Breast Cancer Risk Factors and Associations with Breast Cancer Tumor Characteristics in High Risk Populations

Meghan E. Work

Submitted in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy
under the Executive Committee
of the Graduate School of Arts and Sciences

COLUMBIA UNIVERSITY
2018

Abstract

Breast Cancer Risk Factors and Associations with Breast Cancer Tumor Characteristics in High Risk Populations

Meghan E. Work

Background: Estrogen receptor (ER)- and progesterone receptor (PR)-negative (ER-PR-) breast cancer is associated with higher grade and poorer prognosis compared with other breast cancer subtypes. High parity, coupled with lack of breastfeeding, has been associated with an increased risk of ER-PR- cancer. The mechanism of this etiology is unclear, and may be obfuscated by ER and PR correlation with each other as well as other prognostic tumor characteristics.

Methods: Using population-based and clinic-based ascertained cases and controls from the Breast Cancer Family Registry, I examined reproductive risk factors, including parity, breastfeeding, and oral contraceptive (OC) use, in relation to ER and PR status, using polytomous logistic regression (for the population-based data) and the method of generalized estimating equations (GEE) (for the clinic-based data) as well as the pseudo-conditional likelihood approach, which accounts for correlated outcome variables.

Results: High parity (≥ 3 live births) combined with lack of breastfeeding, was positively associated with ER-PR- tumors (odds ratio [OR]=1.57, 95% confidence interval [CI] 1.10-2.24, population-based cases vs. controls) relative to nulliparity. There was no association with ER-PR- tumors and parity in women who breastfed (OR=0.93, 95%CI 0.71-1.22) relative to nulliparous women. Associations with ER-PR- cancer were higher across all races/ethnicities among women who did not breastfeed compared with women who did. Population-based and clinic-based data were generally in agreement (OR=2.07, 95% CI 1.09-3.91, clinic-based cases vs. controls, relative to nulliparity). When adjusted for the correlation of PR-status and grade, to

ER-status, the association between high parity +lack of breastfeeding and ER- status, was maintained. OC use before year 1975 was associated with an increased risk of ER-PR- tumors (OR=1.32, 95% CI 1.04-1.67, population-based data, cases vs. controls) relative to never use of OCs. For women who began OC use in 1975 or later there was no increased risk. Analysis of OC use in clinic-based data agreed with the findings of the population-based data.

Conclusions: My findings support that there are modifiable factors for ER-PR- breast cancer, and that breastfeeding in particular may mitigate the increased risk of ER-PR-cancers seen from multiparity. The mechanism of both risk and risk mitigation may operate primarily through the estrogen, rather than progesterone, pathway.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

Chapter 1—Risk Factors and Breast Cancer Tumor Characteristics: A Review

1A. INTRODUCTION

It is estimated that nearly 237,000 women in the United States were diagnosed with breast cancer in 2014 (the most recent year numbers were available) and more than 42,000 women died from the disease (*source:* U.S. Centers for Disease Control and Prevention). While age-specific rates of invasive breast cancer in women 45 and above decreased between 1999 and 2003 [1], these rates stabilized by 2008, and the rate in 2016 was expected to be similar to 2008 [2]. Meanwhile, rates among women under the age of 40 have increased slightly [3]. Mortality from breast cancer has been reduced in recent years, with increases in screening leading to early detection and treatment, and innovations in treatments for the disease resulting in longer survival times. Despite overall reductions in mortality, disparities in mortality among African-American and Caucasian breast cancer patients have persisted and even increased, such that African-American women are more likely to die from their breast cancer than Caucasians [4]. Studies have indicated that this disparity may partly occur due to different incidence of breast cancer "subtypes" among African Americans and Caucasians, such that African-Americans have higher rates of breast cancer subtypes that are more resistant to treatment and are associated with poorer prognosis [5-8]. In addition, the increasing incidence of breast cancer among younger women is of concern because these women more commonly present with tumor characteristics, such as high tumor grade and ER- and/or PR-negativity, which render the tumor less amenable to currently available treatments and are associated with poorer prognosis [9-11].

