characteristics

Among 1,109 breast cancer patients in this study, the average age at diagnosis was 51 years (SD=11.5 years, range=23-74 years). Approximately half of patients were African American (45%) and premenopausal (42%). The mean BMI for the entire study population was 28.5 kg/m^2 (SD=6.9 kg/m^2, range=14.3-57.9 kg/m^2), with 37% (n=410) considered obese. The mean WHR was 0.82 (SD=0.08, range=0.60-1.34), with 36% (n=399) considered obese with a WHR$\geq$0.85 (cutoff recommended by WHO) (236). Consistent with previous reports (237), BMI and WHR showed relatively low correlation (Pearson's r=0.40, p<0.01) and low agreement for obese classification (kappa coefficient=0.32, p<0.01). However, patient demographics showed similar distribution by BMI and WHR categories (Table A.8 and Table A.7). Patients with higher BMI or WHR tended to be older, African American, alcohol abstainers, lower socioeconomic status (SES) (measured by education and family income), OC users, and have more births. In addition, the high WHR group ($\geq$0.84) had a higher proportion of current smokers.

Luminal tumors comprised the majority of breast cancers (n=714, 64%), followed by basal-like tumors (n=197, 18%), unclassified (n=126, 11%), and HER2-enriched tumors (n=72, 6%). Higher adiposity level (both BMI and WHR) was significantly associated with higher

prevalence of basal-like tumors, but lower prevalence of luminal tumors (Table A.8 and Table A.7). Moreover, high mitotic index was more prevalent among patients with a BMI of 25-<30 kg/m^2 (p=0.02). Compared with BMI, WHR was more related to tumor characteristics, with high WHR group (≥ 0.84) having higher prevalence of large (>5cm, p<0.01) and high stage (stage III&IV, p=0.01) tumors.

5.3.2. Associations between obesity and prognosis

The median follow-up time was 13.5 years, ranging from 0.2 years to 18.7 years. By the end of follow-up, there were 435 deaths, and 62% of deaths were due to breast cancer (n=268). Among breast cancer deaths, 155 (58%) occurred within 5 years of diagnosis, and 76 (28%) occurred between 5 and 10 years. Patients with high BMI or WHR had poorer overall survival (p-value for log rank test<0.01, Figure B.6). The survival difference by BMI group became insignificant and smaller after adjusting for age, race, study phase, SES factors, and lifestyle factors (BMI ≥30 kg/m^2 vs. <25 kg/m^2,adjusted HR=1.19, 95% CI=0.91-1.55, Table A.8), and was further reduced after further adjusting for tumor characteristics (adjusted HR =1.11, 95% CI=0.84-1.45). Compared with BMI, WHR showed a stronger association with all-cause mortality, which was independent of potential confounders (WHR ≥ 0.84 vs. <0.77, adjusted HR=1.50, 95% CI=1.11, 2.05), but was attenuated after further adjustment for tumor characteristics (adjusted HR=1.25, 95% CI=0.91-1.72). Associations of obesity with BC-specific mortality were weaker than those with overall mortality (p-value for log rank tests were 0.20 and 0.15 for BMI and WHR, respectively; Figure B.7), and were not detected in multivariate analyses (Table A.9).

In subtype-stratified analyses, BMI and WHR demonstrated different prognostic association by subtype. As shown in Figure B.6, among patients with basal-like tumors there

was a significant difference in overall survival by BMI, independent of potential confounders and tumor characteristics (Table A.8, BMI≥30 kg/m^2 vs <25 kg/m^2, adjusted HR=2.04, 95% CI=1.01-4.13). This difference remained after adjustment for WHR (adjusted HR=2.57, 95% CI=1.20-5.54). In contrast, WHR had a stronger influence on all-cause mortality in patients with luminal tumor (WHR ≥0.84 vs. <0.77, adjusted HR=1.75, 95% CI=1.20-2.56). Although this association cannot be explained by BMI (HR adjusted for BMI=1.79, 95% CI=1.20-2.68), it was not independent of tumor characteristics (HR additionally adjusted for tumor characteristics=1.33, 95% CI=0.89-1.97).

We further evaluated subtype-specific HRs according to follow-up period, menopausal status, and race. The influence of obesity on all-cause mortality appeared stronger among patients who survived at least 5 years after diagnosis, particularly among patients with basal-like tumors (Table A.10**,** BMI≥30 kg/m^2, adjusted HR=3.15, 95% CI=1.13-8.79). No significant differences were detected by menopausal status or race, although the association of WHR showed some suggestion of modification by menopausal status and race among basal-like cases (Figure B.8), with HRs higher in postmenopausal and White patients.

5.4. Discussion

Our study was in agreement with previous reports of an association between BMI or WHR and all-cause mortality among breast cancer cases overall (119, 225, 238). The association with all-cause deaths was independent of age, race, lifestyle and SES factors, and was attenuated, but not fully explained by tumor characteristics. Interestingly, the influence of obesity on all-cause mortality varied by intrinsic subtype and obesity measure. While BMI predicted mortality in patients with basal-like tumors, WHR predicted mortality in patients with luminal tumors. The association between these measures and breast-cancer specific survival was not significant.

A few previous studies have assessed the relationship between obesity and breast cancer prognosis by intrinsic or molecular subtype (66, 121-125, 239), and these have suggested a heterogeneous effect of obesity. Five studies examined BMI in triple negative breast cancer (TNBC) (66, 121-125). One study in premenopausal women reported increased BC-specific mortality associated with obesity (obese vs normal BMI: HR=1.4, 95% CI=1.0-2.1) among TNBC, but not among luminal tumors (66). In another study of TNBC cases among predominantly African American women, and including both pre- and post- menopausal women, BMI was associated overall survival (HR=1.36, 95% CI=0.77-2.42), but not relapse-free survival (HR=1.01, 95% CI=0.67-1.52) (123). The other three studies, with a majority of White women (both pre- and post-menopausal), did not detect any association between BMI and breast cancer prognosis among TNBC (121, 122, 124). Although the sparse data and differences in population characteristics and covariates limits direct comparison across these studies, our results seem relatively consistent with the findings of previous studies with high proportions of young or African American patients. These findings suggest that the prognostic association of obesity may vary by age and race. However, the sample size of our study and previous studies was not large enough for multi-stratified analysis.

Previous studies have reported a general larger effect of BMI on overall survival than the effect on BC-specific mortality (225), suggesting that non-cancer causes of death contribute to the less favorable outcomes noted for obese patients. However, our study, consistent with some studies (117, 121, 123, 126, 127), did not observe an association of obesity and BC-specific mortality. Based on two large studies (n=18,967 (156) and n=14,709 (240), respectively), obesity was not associated with loco-regional recurrence (156, 240) and five-year distant metastases (156). Loco-regional recurrence and metastases are strong predictors for BC-specific

mortality, and are more frequently observed in basal-like tumor patients and African American patients (44, 92), which may contribute to the null association in the CBCS where the two subpopulations were over-represented. Obese patients are more likely to have comorbidities and tend to die from non-cancer causes before they die from breast cancer. When the "unhealthy" obese people die from non-breast cancer causes, they are deprived the opportunity to die from breast cancer. The obese women left in the risk population for BC-specific mortality are "healthy" and do not possess disadvantages in BC-specific survival compared with non-obese patients. Therefore no association between obesity and breast cancer mortality will be observed.

BMI and WHR are the most commonly used anthropometric measures of general obesity and central obesity, respectively. There is an increasing body of evidence that different adipose tissue depots (e.g. visceral and subcutaneous adipose tissue) differ in both cellular composition and physiology, resulting in distinct roles in disease development and progression (241, 242). Generally visceral/abdominal adipose is considered more metabolically active, and plays more important roles in pathological processes. This perspective is supported, albeit inconsistently, by epidemiologic data. Compared with BMI, WHR/waist circumference was more strongly correlated with growth hormone, insulin growth factor (IGF)-1, insulin resistance, circulating estradiol fractions and leptin in postmenopausal women (243-245). Paralleling differences in disease risk of diabetes and cardiovascular disease (246-249), differences in the effects of BMI versus WHR were also observed in studies of breast cancer risk (250). When considering breast cancer as a whole, meta-analysis showed that BMI decreased risk of premenopausal breast cancer, but increased risk of postmenopausal breast cancer, while WHR was associated with an increased risk of both pre- and postmenopausal breast cancer (250). In the CBCS, WHR, but not BMI, was independently associated with increased risk of luminal A in postmenopausal women,

and basal-like tumor in pre- and postmenopausal women (76, 251). However, to our knowledge no previous studies have observed differential effects of WHR and BMI on basal-like or luminal breast cancer prognosis.

Tumor characteristics may dominate early survival following diagnosis, with little opportunity for lifestyle factors to mitigate the effects of very aggressive tumor phenotypes. This idea is supported by a study where in the first 5 years after diagnosis, there was no association with BMI (HR=1.08, 95% CI=0.96-1.21), but from 5 to 10 years after diagnosis, the risk of developing distant metastases increased significantly (HR=1.46, 95% CI=1.11-1.92) (156). In our study, we observed a similar increment of the association after 5 years. Particularly in basal-like tumors, their overall and BC-specific mortality HRs of BMI after 5 years were 2 and 4 times the HRs in the first five years respectively (BC-specific mortality HRs were statistically insignificant and not shown in the paper). Furthermore, there has been a paradox observed in triple-negative disease (84), such that triple-negative/basal-like patients with strong pathologic complete response have very favorable prognosis despite very high hazard rates in early years following diagnosis. It may be that in this period when disease-specific mortality is lower for basal-like breast cancer, obesity is more influential for overall survival. This underscores that it remains important to study overall survival for breast cancer patients. Understanding the mortality risks of patients who have low risk of disease relapse are important for cancer survivors; patients who move from high to low risk of breast cancer relapse may move into a period where other comorbidities become a greater concern than breast cancer itself.

Our study should be interpreted in light of some limitations. First, the CBCS oversampled young and African American patients, which resulted in a higher proportion of patients with basal-like tumors in our study population. However, even so, stratified analyses by subtype still

suffered from small sample size and imprecise estimates. Second, obesity status in our study was assessed shortly after diagnosis (< 1 year). Anthropometry is likely to change following diagnosis and treatment. Based on an analysis of 12,915 breast cancer patients from four prospective cohorts, the mean weight change was 1.6 kg during a follow-up averaging 8.1 years (189). This weight change during follow-up may not be large enough to induce considerable misclassification of obesity status. Third, treatment data was not collected in Phases I and II of the CBCS, limiting our ability to study the effect of treatment-obesity interaction and treatment in comorbidities. Finally, although distinct biological features and prognosis by subtype have been established in gene expression studies (224), classification of luminal A and B in epidemiologic studies remains problematic. Recent data show that stratification of Luminal A vs. B using HER2 status (as has been done previously in the CBCS study) results in misclassification of both tumor types (212). To avoid this misclassification, we combined luminal A and B in this analysis, which hampered investigating differences in prognostic association of obesity in these two strata.

In conclusion, our study showed the association of adiposity and overall survival, while effects on breast cancer-specific survival are weak to null. Moreover, different adiposity measures should be considered as each measure appears to be associated with different subtype and capture different biological characteristics. Basal-like and luminal breast cancer patients, particularly those that have longer term survival, may have greater risk of mortality due to comorbidities associated with specific types of obesity.