Previous research into breast cancer etiology has identified a host of breast cancer risk factors, some of which are strong predictors of breast cancer but are of low prevalence in both the general and breast cancer population (including genetic factors such as *BRCA1* and *BRCA2* mutation and first-degree family history), and others that may be considered intermediate, but

modifiable, markers of breast cancer risk, such as breast density. In addition to these stronger factors, which confer greater than 2-fold risk, there are a number of established risk factors of high population prevalence that appear to have modest individual effects on breast cancer risk, including reproductive factors such as nulliparity, early age at menarche and late age at first birth, lack of breastfeeding, and use of exogenous hormones, including oral contraceptives and hormone replacement therapies [12, 13]. Because these latter breast cancer risk factors are typically found to be modest in effect (conferring <2 fold increased risk), it can be difficult to rule out bias as an explanation for associations, and findings are often inconsistent across studies. This may be because, rather than being a singular entity, breast cancer is a heterogeneous disease, with different etiologic factors contributing to disease causation to a greater or lesser degree depending on breast cancer type.

Due to greater understanding of tumor biology and of the importance of tumor markers, breast cancer, like many other cancers, is rarely regarded as a single homogenous disease, not only in terms of treatment and prognosis, but also when understanding cancer risk. The current paradigm for many cancers, including breast cancer, is to examine risk factors for cancer subdivided by tumor characteristics or biomarkers, rather than as a single entity. In breast cancer, most such studies have focused on defining breast cancer by the tumor characteristics of estrogen-receptor (ER) and progesterone receptor (PR) expression, and, beginning in the early 2000s, by human epidermal growth factor receptor 2 (HER2) expression, as these tumor characteristics are relevant for determining treatment options and cancer prognosis and proliferation rate. Tumor grade is a characteristic that is less commonly examined according to risk factor; however it is an important prognostic characteristic that has been demonstrated in some studies to differ according to risk factor [5, 14]. High tumor grade has been associated with self-reported family

history of breast cancer [15], and in breast cancers occurring among those with *BRCA1* or *BRCA2* positivity [16].

1B. BACKGROUND

1.B.1 Overview of Breast Cancer Subtypes

In the past 20 years, an increasingly abundant literature has surfaced to describe how known risk factors for breast cancer may differ among the different "subtypes" of breast cancer as defined by ER and PR status, by grade, and by molecular subtype. If risk factors differ among subtypes, then breast cancer should not be considered as a single disease entity whose development is a result of the same set of causal partners, but rather should be considered a heterogeneous condition. Indeed, from a treatment perspective, this division is already accepted: women who are both ER and PR negative are not treated with adjuvant hormonal therapies such as tamoxifen, which are ineffective in such women, while women of any ER/PR status who have breast cancer that presents as HER2+ are successfully treated with trastuzumab [17, 18].

1.B.2 Breast Cancer Subtypes by Hormone Receptor Expression

ER and PR status are the most widely studied markers in breast tissue [19]. While clinical and pathologic differences between breast cancers defined by ER and PR status are established, epidemiologic studies that have compared risk factors for receptor positive vs. receptor negative tumors have had ambivalent findings for some individual risk factors. However, they have established that risk factors for ER and PR status do differ by subtype, indicating the heterogeneous nature of breast cancer. Previous studies, the majority of which are case-control, typically compare ER+ breast cancer cases, and ER- cases, to controls, or consider ER and PR status jointly to arrive at four ER/PR- defined classifications, ER+PR+, ER+PR-, ER-PR+, and ER-PR-, which are typically compared to a control group that serves as a common referent

group. Less commonly, a case-only analysis is conducted, where ER+PR+ will serve as the reference group to which the other categories are compared. Because ER+PR- and ER-PR+ cases are less common, analyses are often underpowered to find statistically significant findings in these classifications. Some clinicians believe that any hormone positivity (ER+, PR+, or positive for both) should be analyzed as a group against those who are both ER and PR negative, however data have indicated that those who are positive for both markers have better treatment outcomes than those positive for only one marker [20, 21].

1.B.3 Breast Cancer "Molecular" Subtypes

The understanding of HER2 as an important tumor marker that aids in determining optimal patient treatment, and the combination of this marker with the presence of ER and PR biomarkers, has resulted in the designation of four "molecular subtypes", originally determined by intrinsic expression, but correlated with ER, PR, and HER2 status as follows: Luminal A (ER and/or PR+, HER2-), which typically comprises over 60% of the population; Luminal B (ER and/or PR+, HER2+), which accounts for about 12% of the population, HER2+ (ER and PR-, HER2+), which accounts for less than 10% of the population, and triple-negative (ER-, PR-, and HER2-) types, which accounts for about 15% of the population [5, 22]. Although sometimes the term "basal-like" is used interchangeably with triple-negative cancer, whether a tumor is basal-like is determined through tissue microarray or its IHC surrogate of CK5/6 CK14 and or EGFR expression, while determination of ER, PR, and HER2 negativity occurs through immunohistochemistry [23]. While there is overlap, not all triple-negative tumors are basal-like; the correlation between triple-negative and basal-like breast cancers is 70-80% according to studies [24, 25]. The term "triple-negative" will be used throughout this dissertation to connote that the subtype is determined immunohistochemically. The respective importance of various

risk factors, treatment effectiveness, and cancer outcome differ across these types [5, 6, 22, 26-30]. *Figure 1-1* represents the categorization of breast cancer into molecular subtypes.

1.B.4 Other options for classifying tumors

Although used less frequently, there are other appropriate sub-classifications of breast cancer for the purposes of examining risk factors by subtype. Tumors can be classified by histologic type [31-34], or by other intrinsic features that are associated with prognosis, such as tumor grade or tumor size, or features of the cancer, such as presence of positive nodes or cancer stage. However, these latter two features are largely predicated on when the tumor is diagnosed, rather than features that are intrinsic to the tumor, so examining risk factor associations with a characteristic such as stage may be complicated by factors associated with when the tumor is diagnosed (such as healthcare-seeking behavior) rather than an intrinsic function of the tumor. Lymph node status is positively correlated with grade and tumor size, however the relationship with ER and PR status is less clear; one study indicated that 46% of ER and PR positive tumors had positive nodes, while 53% of tumors that were ER and PR negative had positive nodes, indicating any correlation between ER and PR and nodal may be weak or nonexistent [35]. Additionally, expression of other receptors on tumor tissue may be of value for classification; expression of cell-cycle proteins, for example, is associated with poorer prognosis, and risk factors for cancers that do and do not express these proteins may differ [36].

1.B.5 Tumor Grade

In this dissertation, I will examine tumor grade, an important prognostic feature of breast cancer. Grade has been demonstrated to independently affect prognosis, regardless of tumor size or number of positive nodes (which represent other prognostic indicators) [37]. Tumor grade classifies cancer cells according to their appearance under a microscope (how abnormal they

look compared to normal breast tissue) and how quickly the tumor is likely to grow and spread. The Scarff-Bloom-Richardson system is the most common type of classification system used, and will be used for the data in this dissertation. In this scoring system, pathologists observe three features of the cancer cells to determine tumor grade: (1) the frequency of cell mitosis, (2) tubule formation (the percentage of cancer composed of tubular structures) and (3) nuclear pleomorphism (also known as nuclear grade), which describes the change in cell size and uniformity. Each of these features is assigned a score ranging from 1 to 3 (with 1 indicating a more normative situation, e.g., slower mitosis, fewer tubular structures, more uniform cells, and 3 indicating a more radical situation). The scores of each the cell's features are then added together for a final sum that ranges from 3 to 9. A tumor with a final sum of 3, 4, or 5 is considered to have a tumor grade of 1 (i.e., well-differentiated). A sum of 6 or 7 is Grade 2, or moderately differentiated, and a sum of 8 or 9 is Grade 3, considered poorly differentiated. *Table 1-1* describes the components of tumor grade.

1.B.6 Breast Cancer Subtypes: The Role of Family History

Family history is a well-established breast cancer risk factor, typically conferring a two-fold increased risk of breast cancer for women with one affected first-degree relative [38, 39]; this association can increase to a four-fold risk for women with 3 or more affected relatives [38]. Family history has been associated with all subtypes of breast cancer defined by hormone receptor status, both ER+ and ER-, PR+ and PR- [15, 40-47]. Therefore, family history appears to be a consistent risk factor for breast cancer across subtypes.

There has been little exploration of how family history may modify the effect of risk factors on different tumor subtypes. This may partially result from the fact that, in average risk populations,

only 5-10% of cancer patients will have a positive first-degree family history of breast cancer, limiting the power to conduct sub-analyses on persons with family history.