CHAPTER 6: DISCUSSION

6.1. Main findings

In this cohort study of 1,140 patients with invasive breast cancer from the Phase I and II of the CBCS study, we evaluated the influence of parity and obesity on overall and BC-specific survival. Parity (measured by number of full-term birth and recency of last birth) was significantly associated with poor BC-specific survival, while obesity measures (BMI and WHR) were significantly associated with poor overall survival. These associations were independent of age, race, SES factors, and lifestyle factors (in obesity analysis), although the associations were attenuated after adjusting for tumor characteristics.

The influence of parity and obesity on prognosis was distinct for basal-like and luminal tumors. Both multiparty and birth recency had stronger effect on breast cancer-specific survival among luminal patients than among basal-like patients. High BMI ($\geq$30 kg/m^2) was associated with higher all-cause mortality among patients with basal-like tumor, while high WHR ($\geq$0.84) was associated with higher mortality among patients with luminal tumor.

It has been argued that the first few years of survivorship are determined most strongly by tumor characteristics, while the effects of behavioral or other patient characteristics may play a stronger role five or more years after diagnosis (213). Our study provides some evidence for this pattern, with more pronounced effects of parity and obesity conditioning upon survival to 5 years post-diagnosis.

6.2. Biological hypotheses for distinct parity- and obesity-associated survival by subtype

The prognostic influence of parity and obesity in breast cancer has been reported previously, and the underlying mechanisms are proposed and reviewed (138, 210, 226, 228). However, biological explanations for their different effect by intrinsic subtype are under-explored.

The association of parity with breast cancer is complex, and the underlying biological basis is likely through multiple pathways. In the long run, parity confers a protection against the development of breast cancer by inducing differentiation and apoptosis of mammary stem cells (252). However, it also has a possible promoting effect on breast cancer (218, 253). High levels of pregnancy hormones may change the course of the disease, acting as a selection force by stimulating growth and promotion of existing tumor cells. Likely, this hormone-related pathway specifically influences ER-positive tumors. This hormone-drive hypothesis is supported by the stronger parity association among luminal tumors than basal-like tumors in our study.

A second parity-associated hypothesis relates to a role for the involuting microenvironment in tumor promotion. Studies demonstrate that during post-lactational involution, immune cells infiltrate in breast tissue and the microstructure is remodeled (138, 254), which may create a permissive environment for breast cancer promotion and progression (255). Interestingly, in a recent gene expression study, we detected a pregnancy-associated inflammatory signature among ER-positive breast cancers, but not among ER-negative tumors (222). These findings highlight some parity-associated changes in microenvironment may particularly favor ER-positive/luminal tumors.

Third, the differences in parity-associated breast cancer aggressiveness by subtype may reflect fundamental differences in progression patterns between the two cancer types. Anderson

et al. have clearly demonstrated that the hazard rate for mortality is higher for ER-negative than for ER-positive breast cancers in the first three years following diagnosis (221). If hazards are uniformly high among ER-negative and/or basal-like breast cancers, then parity may do little to alter this course. However, we also evaluated progression conditional upon five years of survival. The results, showing no significant difference in effect of parity on basal-like progression before and after the first five years after diagnosis, suggest that the phenomenon of pregnancy-associated breast cancer aggressiveness may be limited to luminal breast cancers.

Our observation of a specific luminal-promoting effect of parity may seem to contradict the high proportion of ER-negative/triple-negative tumors in pregnancy-associated breast cancer (256, 257). However, it is important to distinguish etiologic heterogeneity and subtype-specific effects on progression. While the natural history of tumorigenesis often finds parallels between etiologic and progressions factors, it is also often the case that something which lowers barriers to carcinogenesis confers no additional advantage once the tumor is formed.

The mechanisms underlying the association between obesity and breast cancer have been well studied, including insulin and insulin-like growth factors, sex hormone, sex steroids, adipokines, epithelial-mesenchymal transition, and pro-inflammatory microenvironment (226, 228, 258). However, how these mechanisms differentially contribute to the etiology and progression of each subtype has not been well characterized. In general, it is believed that increased production of estrogens in the adipose tissue and decreased sex hormone-binding globulin are more specific to hormone receptor-positive tumors, while likely other pathways work for all subtypes (259). In our previous study, we have observed infiltration of macrophages and up-regulated gene expression of immune response pathways in normal tissues of obese women (260). This pro-inflammatory microenvironment, orchestrated with adipokines, may

increase tumor-related angiogenesis and facilitate tumor invasion and metastasis (261, 262). This pathway may be particularly important for basal-like tumors, given early blood-borne dissemination is more common in this subtype (91).

In the current research, we detected an association of obesity with overall survival, but not BC-specific survival. The stronger effect of obesity on overall mortality than BC-specific mortality has also been observed in previous studies (263, 264). These findings suggest that obesity may influence mortality after breast cancer via tumor-independent mechanisms. Obesity-associated comorbidities and obesity-treatment interaction are potentially the key contributors to the difference between overall and BC-specific association.

Based on our analysis, the prognostic influence of obesity on all-cause mortality varied not only by subtype, but also by obesity measure. The different metrics of adiposity has been recognized to reflect different underlying characteristics of obesity. Different adipose tissue depots (e.g. visceral and subcutaneous adipose tissues) are different in cellular composition and physiology, resulting in their heterogeneous phenotypic properties and roles in disease course (241, 242). Generally visceral/abdominal adipose is considered more metabolically active, and plays more important role in pathological status (e.g. chronic inflammation, insulin resistance) (241, 242). These biological differences were mirrored by the results of epidemiological studies, where compared with BMI, WHR or waist circumference was more correlated with growth hormone, insulin growth factor (IGF)-1, insulin resistance, circulating estradiol fractions and leptin (243-245). While different metrics of adiposity has been intensively studied in etiology of diabetes and cardiovascular disease (246-249), unfortunately, it has received limited attention in breast cancer studies. Specific explanations for the different prognostic association by obesity measures still need further investigation.

6.3. Significance

The significance of our research is that it addresses several important challenges in epidemiology. First, we are evaluating risk factors for a role in progression, acknowledging that some factors may continue to exert effects in the same direction during progression and others will not. Second, we are using anthropometric variables to make inferences about underlying biological processes. Finally, in light of what these data show, priorities for reducing the burden of breast cancer can be inferred.

Parity and obesity are established breast cancer risk factors that have consistently shown distinct etiologic associations with intrinsic subtype. While etiology and progression are considered separately in studies, the whole disease course of breast cancer is actually a continuous process. Therefore it is important to consider whether factors that play a role in initiation also affect breast cancer progression. Our results showed that the both parity and obesity have effects during progression that are distinct from their effects on etiology. That is, parity reduces risk of luminal breast cancer but increases mortality. Obesity on the other hand, influences breast cancer etiology, but does not appear to strongly influence breast cancer-specific survival.

This research used anthropometric and reproductive factors to investigate critical pathways in carcinogenesis. By comparing the results for obesity and parity, we can infer whether each of these interacts with tumor biology or through other mechanisms. While parity significantly influenced BC-specific survival implying an effect on tumor biology, obesity showed no relationship with breast cancer specific survival. Moreover, the effects of parity are not mediated solely by established measures of tumor characteristics (e.g. tumor size, lymph node status) since this association was independent of these variables.

In spite of the limited role for obesity in tumor progression, our data shows that obesity remains an important predictor for overall outcome, likely through the pathways not mediated by tumor (e.g. host health condition). These findings are consistent with our early hypotheses. In the section of *3.4 Linkage between risk factors and prognosis*, we hypothesized that "obesity could influence breast cancer prognosis by altering susceptibility to more aggressive subtypes", therefore "obesity is not associated with breast cancer-specific mortality," and that parity's "association with breast cancer prognosis is only through breast cancer, without a direct pathway".

These results of parity and obesity provide important indication for breast cancer prevention and management. Considering parity-prognosis association will be significant to optimize treatment and to plan pregnancy in young breast cancer survivors. Compared with parity, obesity-prognosis association has more public health value, since obesity intervention reduces risk for both occurrence and mortality.

6.4. Future directions

An important future epidemiologic direction is to consider the role of exposures that occur after diagnosis. For example, both parity and obesity may interact with treatment (265, 266), which may further affects the prognostic association. Moreover, studies demonstrated weight changes after diagnosis due to age, treatment or lifestyle changes, with estimated 50-96% of breast cancer patients gaining weight, particularly during chemotherapy (188). Whether after-diagnosis weight change varies by subtype and how it influences prognosis cannot be answered by our data. A future study with detailed records on treatment and longitudinal data on parity, obesity, and comorbidities will help explain the distinct prognostic associations of parity and obesity we observed in this study.

Future biological research should focus on well-delineated pathways specific for each subtype. This study is the first one that found that prognostic association of obesity varied by both subtype and obesity measures. This finding needs to be validated in model systems. BMI and WHR are anthropometric measures of general and central obesity respectively. They reflect the difference between visceral and subcutaneous adipose tissue, but cannot accurately capture the biological difference. In order to distinguish the different role by obesity type in breast cancer development and progression, studies with more accurate methods measuring different type of adiposity and biomarkers describing the different pathways will be critical. Last, most of the obesity-associated biomarkers currently used in large epidemiological studies (e.g. CRP, IL-6) (238) are originally developed in the settings of cardiovascular disease or diabetes. Although cancer may share some mechanisms with these diseases, more likely obesity and cancer have some specific or preferable pathways. Therefore obesity-associated biomarkers aimed to describe the obesity-cancer linkage need further development.

APPENDIX A: TABLES

Table A.1: Characteristics of study population by parity, in the CBCS Phases I and II [a].

Characteristics		Overall (n=1140)	Nulliparous (n=173)	1-2 (n=551)	≥3 (n=416)	P-value
Age (years)						
	Mean (SD)	50.64 (12)	49.07 (12.40)	47.91 (10.63)	54.92 (10.98)	<0.01
	<40	194 (17)	40 (23)	114 (21)	40 (10)	<0.01
	40-49	436 (38)	68 (39)	256 (46)	112 (27)	
	50-59	227 (20)	28 (16)	91 (17)	108 (26)	
	≥60	283 (25)	37 (21)	90 (16)	156 (38)	
Menopausal status						
	Premenopausal	556 (49)	92 (53)	323 (59)	141 (34)	<0.01
	Postmenopausal	584 (51)	81 (47)	228 (41)	275 (66)	
Race						
	White	622 (55)	99 (57)	348 (63)	175 (42)	<0.01
	African American	518 (45)	74 (43)	203 (37)	241 (58)	
BMI (kg/m^2)						
	Mean (SD)	28.51 (6.89)	27.13 (6.82)	27.83 (6.54)	29.99 (7.14)	<0.01
	<25	395 (35)	73 (43)	214 (39)	108 (27)	<0.01
	25-<30	308 (28)	44 (26)	160 (29)	104 (26)	
	≥30	411 (37)	51 (30)	169 (31)	191 (47)	
WHR						
	Mean (SD)	0.82 (0.08)	0.81 (0.08)	0.81 (0.08)	0.84 (0.08)	<0.01
	<0.77	284 (25)	54 (32)	160 (29)	70 (17)	<0.01
	0.77-<0.84	389 (35)	53 (31)	206 (38)	130 (32)	
	≥0.84	451 (40)	63 (37)	180 (33)	208 (51)	

Table A.1: Characteristics of study population by parity, in the CBCS Phases I and II [a].

Characteristics	Overall (n=1140)	Nulliparous (n=173)	1-2 (n=551)	≥3 (n=416)	P-value
Years since last full-term pregnancy among parous women					
<5	76 (8)	-	58 (11)	18 (4)	<0.01
5-<10	89 (9)	-	61 (11)	28 (7)	
≥10	801 (83)	-	432 (78)	369 (89)	
Family history of breast cancer[b]					
Yes	180 (16)	24 (14)	90 (17)	66 (17)	0.75
No	927 (84)	144 (86)	450 (83)	333 (83)	
Education					
Lower than high school	199 (17)	18 (10)	58 (11)	123 (30)	<0.01
High school/post high school	627 (55)	84 (49)	317 (58)	226 (54)	
College and above	314 (28)	71 (41)	176 (32)	67 (16)	
Family income (thousand US dollar)					
<15	244 (23)	32 (20)	78 (15)	134 (35)	<0.01
15-<30	259 (25)	45 (28)	117 (23)	97 (26)	
30-<50	258 (24)	37 (23)	139 (27)	82 (22)	
≥50	293 (28)	44 (28)	183 (35)	66 (17)	
Smoking					
Never	599 (53)	90 (52)	294 (53)	215 (52)	0.79
Former	353 (31)	51 (29)	174 (32)	128 (31)	
current	188 (16)	32 (19)	83 (15)	73 (18)	
Alcohol					
No	351 (31)	48 (28)	151 (27)	152 (37)	<0.01
Yes	788 (69)	124 (72)	400 (73)	264 (63)	
Physical activity					

Table A.1: Characteristics of study population by parity, in the CBCS Phases I and II [a].

Characteristics		Overall (n=1140)	Nulliparous (n=173)	1-2 (n=551)	≥3 (n=416)	P-value
	no	560 (49)	81 (47)	262 (48)	217 (52)	0.29
	yes	580 (51)	92 (53)	289 (52)	199 (48)	
HRT						
	Never	821 (72)	125 (72)	398 (72)	298 (72)	0.95
	Former	221 (19)	32 (19)	110 (20)	79 (19)	
	Current	96 (8)	16 (9)	43 (8)	37 (9)	
OC						
	Never	382 (34)	65 (38)	129 (23)	188 (45)	<0.01
	Ever	754 (66)	107 (62)	421 (77)	226 (55)	
Lymph node status						
	Positive	448 (40)	50 (29)	223 (41)	175 (42)	0.01
	Negative	686 (60)	120 (71)	326 (59)	240 (58)	
Intrinsic subtype						
	Luminal	731 (64)	118 (68)	349 (63)	264 (63)	0.58
	Basal-like	205 (18)	24 (14)	109 (20)	72 (17)	
	Her2-positive	73 (6)	13 (8)	31 (6)	29 (7)	
	Normal-like	131 (11)	18 (10)	62 (11)	51 (12)	
Tumor size (cm)						
	≤2	540 (48)	84 (49)	251 (47)	205 (50)	0.53
	>2-5	468 (42)	68 (40)	239 (45)	161 (39)	
	>5	106 (10)	18 (11)	46 (9)	42 (10)	
Tumor stage (AJCC/UICC Stage Grouping)						
	I	414 (37)	69 (41)	193 (36)	152 (38)	0.83
	II	559 (50)	80 (48)	277 (52)	202 (50)	

Table A.1: Characteristics of study population by parity, in the CBCS Phases I and II [a].

Characteristics		Overall (n=1140)	Nulliparous (n=173)	1-2 (n=551)	≥3 (n=416)	P-value
	III+IV	136 (12)	19 (11)	66 (12)	51 (13)	
Histology group						
	Ductal	939 (82)	148 (86)	453 (82)	338 (81)	0.46
	Others	201 (18)	25 (14)	98 (18)	78 (19)	
Nuclear grade[c]						
	Pleomorphism	211 (43)	33 (42)	112 (46)	66 (39)	0.43
	Slight/moderate	280 (57)	45 (58)	133 (54)	102 (61)	
Histologic grade[c]						
	Well/moderate	173 (35)	27 (35)	81 (33)	65 (39)	0.50
	Poor	318 (65)	51 (65)	164 (67)	103 (61)	
Mitotic index[c]						
	Low	265 (54)	44 (56)	130 (54)	91 (54)	0.90
	High	224 (46)	34 (44)	113 (46)	77 (46)	

[a] P-values for the comparisons across parity groups were calculated by t test for continuous variables and x2 test for categorical variables except that when expected cell count was less than 5, they were calculated by Fisher exact test. Missing values were excluded from percentage calculations.
[b] First degree.
[c] Only available in Phase I.

Table A.2: Characteristics of study population by last birth recency group, in the CBCS Phases I and II[a].

Characteristics	Overall (n=1139)	Nulliparous (n=173)	<5 years (n=76)	5-<10 years (n=89)	≥ 10 years (n=801)	P-value
Age (years)						
Mean (SD)	50.62 (11.49)	49.07 (12.4)	35.43 (5.03)	39.46 (5.23)	53.64 (10.20)	<.01
<40	194 (17)	40 (23)	59 (78)	44 (49)	51 (6)	
40-49	436 (38)	68 (39)	17 (22)	44 (49)	307 (38)	
50-59	227 (20)	28 (16)	0 (0)	1 (1)	198 (25)	
≥60	282 (25)	37 (21)	0 (0)	0 (0)	245 (31)	
Menopausal status						
Premenopausal	556 (49)	92 (53)	72 (95)	84 (94)	308 (38)	<.01
Postmenopausal	583 (51)	81 (47)	4 (5)	5 (6)	493 (62)	
Race						
White	622 (55)	99 (57)	50 (66)	52 (58)	421 (53)	0.10
African American	517 (45)	74 (43)	26 (34)	37 (42)	380 (47)	
BMI (kg/m^2)						
Mean (SD)	28.51 (6.89)	27.13 (6.82)	26.69 (7.12)	26.91 (6.65)	29.15 (6.82)	<.01
<25	395 (35)	73 (43)	38 (51)	35 (41)	249 (32)	<.01
25-<30	308 (28)	44 (26)	16 (22)	29 (34)	219 (28)	
≥30	411 (37)	51 (30)	20 (27)	22 (26)	318 (40)	
WHR						
Mean (SD)	0.82 (0.08)	0.81 (0.08)	0.79 (0.09)	0.79 (0.07)	0.83 (0.08)	<.01
<0.77	284 (25)	54 (32)	30 (41)	31 (36)	169 (21)	<.01
0.77-<0.84	389 (35)	53 (31)	28 (38)	33 (38)	275 (35)	
≥0.84	451 (40)	63 (37)	15 (21)	23 (26)	350 (44)	
Full term pregnancy						
nulliparous	173 (15)	173 (100)	0 (0)	0 (0)	0 (0)	<.01

Table A.2: Characteristics of study population by last birth recency group, in the CBCS Phases I and II[a].

Characteristics	Overall (n=1139)	Nulliparous (n=173)	<5 years (n=76)	5-<10 years (n=89)	≥ 10 years (n=801)	P-value
1-2	551 (48)	0 (0)	58 (76)	61 (69)	432 (54)	
≥ 3	415 (36)	0 (0)	18 (24)	28 (31)	369 (46)	
Family history of breast cancer[b]						
Yes	180 (16)	24 (14)	10 (14)	10 (11)	136 (18)	0.33
No	926 (84)	144 (86)	63 (86)	79 (89)	640 (82)	
Education						
Lower than high school	198 (17)	18 (10)	4 (5)	6 (7)	170 (21)	<.01
High school/post high school	627 (55)	84 (49)	35 (46)	45 (51)	463 (58)	
College and above	314 (28)	71 (41)	37 (49)	38 (43)	168 (21)	
Family income (thousand US dollar)						
<15	259 (25)	32 (20)	14 (19)	9 (11)	188 (26)	<.01
15-<30	258 (24)	45 (28)	11 (15)	22 (27)	181 (25)	
30-<50	293 (28)	37 (23)	19 (25)	19 (23)	183 (25)	
≥50	243 (23)	44 (28)	31 (41)	33 (40)	185 (25)	
Smoking						
Never	599 (53)	90 (52)	51 (67)	57 (64)	401 (50)	0.01
Former	352 (31)	51 (29)	21 (28)	19 (21)	261 (33)	
current	188 (17)	32 (19)	4 (5)	13 (15)	139 (17)	
Alcohol						
No	350 (31)	48 (28)	14 (18)	23 (26)	265 (33)	0.03
Yes	788 (69)	124 (72)	62 (82)	66 (74)	536 (67)	
Physical activity						
no	559 (49)	81 (47)	40 (53)	41 (46)	397 (50)	0.77

Table A.2: Characteristics of study population by last birth recency group, in the CBCS Phases I and II[a].

Characteristics	Overall (n=1139)	Nulliparous (n=173)	<5 years (n=76)	5-<10 years (n=89)	≥ 10 years (n=801)	P-value
yes	580 (51)	92 (53)	36 (47)	48 (54)	404 (50)	
HRT						
Never	820 (72)	125 (72)	74 (97)	83 (93)	538 (67)	<.01
Former	221 (19)	32 (19)	0 (0)	3 (3)	186 (23)	
Current	96 (8)	16 (9)	2 (3)	3 (3)	75 (9)	
OC						
Never	381 (34)	65 (38)	4 (5)	11 (12)	301 (38)	<.01
Ever	754 (66)	107 (62)	72 (95)	78 (88)	497 (62)	
Lymph node status						
Positive	447 (39)	50 (29)	42 (55)	40 (45)	315 (39)	<.01
Negative	686 (61)	120 (71)	34 (45)	49 (55)	483 (61)	
Intrinsic subtype						
Luminal	730 (64)	118 (68)	42 (55)	41 (46)	529 (66)	0.01
Basal-like	205 (18)	24 (14)	14 (18)	26 (29)	141 (18)	
Her2-positive	73 (6)	13 (8)	7 (9)	7 (8)	46 (6)	
Normal-like	131 (12)	18 (10)	13 (17)	15 (17)	85 (11)	
Tumor size (cm)						
≤2	540 (49)	84 (49)	32 (43)	37 (42)	387 (50)	0.72
>2-5	467 (42)	68 (40)	33 (45)	43 (49)	323 (41)	
>5	106 (10)	18 (11)	9 (12)	8 (9)	71 (9)	
Tumor stage (AJCC/UICC Stage Grouping)						
I	414 (37)	69 (41)	21 (28)	30 (34)	294 (38)	0.24
II	558 (50)	80 (48)	39 (53)	51 (58)	388 (50)	

Table A.2: Characteristics of study population by last birth recency group, in the CBCS Phases I and II[a].