Familial breast cancer (FBC) is defined as either breast cancer that occurs among patients carrying mutations in the two known breast cancer susceptibility genes: *BRCA1* and *BRCA2*, or that which occurs in cases with family history of the disease, that do not carry a known susceptibility gene (typically in a first or 2nd degree relative). While as many as 90% of persons carrying *BRCA* mutations will ultimately be diagnosed with breast or a related cancer [48], most FBC patients do not carry mutations in these genes. Examination of how breast cancer subtypes and other tumor characteristics associate with family history, particularly among those who are not *BRCA1* or *2* positive, has been limited, and available studies have taken different approaches to examine this issue *(See Table 1-2).*

Perhaps a more important question regarding the role of family history on breast cancer risk is what role environmental risk factors play in breast cancer risk in the presence or absence of family history. In my comprehensive review (see below), I have separated studies of "high-risk" women and risk factors for breast cancer defined by tumor subtype, from studies of "average-risk" women. Included within the higher-risk group are studies that examine risk factors by tumor subtype among women with a first-degree family history of breast cancer. If we can understand how breast cancer risk factors affect risk in the presence of family history, women may be better able to make decisions that allow for reduction in overall risk. If, in addition, we can understand the role that these risk factors play in the etiology of subtypes with prognostic ramifications, in the presence or absence of family history, we could further tailor behavioral modifications to reduce the risk of those types of breast cancer associated with poorer survival, such as ER-PR- or triple-negative breast cancers.

Previous studies that have examined environmental breast cancer risk factors among women with a family history of BC include the Minnesota Breast Cancer Family Study, a study of 544 case families that found evidence that family history modifies the association between adolescent obesity, alcohol consumption, and OC use, and breast cancer risk. However, this study did not examine hormone-receptor defined subtypes of breast cancer. Other studies have compared strengths of associations between risk factors and breast cancer for women with a family history compared to those for women with no family history [49], but again, these associations have been examined using the outcome of invasive breast cancer as one homogenous condition, rather than via receptor-defined subtypes.

This review will separate studies that examine women at high risk of breast cancer based on factors such as family history, age at diagnosis, and *BRCA1* or *BRCA2* status from studies that examine risk factors in average-risk populations. A population will be considered to be "high risk" if the examined population is premenopausal or consists entirely of women age 55 or younger, consists of a *BRCA1* or *BRCA2* positive population, or has a first-degree family history prevalence of breast cancer of 20% or more of the population (the 20% mark is similar to the first-degree family history proportion within the population-based sites of the Breast Cancer Family Registry, the population examined in this dissertation). The review will primarily cover known reproductive and hormonal risk factors for breast cancer.

1C. COMPREHENSIVE REVIEW OF CURRENT LITERATURE ON RISK FACTORS AND BREAST CANCER TUMOR CHARACTERISTICS

1.C.1 Literature Search Criteria of Risk Factor Associations with Tumor Characteristics

1.C.1.a Literature Search for reproductive and hormonal risk factors associated with molecular subtype or similar

To date, there has been one systematic review of reproductive risk factor associations with "molecular" subtypes, defined immunohistochemically by ER, PR, and HER2 status [30], as well as a review that included the association with reproductive risk factors and HR+, HER2+ and triple negative cancer subtypes [50] and some reviews (covering etiology, genetics, treatment, prognosis) specifically of the triple-negative subtype [23, 51-53].

For the purposes of this dissertation, I conducted a literature review of all publications examining risk factor relationships with the molecular subtypes Luminal A, Luminal B, HER2+, and Triple-Negative breast cancer. Because the term "triple-negative" breast cancer did not appear until October of 2005 [23], this comprehensive review primarily covers literature published between October 1, 2005, and September 1, 2017. Prior to this date, there were a few publications that examined risk factors in relation to HER2 expression independent of ER/PR status [26, 54, 55], and these publications are included in the review.