Characteristics	Overall (n=1139)	Nulliparous (n=173)	<5 years (n=76)	5-<10 years (n=89)	≥ 10 years (n=801)	P-value
III+IV	136 (12)	19 (11)	14 (19)	7 (8)	96 (12)	
Histology group						
Ductal	938 (82)	148 (86)	69 (91)	72 (81)	649 (81)	0.11
Others	201 (18)	25 (14)	7 (9)	17 (19)	152 (19)	
Nuclear grade[c]						
Pleomorphism	211 (43)	33 (42)	18 (55)	22 (56)	138 (41)	0.14
Slight/moderate	279 (57)	45 (58)	15 (45)	17 (44)	202 (59)	
Histologic grade[c]						
Well/moderate	172 (35)	27 (35)	4 (12)	15 (38)	126 (37)	0.04
Poor	318 (65)	51 (65)	29 (88)	24 (62)	214 (63)	
Mitotic index[c]						
Low	264 (54)	44 (56)	13 (39)	17 (44)	190 (56)	0.15
High	224 (46)	34 (44)	20 (61)	22 (56)	148 (44)	

[a] P-values for the comparisons across birth recency were calculated by t test for continuous variables and x2 test for categorical variables except that when expected cell count was less than 5, they were calculated by Fisher exact test. Missing values were excluded from percentage calculations.

[b] First degree

[c] Only available in Phase I

Table A.3: HRs of BC-specific mortality associated with parity and birth recency, in the CBCS Phases I and II[a]

Variable	Deaths/N	Model 1 HR (95%CI)	Model 2 HR (95%CI)	Model 3 HR (95%CI)
		Parity		
All BC patients				
nulliparous	31/173	1.32 (0.89, 1.95)	1.44 (0.94, 2.21)	1.16 (0.76, 1.78)
1-2	132/551	1.77 (1.18, 2.66)	1.76 (1.13, 2.73)	1.42 (0.91, 2.23)
3+	113/416			
Basal-like		1	1	1
nulliparous	6/24	1.05 (0.43, 2.56)	1.28 (0.49, 3.39)	1.44 (0.52, 4.03)
1-2	30/109	1.52 (0.61, 3.82)	1.56 (0.58, 4.21)	1.45 (0.52, 4.05)
3+	26/72			
Luminal		1	1	1
nulliparous	13/118	2.02 (1.12, 3.64)	2.12 (1.14, 3.91)	1.46 (0.78, 2.75)
1-2	78/349	2.54 (1.38, 4.68)	2.34 (1.22, 4.47)	1.56 (0.81, 3.03)
3+	62/264	1.32 (0.89, 1.95)	1.44 (0.94, 2.21)	1.16 (0.76, 1.78)
		Birth recency		
All BC patients				
nulliparous	31/173	1	1	1
<5 years	28/76	1.83 (1.07, 3.13)	1.90 (1.10, 3.34)	1.37 (0.77. 2.45)
5-<10 years	27/89	1.42 (0.84, 2.40)	1.45 (0.83, 2.55)	1.09 (0.61, 1.95)
10+ years	190/801	1.43 (0.97, 2.11)	1.51 (0.99, 2.29)	1.26 (0.82, 1.93)
Basal-like				
nulliparous	6/24	1	1	1
<5 years	5/14	1.40 (0.41, 4.72)	1.55 (0.43, 5.56)	1.38 (0.38, 5.08)
5-<10 years	8/26	1.07 (0.36, 3.21)	1.21 (0.38, 3.89)	0.84 (0.24, 2.89)
10+ years	43/141	1.22 (0.51, 2.94)	1.41 (0.54, 3.68)	1.69 (0.62, 4.63)
Luminal				
nulliparous	13/118	1	1	1
<5 years	18/42	3.66 (1.73, 7.75)	3.78 (1.74, 8.19)	2.35 (1.05, 5.27)
5-<10 years	14/41	2.91 (1.35, 6.27)	2.84 (1.23, 6.57)	2.10 (0.89, 4.94)
10+ years	108/529	1.94 (1.08, 3.47)	1.90 (1.03, 3.50)	1.30 (0.69, 2.43)

[a] Model 1 was adjusted for age, race, and study phase; model 2 was additionally adjusted for income and education; model 3 was additionally adjusted for tumor stage, tumor size, lymph node status, and histological type.

Table A.4: HRs of all-cause mortality associated with parity and birth recency, in the CBCS Phases I and II[a]

Variable	Deaths/N	Model 1 HR (95%CI)	Model 2 HR (95%CI)	Model 3 HR (95%CI)
		Parity		
All BC patients				
nulliparous	65/173	1	1	1
1-2	195/551	0.97 (0.73, 1.28)	1.03 (0.76, 1.39)	0.91 (0.67, 1.24)
3+	190/416	1.09 (0.82, 1.45)	1.05 (0.77, 1.43)	0.93 (0.67, 1.28)
Basal-like				
nulliparous	12/24	1	1	1
1-2	38/109	0.67 (0.34, 1.30)	0.84 (0.40, 1.68)	0.90 (0.42, 1.94)
3+	36/72	0.97 (0.48, 1.94)	0.96 (0.45, 2.03)	0.91 (0.42, 1.99)
Luminal				
nulliparous	40/118	1	1	1
1-2	125/349	1.14 (0.80, 1.63)	1.16 (0.80, 1.69)	0.99 (0.67, 1.46)
3+	118/264	1.17 (0.81, 1.69)	1.08 (0.74, 1.60)	0.92 (0.61, 1.39)
		Birth recency		
All BC patients				
nulliparous	65/173	1	1	1
<5 years	30/76	1.29 (0.81, 2.05)	1.31 (0.81, 2.12)	1.03 (0.63, 1.71)
5-<10 years	31/89	1.05 (0.68, 1.64)	1.12 (0.70, 1.78)	0.95 (0.58, 1.54)
10+ years	323/801	0.99 (0.75, 1.29)	1.00 (0.75, 1.33)	0.90 (0.66, 1.22)
Basal-like				
nulliparous	12/24	1	1	1
<5 years	6/14	1.00 (0.36, 2.74)	1.06 (0.37, 3.04)	0.98 (0.33, 2.90)
5-<10 years	8/26	0.64 (0.25, 1.63)	0.76 (0.29, 2.05)	0.57 (0.20, 1.64)
10+ years	60/141	0.77 (0.40, 1.49)	0.86 (0.42, 1.76)	0.98 (0.47, 2.06)
Luminal				
nulliparous	40/118	1	1	1
<5 years	18/42	1.96 (1.07, 3.61)	2.01 (1.07, 3.76)	1.52 (0.79, 2.94)
5-<10 years	17/41	1.81 (1.00, 3.27)	1.86 (0.97, 3.56)	1.62 (0.83, 3.15)
10+ years	207/529	1.06 (0.75, 1.49)	1.02 (0.71, 1.46)	0.87 (0.59, 1.28)

[a] Model 1 was adjusted for age, race, and study phase; model 2 was additionally adjusted for income and education; model 3 was additionally adjusted for tumor stage, tumor size, lymph node status, and histological type.

Table A.5: HRs of BC-specific mortality associated with parity and birth recency, by follow-up time, in the CBCS Phases I and II[a].

Parity	HR (95%CI)		Birth recency	HR (95%CI)	
	≤ 5 years	> 5 years		≤ 5 years	> 5 years
All BC patients			All BC patients		
nulliparous	1	1	nulliparous	1	1
1-2	1.22 (0.75, 2.00)	1.49 (0.78, 2.85)	<5 years	1.59 (0.81, 3.13)	2.28 (0.99, 5.23)
3+	1.43 (0.86, 2.39)	2.41 (1.25, 4.65)	5-<10 years	1.16 (0.58, 2.31)	1.89 (0.84, 4.25)
			10+ years	1.28 (0.79, 2.08)	1.73 (0.84, 4.25)
Basal-like			Basal-like		
nulliparous	1	1	nulliparous	1	1
1-2	1.03 (0.39, 2.72)	1.13 (0.13, 9.71)	<5 years	1.64 (0.46, 5.86)	-
3+	1.28 (0.46, 3.52)	2.90 (0.35, 23.90)	5-<10 years	0.95 (0.28, 3.24)	1.68 (0.15, 18.84)
			10+ years	1.09 (0.42, 2.85)	1.93 (0.24, 15.24)
Luminal			Luminal		
nulliparous	1	1	nulliparous	1	1
1-2	1.47 (0.68, 3.17)	2.91 (0.15, 7.35)	<5 years	1.86 (0.63, 5.53)	6.65 (2.29, 19.33)
3+	1.60 (0.72, 3.57)	4.08 (1.59, 10.47)	5-<10 years	1.68 (0.54, 5.19)	4.91 (1.63, 14.75)
			10+ years	1.44 (0.68, 3.05)	2.78 (1.11, 6.96)

[a]The association was adjusted for age, race, and study phase.

Table A.6: Characteristics of study population by BMI group, in the CBCS Phases I and II[a].

Characteristics	Overall (n=1109)	<25 kg/m^2 (n=395)	25-<30 kg/m^2 (n=308)	30+ kg/m^2 (n=411)	P-value
Age (years)					
Mean (SD)	50.60 (11.45)	48.64 (11.72)	51.47 (11.15)	51.82 (11.19)	<0.01
<40	188 (17)	90 (23)	42 (14)	56 (14)	<0.01
40-49	427 (39)	163 (41)	116 (38)	148 (36)	
50-59	220 (20)	56 (14)	68 (22)	96 (23)	
≥60	274 (25)	84 (21)	80 (26)	110 (27)	
Menopausal status					
Premenopausal	541 (49)	209 (53)	138 (45)	194 (47)	0.08
Postmenopausal	568 (51)	184 (47)	168 (55)	216 (53)	
Race					
White	612 (55)	301 (77)	173 (57)	138 (34)	<0.01
African American	497 (45)	92 (23)	133 (43)	272 (66)	
Number of full-term pregnancy					
Nulliparous	168 (15)	73 (18)	44 (14)	51 (12)	<0.01
1-2	541 (49)	214 (54)	158 (52)	169 (41)	
≥3	400 (36)	106 (27)	104 (34)	190 (46)	
Years since last full-term pregnancy among parous women					
<5	72 (8)	38 (12)	15 (6)	19 (5)	<0.01
5-<10	86 (9)	35 (11)	29 (11)	22 (6)	
≥10	783 (83)	249 (77)	218 (83)	318 (89)	
Family history of breast cancer[b]					
Yes	176 (16)	57 (15)	50 (17)	69 (17)	0.66
No	901 (84)	326 (85)	246 (83)	331 (83)	
Education					
Lower than high school	187 (17)	41 (10)	42 (14)	104 (25)	<0.01

Table A.6: Characteristics of study population by BMI group, in the CBCS Phases I and II[a].

Characteristics	Overall (n=1109)	<25 kg/m^2 (n=395)	25-<30 kg/m^2 (n=308)	30+ kg/m^2 (n=411)	P-value
High school/post high school	616 (56)	197 (50)	184 (60)	235 (57)	
College and above	306 (28)	155 (39)	80 (26)	71 (17)	
Family income (thousand US dollar)					
<15	235 (23)	65 (18)	56 (20)	114 (29)	<0.01
15-<30	253 (25)	61 (17)	70 (25)	122 (31)	
30-<50	250 (24)	86 (24)	77 (28)	87 (22)	
≥50	291 (28)	152 (42)	73 (26)	66 (17)	
Smoking					
Never	585 (53)	205 (52)	147 (48)	233 (57)	0.11
Former	345 (31)	117 (30)	107 (35)	121 (29)	
current	184 (17)	73 (18)	54 (18)	57 (14)	
Physical activity					
yes	566 (51)	213 (54)	159 (52)	194 (47)	0.14
no	543 (49)	180 (46)	147 (48)	216 (53)	
Alcohol consumption					
No	344 (31)	79 (20)	99 (32)	166 (40)	<0.01
Yes	764 (69)	313 (80)	207 (68)	244 (60)	
HRT					
Never	794 (72)	276 (70)	210 (69)	308 (75)	0.06
Former	218 (20)	87 (22)	69 (23)	62 (15)	
Current	95 (9)	29 (7)	27 (9)	39 (10)	
OC					
Never	368 (33)	104 (27)	110 (36)	154 (38)	<0.01
Ever	737 (67)	287 (73)	194 (64)	256 (62)	

Table A.6: Characteristics of study population by BMI group, in the CBCS Phases I and II[a].