A PubMed search using the terms:

"molecular subtype" [Title/Abstract] OR ("molecular" [Title/Abstract] AND "subtype"[Title]) AND ("breast neoplasms"[Title] OR ("breast"[Title] AND "neoplasms"[Title]) OR ("breast"[Title] AND "cancer" [Title]) OR "breast cancer" [Title]) AND ("women"[MeSH

Terms] OR "women"[Title/Abstract] OR "female"[Title/Abstract]) AND (“risk” [Title/Abstract] or “odds”[Title/Abstract] or “rate”[Title/Abstract]) AND “English”[lang]

yielded **63** studies. After excluding clinical trials and studies that dealt with prognosis, treatment, survival, recurrence, genetic factors/polymorphisms, and studies that examined risk factors outside of the scope of this dissertation (e.g. mammographic density, diet, non-steroidal anti-inflammatory use), or publications that were comments or letters, **13** studies remained for inclusion in the literature review. I then replaced the term “molecular subtype” in the above search with “triple-negative” and limited the search to title, which yielded **8** additional pertinent studies, then changed triple-negative to “basal-like” which yielded **2** additional pertinent studies. Replacing “triple-negative” with ER-PR-HER2- yielded no additional pertinent studies, and replacing “triple-negative” with “luminal” yielded no additional pertinent studies. Finally, I conducted a search that incorporated each risk factor of interest separately, to capture any remaining studies that had been missed, for example:

“triple-negative” [Title/Abstract] OR (“molecular” [Title/Abstract] AND “subtype”[Title]) AND ("breast neoplasms"[Title] OR ("breast"[Title] AND "neoplasms"[Title]) OR ("breast"[Title] AND "cancer" [Title]) OR "breast cancer" [Title]) AND ("women"[MeSH Terms] OR "women"[Title/Abstract] OR "female"[Title/Abstract]) AND (“**parity**”[MeSH Terms] or “**parity**”[Title/Abstract]) AND “English”[lang]

For each search, the relevant risk factor, such as “parity” (the bolded term in the search string above) was inserted, and “luminal” was substituted for “triple-negative” such that 2 searches were conducted for each risk factor. This process yielded a total of **11** additional relevant papers. In total, I found 13+8+2+11=**34** studies that met the initial criteria for review. The References

section of each study was examined to yield additional studies for review, however no additional studies were found. I additionally limited the review to those studies with a total sample size (cases and controls, where relevant) of 500 or more subjects, as studies with a smaller overall sample size are likely to be underpowered to detect associations in rarer subtypes (are likely to have fewer than 50 cases of HER2+, for example). This criterion eliminated 4/34 studies, yielding a body of **30** studies for review.

1.C.1.b Literature Search for reproductive and hormonal risk factors associated with ER and/or PR Status

The 30 studies ascertained above are the basis of information on risk factor associations with breast cancer classified by ER, PR, and HER2 status into the molecular subtypes denoted in *Figure 1-1*. I additionally reviewed the literature to ascertain papers on risk factors associated with breast cancer defined by ER, PR, or joint ER/PR status.

A comprehensive review on this topic was published in 2004 by M. Althuis *et al*, in the journal *Cancer Epidemiology Biomarkers and Prevention [19]*. An additional review, specifically for breastfeeding, was published in 2015 by F. Islami *et al*, in *Annals of Oncology*. Finally, a review of reproductive risk factors for HR+ tumors as well as HER2+ and triple negative tumors was published in 2014 (Anderson *et al*, *Breast Cancer Research and Treatment*). A summary of these reviews, and an additional literature review of all publications examining risk factor relationships with ER status, PR status, and joint ER/PR status published between Feb. 1, 2004 (the last date of inclusion for the Althuis review) and September 1, 2017 was combined with the review of molecular subtypes. Specifically for breastfeeding, since a recent review has been published, a review is included only for articles published after the last date of paper inclusion for the Islami

review (8/27/14). In order to identify articles published on ER/PR, or joint ER/PR status between the dates specified, a PubMed search using the terms:

(estrogen[Title/Abstract] OR progesterone[Title/Abstract] AND receptor[Title/Abstract] AND breast[Title/Abstract] AND cancer[Title/Abstract] AND **parity[Title/Abstract]**) AND (risk[Title/Abstract] OR odds[Title/Abstract] OR rate[Title/Abstract]) AND (English[lang] AND ("2004/02/01"[PDAT]: "2017/09/01"[PDAT]))