Characteristics	Overall (n=1109)	<25 kg/m^2 (n=395)	25-<30 kg/m^2 (n=308)	30+ kg/m^2 (n=411)	P-value
Lymph node status					
Positive	439 (40)	144 (37)	130 (43)	165 (41)	0.27
Negative	665 (60)	248 (63)	175 (57)	242 (59)	
Intrinsic subtype					
Luminal	714 (64)	266 (68)	197 (64)	251 (61)	0.02
Basal-like	197 (18)	53 (13)	61 (20)	83 (20)	
Her2-positive	72 (6)	23 (6)	25 (8)	24 (6)	
Normal-like	126 (11)	51 (13)	23 (8)	52 (13)	
Tumor size (cm)					
≤2	526 (49)	205 (54)	141 (47)	180 (45)	0.09
>2-5	455 (42)	151 (39)	127 (43)	177 (44)	
>5	102 (9)	27 (7)	30 (10)	45 (11)	
Tumor stage (AJCC/UICC Stage Grouping)					
I	403 (37)	157 (41)	116 (39)	130 (33)	0.09
II	543 (50)	189 (49)	141 (47)	213 (53)	
III+IV	133 (12)	38 (10)	40 (13)	55 (14)	
Histology group					
Ductal	911 (82)	312 (79)	256 (84)	343 (84)	0.21
Others	198 (18)	81 (21)	50 (16)	67 (16)	
Nuclear grade[c]					
Pleomorphism	204 (43)	79 (42)	57 (43)	68 (44)	0.93
Slight/moderate	275 (57)	111 (58)	76 (57)	88 (56)	
Histologic grade[c]					
Well/moderate	309 (65)	125 (66)	85 (64)	99 (63)	0.89
Poor	170 (35)	65 (34)	48 (36)	57 (37)	
Mitotic index [ǂ]					
Low	259 (54)	115 (61)	58 (44)	86 (55)	0.02

Table A.6: Characteristics of study population by BMI group, in the CBCS Phases I and II[a].

Characteristics	Overall (n=1109)	<25 kg/m^2 (n=395)	25-<30 kg/m^2 (n=308)	30+ kg/m^2 (n=411)	P-value
High	218 (46)	75 (39)	73 (56)	70 (45)	

[a] P values for the comparisons across intrinsic subtypes were calculated by t test for continuous variables and x2 test for categorical variables except that when expected cell count was less than 5, they were calculated by Fisher exact test. Missing values were excluded from percentage calculations.

[b] First degree

[c] Only available in Phase I.

Table A.7: Characteristics of study population by WHR tertiles, in the CBCS Phases I and II[a].

Characteristics	Overall (n=1109)	<0.77 (n=282)	0.77-<0.84 (n=382)	0.84+ (n=445)	P-value
Age (years)					
Mean (SD)	50.60 (11.45)	46.99 (10.46)	50.33 (11.50)	53.75 (11.01)	<0.01
<40	188 (17)	83 (29)	62 (16)	43 (10)	<0.01
40-49	427 (39)	124 (44)	159 (42)	144 (32)	
50-59	220 (20)	36 (13)	70 (18)	114 (26)	
≥60	274 (25)	39 (14)	91 (24)	144 (32)	
Menopausal status					
Premenopausal	541 (49)	180 (64)	197 (52)	164 (37)	<0.01
Postmenopausal	568 (51)	102 (36)	185 (48)	281 (63)	
Race					
White	612 (55)	222 (79)	228 (60)	162 (36)	<0.01
African American	497 (45)	60 (21)	154 (40)	283 (64)	
Number of full-term pregnancy					
Nulliparous	168 (15)	53 (19)	52 (14)	63 (14)	<0.01
1-2	541 (49)	160 (57)	204 (53)	177 (40)	
≥3	400 (36)	69 (24)	126 (33)	205 (46)	
Years since last full-term pregnancy among parous women					
<5	72 (8)	30 (13)	27 (8)	15 (4)	<0.01
5-<10	86 (9)	31 (14)	32 (10)	23 (6)	
≥10	783 (83)	168 (73)	271 (82)	345 (90)	
Family history of breast cancer[b]					
Yes	176 (16)	239 (86)	318 (83)	356 (82)	0.52
No	901 (84)	40 (14)	63 (17)	76 (18)	
Education					
Lower than high school	187 (17)	12 (4)	52 (14)	123 (28)	<0.01

Table A.7: Characteristics of study population by WHR tertiles, in the CBCS Phases I and II[a].

Characteristics	Overall (n=1109)	<0.77 (n=282)	0.77-<0.84 (n=382)	0.84+ (n=445)	P-value
High school/post high school	616 (56)	145 (51)	213 (56)	258 (58)	
College and above	306 (28)	125 (44)	117 (31)	64 (14)	
Family income (thousand US dollar)					
<15	235 (23)	29 (11)	69 (19)	137 (34)	<0.01
15-<30	253 (25)	46 (17)	85 (24)	122 (30)	
30-<50	250 (24)	62 (23)	90 (25)	98 (24)	
≥50	291 (28)	128 (48)	116 (32)	47 (12)	
Smoking					
Never	585 (53)	166 (58)	211 (54)	215 (48)	<0.01
Former	345 (31)	92 (32)	120 (31)	135 (30)	
current	184 (17)	26 (9)	58 (15)	101 (22)	
Alcohol consumption					
No	344 (31)	52 (18)	123 (32)	169 (38)	<0.01
Yes	764 (69)	230 (82)	258 (68)	276 (62)	
Physical activity					
no	566 (51)	130 (46)	182 (48)	231 (52)	0.25
yes	543 (49)	152 (54)	200 (52)	214 (48)	
HRT					
Never	794 (72)	208 (74)	267 (70)	319 (72)	0.35
Former	218 (20)	58 (21)	77 (20)	83 (19)	
Current	95 (9)	16 (6)	37 (10)	42 (9)	
OC					
Never	368 (33)	54 (19)	115 (30)	199 (45)	<0.01
Ever	737 (67)	226 (81)	266 (70)	245 (55)	

Table A.7: Characteristics of study population by WHR tertiles, in the CBCS Phases I and II[a].

Characteristics	Overall (n=1109)	<0.77 (n=282)	0.77-<0.84 (n=382)	0.84+ (n=445)	P-value
Lymph node status					
Positive	439 (40)	103 (36)	153 (40)	183 (41)	0.45
Negative	665 (60)	178 (64)	227 (60)	260 (59)	
Intrinsic subtype					
Luminal	714 (64)	199 (71)	236 (62)	279 (63)	0.01
Basal-like	197 (18)	36 (13)	80 (21)	81 (18)	
Her2-positive	72 (6)	20 (7)	30 (8)	22 (5)	
Normal-like	126 (11)	27 (10)	36 (9)	63 (14)	
Tumor size (cm)					
≤2	526 (49)	154 (56)	179 (48)	193 (44)	<0.01
>2-5	455 (42)	107 (39)	158 (42)	190 (44)	
>5	102 (9)	13 (5)	38 (10)	51 (12)	
Tumor stage (AJCC/UICC Stage Grouping)					
I	403 (37)	115 (42)	142 (38)	146 (34)	0.01
II	543 (50)	139 (51)	188 (50)	216 (50)	
III+IV	133 (12)	20 (7)	45 (12)	68 (16)	
Nuclear grade[c]					
Pleomorphism	204 (43)	59 (44)	92 (56)	76 (57)	0.72
Slight/moderate	275 (57)	76 (56)	73 (44)	59 (44)	
Histologic grade[c]					
Well/moderate	170 (35)	47 (35)	53 (32)	70 (39)	0.39
Poor	309 (65)	88 (65)	112 (68)	109 (61)	
Histology group					
Ductal	911 (82)	224 (79)	307 (80)	380 (85)	0.07
Others	198 (18)	58 (21)	75 (20)	65 (15)	
Mitotic index[c]					

Table A.7: Characteristics of study population by WHR tertiles, in the CBCS Phases I and II[a].

Characteristics		Overall (n=1109)	<0.77 (n=282)	0.77-<0.84 (n=382)	0.84+ (n=445)	P-value
	Low	259 (54)	79 (59)	84 (51)	96 (54)	0.38
	High	218 (46)	55 (41)	81 (49)	82 (46)	

[a] The cutoff points of tertiles were 0.77 and 0.84 based on WHR distribution of controls of CBCS Phase I&II. P-values for the comparisons across intrinsic subtypes were calculated by t test for continuous variables and x2 test for categorical variables except that when expected cell count was less than 5, they were calculated by Fisher exact test. Missing values were excluded from percentage calculations.
[b] First degree
[c] Only available in Phase I.

Table A.8: HRs for overall mortality associated with BMI and WHR, in the CBCS Phases I and II[a]

Variable	Deaths/N	Model 1 HR (95%CI)	Model 2 HR (95%CI)	Model 3 HR (95%CI)
		BMI		
All BC patients				
<25 kg/m^2	129/395	1	1	1
25-<30 kg/m^2	125/308	1.18 (0.91, 1.51)	1.28 (0.98, 1.68)	1.14 (0.86, 1.50)
≥30 kg/m^2	184/411	1.23 (0.96, 1.57)	1.19 (0.91, 1.55)	1.11 (0.84, 1.45)
Basal-like				
<25 kg/m^2	16/53	1	1	1
25-<30 kg/m^2	27/62	1.49 (0.79, 2.81)	1.90 (0.93, 3.85)	1.65 (0.79, 3.45)
≥30 kg/m^2	41/83	1.92 (1.04, 3.54)	2.25 (1.14, 4.46)	2.04 (1.01, 4.13)
Luminal				
<25 kg/m^2	84/268	1	1	1
25-<30 kg/m^2	80/198	1.22 (0.89, 1.67)	1.33 (0.95, 1.85)	1.24 (0.88, 1.74)
≥30 kg/m^2	111/251	1.17 (0.86, 1.61)	1.12 (0.80, 1.57)	1.01 (0.71, 1.44)
		WHR		
All BC patients				
<0.77	79/284	1	1	1
0.77-<0.84	142/389	1.30 (0.98, 1.72)	1.25 (0.92, 1.68)	1.08 (0.79, 1.47)
≥0.84	221/451	1.68 (1.27, 2.23)	1.50 (1.11, 2.05)	1.25 (0.91, 1.72)
Basal-like				
<0.77	13/36	1	1	1
0.77-<0.84	37/81	1.37 (0.70, 2.65)	1.43 (0.69, 2.93)	1.26 (0.61, 2.62)
≥0.84	34/83	1.11 (0.56, 2.23)	0.88 (0.40, 1.95)	0.87 (0.39, 1.93)
Luminal				
<0.77	53/201	1	1	1
0.77-<0.84	77/240	1.15 (0.81, 1.66)	1.09 (0.74, 1.60)	0.89 (0.60, 1.33)
≥0.84	148/282	1.97 (1.40, 2.78)	1.75 (1,20, 2.56)	1.33 (0.89, 1.97)

[a] Model 1 was adjusted for age, race, and study phase; model 2 was additionally adjusted for income, education, physical activity, alcohol intake, smoking, and parity; model 3 was additionally adjusted for tumor stage, tumor size, lymph node status, and histological type.

Table A.9: HRs for BC-specific mortality associated with BMI and WHR, in the CBCS Phases I and II[a]

Variable	Deaths/N	Model 1 HR (95%CI)	Model 2 HR (95%CI)	Model 3 HR (95%CI)
		BMI		
All BC patients				
<25 kg/m^2	84/395	1	1	1
25-<30 kg/m^2	79/308	1.22 (0.89, 1.68)	1.27 (0.91, 1.78)	1.07 (0.76, 1.52)
≥30 kg/m^2	106/411	1.16 (0.85, 1.59)	1.06 (0.75, 1.48)	0.97 (0.68, 1.37)
Basal-like				
<25 kg/m^2	10/53	1	1	1
25-<30 kg/m^2	20/62	1.93 (0.89, 4.17)	2.03 (0.85, 4.84)	1.40 (0.56, 3.48)
≥30 kg/m^2	30/83	2.06 (0.97, 4.36)	2.21 (0.94, 5.21)	1.67 (0.69, 4.05)
Luminal				
<25 kg/m^2	53/268	1	1	1
25-<30 kg/m^2	42/198	1.12 (0.74, 1.70)	1.16 (0.74, 1.81)	1.04 (0.66, 1.64)
≥30 kg/m^2	55/251	1.05 (0.69, 1.60)	0.95 (0.60, 1.49)	0.84 (0.52, 1.36)
		WHR		
All BC patients				
<0.77	61/284	1	1	1
0.77-<0.84	94/389	1.21 (0.87, 1.69)	1.09 (0.77, 1.54)	0.95 (0.66, 1.36)
≥0.84	118/451	1.37 (0.97, 1.91)	1.14 (0.79, 1.65)	0.91 (0.62, 1.34)
Basal-like				
<0.77	8/36	1	1	1
0.77-<0.84	29/81	1.64 (0.73, 3.69)	1.49 (0.64, 3.46)	1.35 (0.58, 3.15)
≥0.84	24/83	1.25 (0.53, 2.97)	0.98 (0.38, 2.49)	0.94 (0.37, 2.41)
Luminal				
<0.77	41/201	1	1	1
0.77-<0.84	44/240	0.98 (0.63, 1.51)	0.92 (0.57, 1.47)	0.75 (0.46, 1.22)
≥0.84	67/282	1.41 (0.92, 2.17)	1.15 (0.71, 1.85)	0.82 (0.50, 1.36)

[a] Model 1 was adjusted for age, race, and study phase; model 2 was additionally adjusted for income, education, physical activity, alcohol intake, smoking, and parity; model 3 was additionally adjusted for tumor stage, tumor size, lymph node status, and histological type.

Table A.10: HRs of overall deaths associated with BMI and WHR, by follow-up time, in the CBCS Phases I and II[a].

BMI	HR (95%CI)		WHR	HR (95%CI)	
	≤ 5 years	> 5 years		≤ 5 years	> 5 years
All BC patients			All BC patients		
<25 kg/m^2	1	1	≥ 0.77	1	1
25-<30 kg/m^2	1.04 (0.72, 1.50)	1.25 (0.90, 1.72)	0.77-<0.84	1.27 (0.85, 1.89)	1.20 (0.83, 1.73)
≥30 kg/m^2	1.11 (0.62, 1.44)	1.28 (0.94, 1.74)	≥0.84	1.34 (0.91, 1.98)	1.82 (1.29, 2.58)
Basal-like			Basal-like		
<25 kg/m^2	1	1	≥ 0.77	1	1
25-<30 kg/m^2	1.22 (0.59, 2.53)	2.10 (0.71, 6.23)	0.77-<0.84	1.23 (0.58, 2.62)	1.49 (0.52, 4.26)
≥30 kg/m^2	1.44 (0.72, 2.87)	3.15 (1.13, 8.79)	≥0.84	0.95 (0.43, 2.08)	1.31 (0.45, 3.80)
Luminal			Luminal		
<25 kg/m^2	1	1	≥ 0.77	1	1
25-<30 kg/m^2	1.13 (0.67, 1.90)	1.21 (0.83, 1.75)	0.77-<0.84	0.94 (0.52, 1.73)	1.18 (0.77, 1.79)
≥30 kg/m^2	1.07 (0.65, 1.74)	1.15 (0.80, 1.66)	≥0.84	1.63 (0.96, 2.78)	1.98 (1.33, 2.94)

[a] The association was adjusted for age, race, and study phase.

APPENDIX B: FIGURES

Figure B.1: Overall survival by parity and birth recency, overall, among luminal tumors, and among basal- tumors.

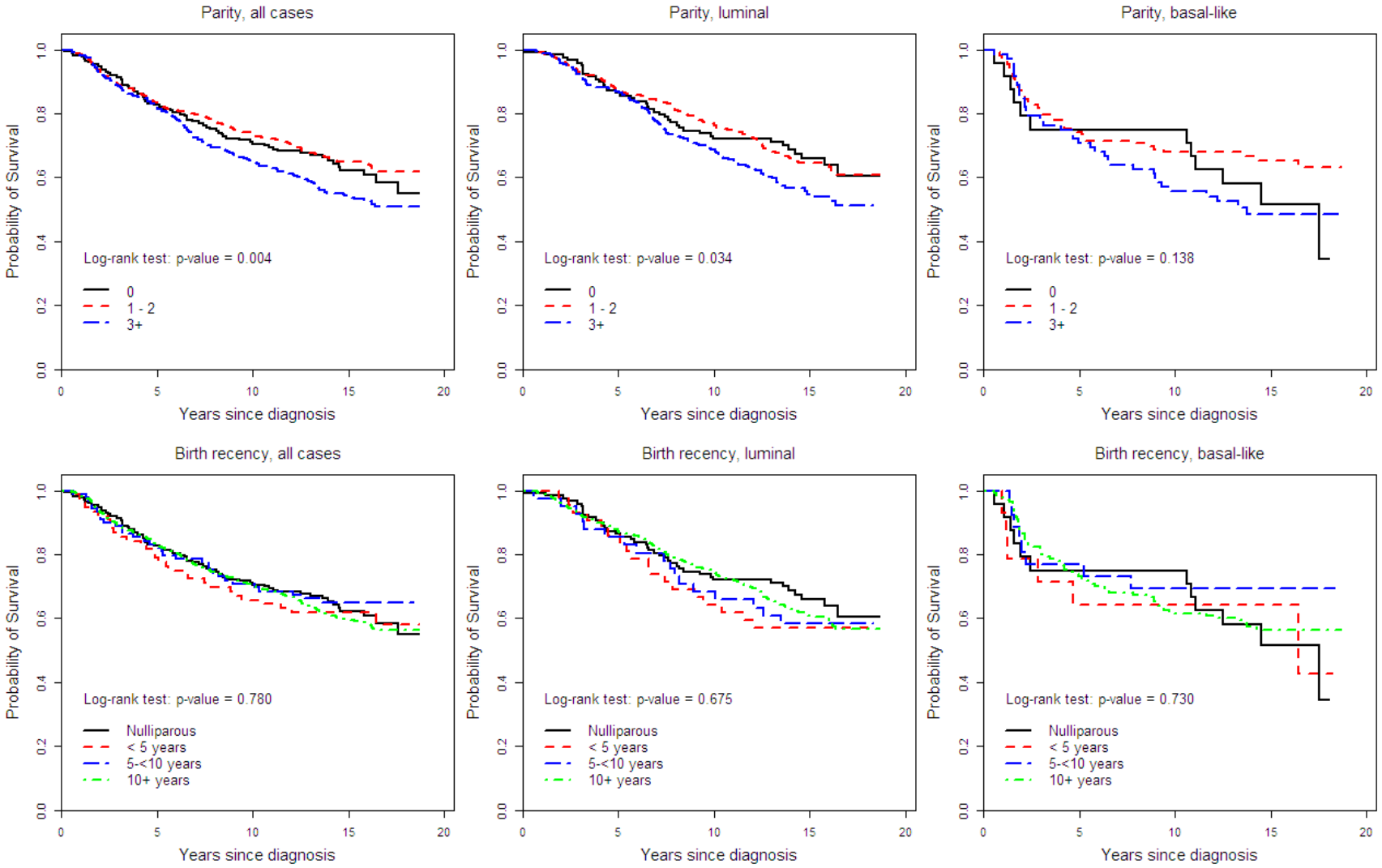


Figure B.2: BC-specific survival by parity and last birth recency, overall, among luminal tumors, and among basal- tumors.

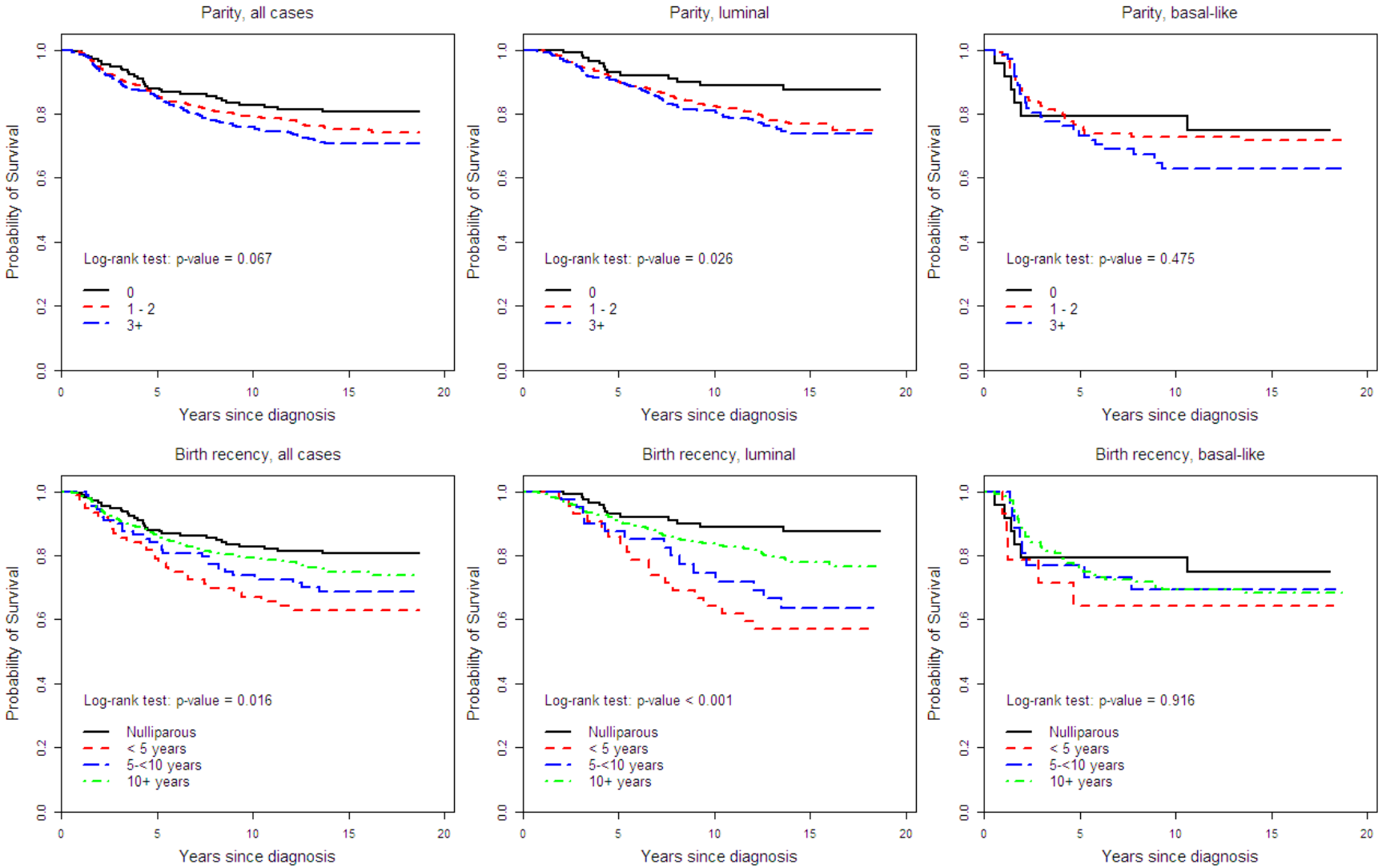
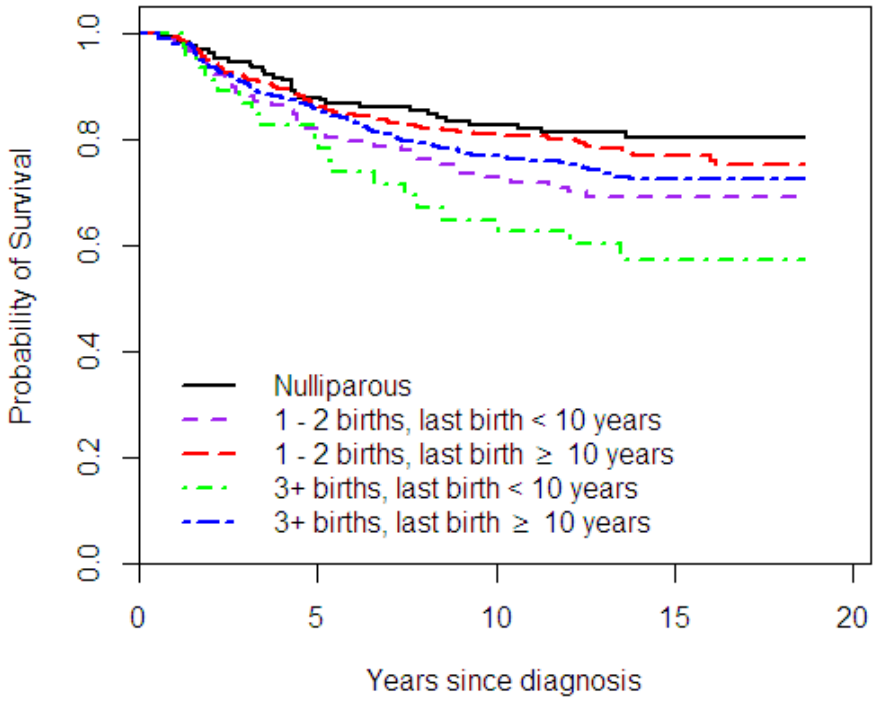


Figure B.3: BC-specific survival by multiparity-recency groups, in the CBCS Phases I and II.

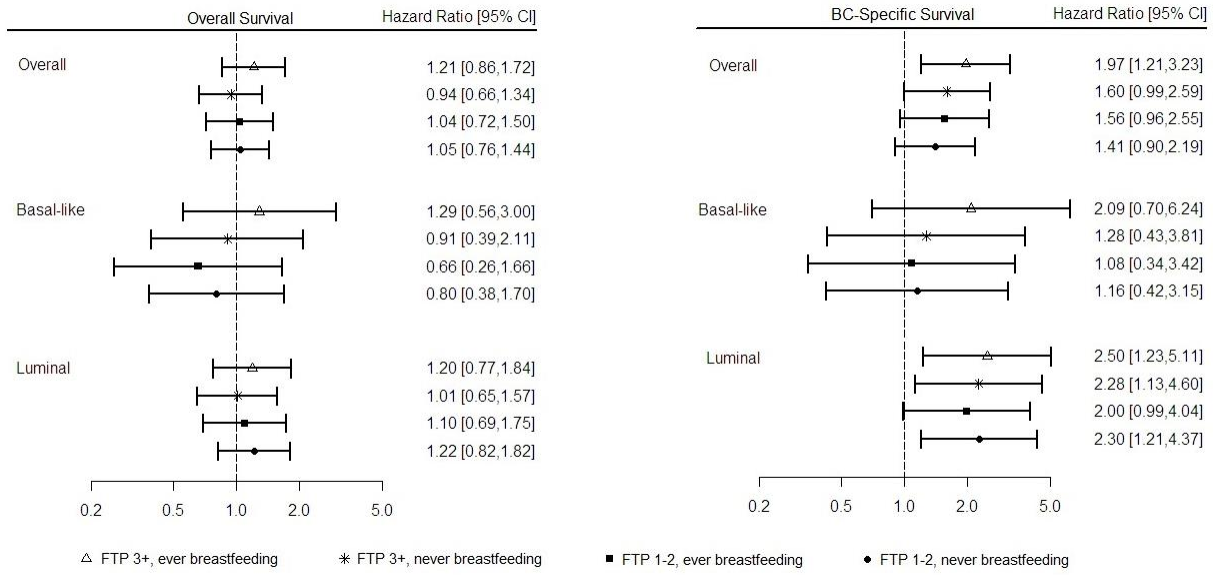


HRs of BC-specific mortality associated with parity and birth recency[a].

Nulliparous (reference, HR=1)	1-2	3+	HRs regardless parity
Time since last birth ≥10 years	1.42 (0.92, 2.21)	1.47 (0.87, 2.50)	1.51 (1.00, 2.30)
Time since last birth <10 years	1.69 (1.06, 2.67)	2.02 (1.09, 3.73)	1.65 (1.01, 2.68)
HRs regardless recency	1.44 (0.94, 2.19)	1.76 (1.13, 2.73)	

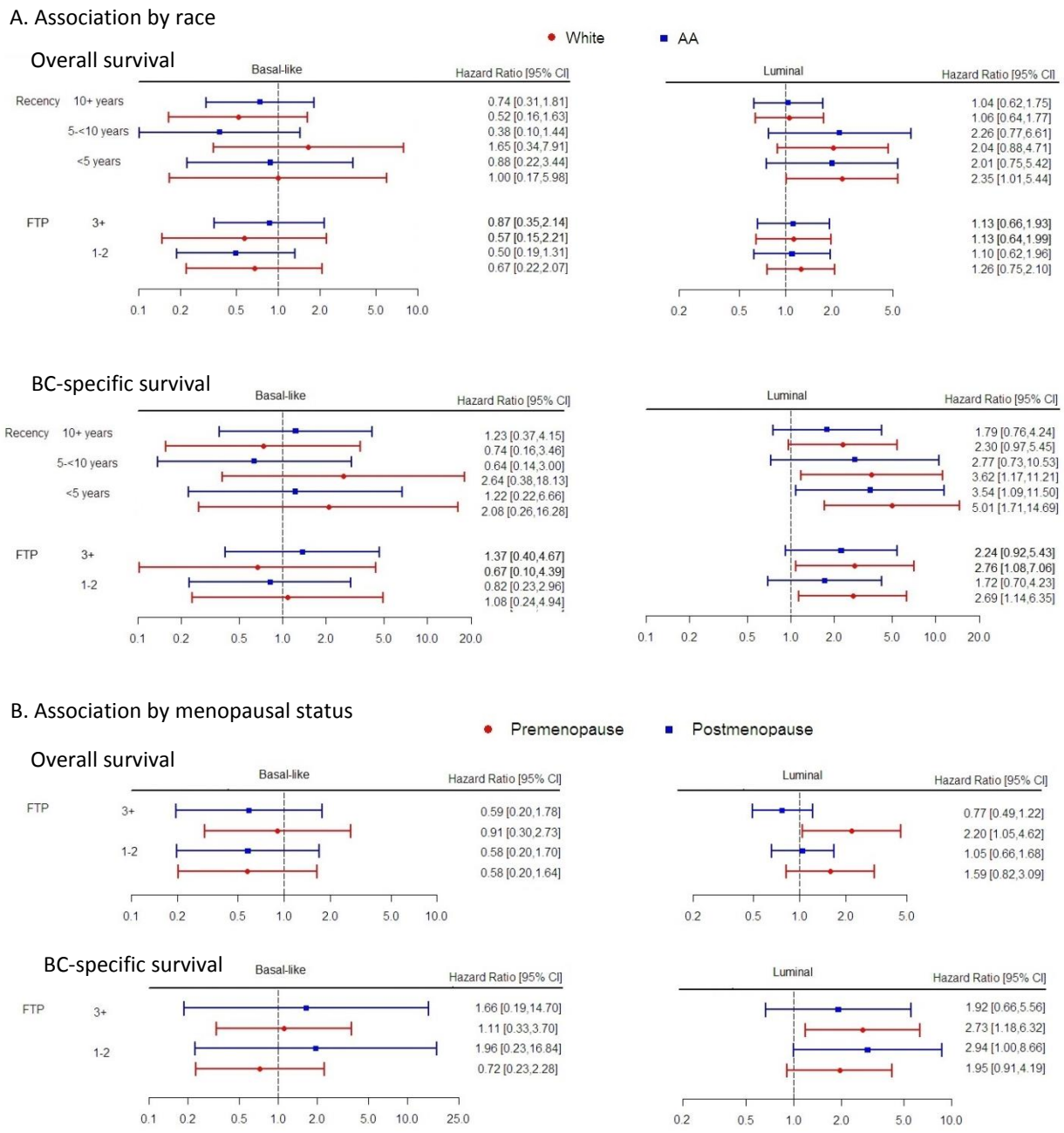
[a]HRs were adjusted for age, race, study phase, income and education

Figure B.4: HRs of overall and BC-specific death associated with variables of parity-breastfeeding and last birth-breastfeeding, respectively.



HRs were adjusted for age, race, study phase, and SES factors.

Figure B.5: HRs of overall and BC-specific deaths associated with parity and last birth, by race and menopausal status, in patients with luminal and basal-like tumors respectively.



The HRs were adjusted for age, race (in associations by menopausal status only), study phase, and SES factors.

Figure B.6: Overall survival by BMI and WHR, overall, among luminal tumors, and among basal- tumors.

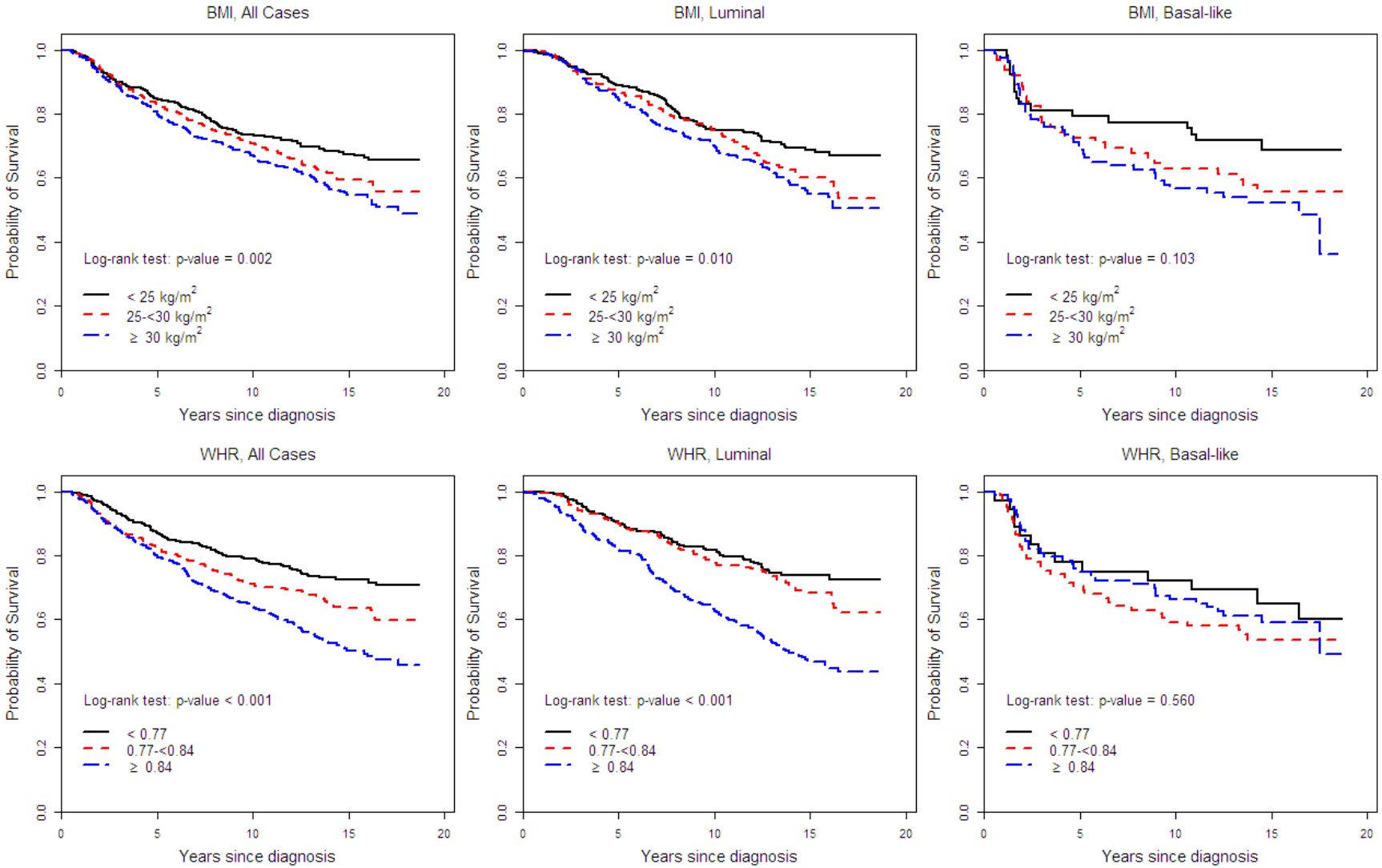


Figure B.7: BC-specific survival by BMI and WHR, overall, among luminal tumors, and among basal- tumors.

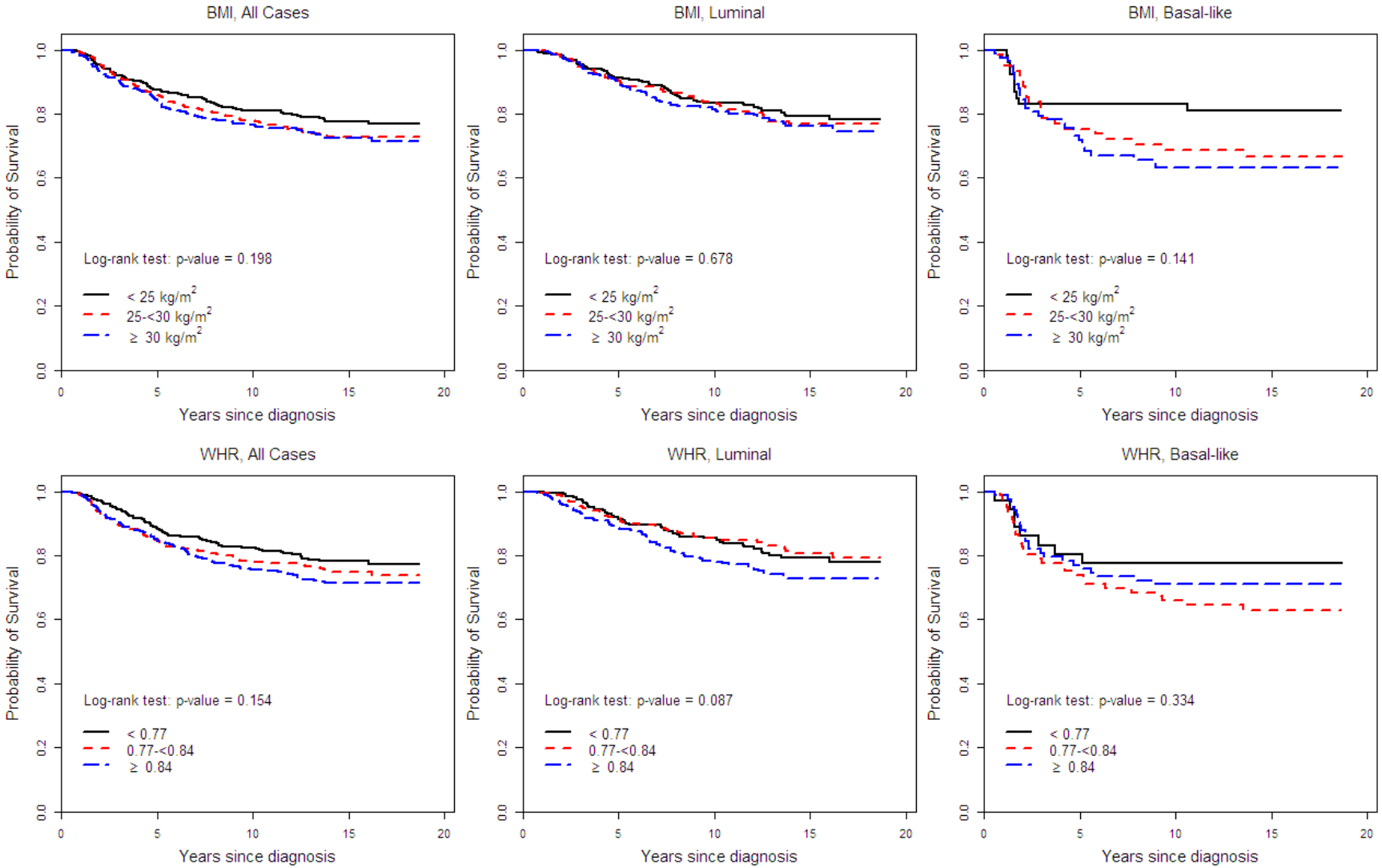
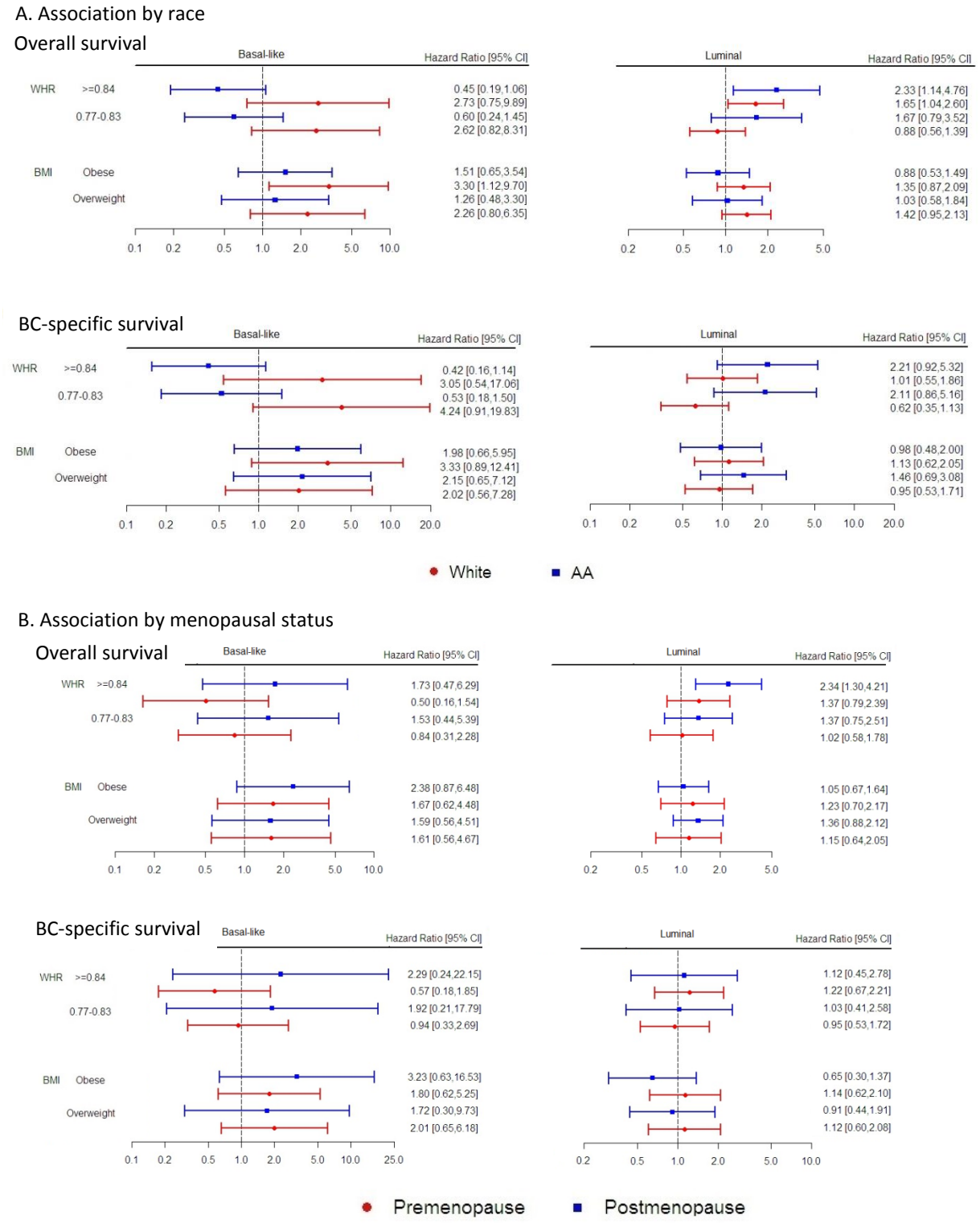


Figure B.8: HRs of overall and BC-specific deaths associated with parity and birth recency, by race and menopausal status, in patients with luminal and basal-like tumors respectively.



The HRs were adjusted for age, race (in associations by menopausal status only), study phase, and SES factors.

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