was conducted. The bolded term ("parity" in the example above) was replaced with each risk factor of interest, and a separate search run for each risk factor (parity, breastfeeding/lactation, age at first birth, age at menarche, oral contraceptive use, hormone replacement therapy) of interest, as well as for factors that may additionally be examined or adjusted for in the model (age, race, menopausal status, education, body mass index [BMI], smoking, alcohol use). Papers that classified breast cancer using the "molecular subtypes" rather than just ER and PR status were examined separately and reviewed using the criteria noted in section *1.C.1.a* above. This method yielded **102** studies for "parity", **42** studies for "breastfeeding" or "lactation", 47 studies for "contraceptive", 85 studies for "menarche", **29** studies for "age at first birth" and **150** studies for "exogenous hormone" or "hormone replacement therapy". After excluding studies that dealt with prognosis, treatment, survival, recurrence, genetic factor/polymorphisms, studies that examined risk factors outside of the scope of this dissertation (e.g. mammographic density, diet, non-steroidal anti-inflammatory use), studies that were redundant across searches, and comments or letters, the search terms above yielded a total of **56** pertinent studies (including reviews). These 56 studies are distinct from those found using the molecular subtype search terms.

Papers meeting these criteria were then examined to determine if they consisted of a sample of at least 500 subjects, and bibliographies examined to yield additional studies. Five of fifty-six papers included fewer than 500 subjects, however two of these papers (Largent *et al*, 2005 and Jia *et al*, 2015) examined risk factors and ER status in women 35 years of age or younger. Thus, I did not exclude these papers because they examined a high risk population. Examination of bibliographies yielded two additional pertinent references. Thus, **55** papers on ER/PR status published since the *Althuis* review are included in the comprehensive review.

1.C.1.c Literature Search for reproductive and hormonal risk factors associated with tumor grade

Tumor grade is under-examined in terms of its relation to typical breast cancer risk factors. No previous review of risk factor associations with tumor grade exists. To search for literature associating risk factors of interest with tumor grade, I conducted a literature search in PubMed using the following search terms:

Breast [Title/Abstract] AND cancer [Title/Abstract] AND "**parity**" [Title/Abstract] AND grade [Title/Abstract]

"Parity" was replaced in consecutive searches with the other risk factors of interest, as was done for the previous searches. **Thirteen** total pertinent studies examining tumor grade were found. The bibliographies of these studies were examined to yield additional references, revealing one other pertinent reference, for a total of **14** studies. Because of the overall paucity of papers in this field, I did not limit the sample size for inclusion of papers examining the association with relevant risk factors and grade.

1.C.2 Findings of Review

The results of the review of hormonal and reproductive risk factors for average-risk populations are summarized by risk factor of interest in Table *1-3*. The results of the comprehensive review for high-risk population are summarized in Table *1-4*. While the table of study findings of average-risk populations includes summary across multiple studies, *Table 1-4*, of high-risk populations, describes results of individual studies. To my knowledge, the below review is the first to review the literature regarding breast cancer risk factors and tumor characteristics, specifically within higher-risk populations, as well as the first review of risk factors on tumor grade.

1.C.2.a Risk Factor: Parity

ER, PR, and joint ER/PR status: The results of the Althuis *et al* review found that any parity, compared with nulliparity, reduced risk of ER positive, but not ER negative, tumors in most instances, with risk estimates ranging from 0.5-0.8 (compared with controls), and the greatest reduction in risk found for multiparous women [19]. The review's findings regarding whether PR status is affected by parity appear to indicate that PR+ women are more likely to be nulliparous [19]. Of the studies in average-risk populations published since Althuis that examine ER status, ten have supported that parity reduces ER+, but not ER- breast cancer when compared to a control group [56-65]. The review of HR+ cancers found that an inverse association between parity and HR+ breast cancer was present in 19 of 22 studies [50]. Only Iwasaki *et al* examined PR status alone, and found it unrelated to parity [66]. Four studies, by Nichols *et al*, (premenopausal women), Jia *et al* (women under age 36), Largent *et al* (women under age 35) and Bertrand *et al* (African-American women under age 45) examined parity and ER status in a

high-risk category of women [55, 67-69] . Nichols and Bertrand found results regarding ER-status similar to those found in average risk populations, while Jia found increased likelihood of ER+ tumors, with higher parity, and Largent found no association between parity and ER status *(Table 1-3 and Table 1-4).*

Regarding joint ER/PR status, Althuis *et al* found equivocal results in regards to parity, however, some of the differences in findings may have been attributable to differences in age distribution of the population studied, or selection bias associated with missing receptor status [63]. Since 2004, the additional studies that have examined the relation between parity and joint ER/PR status, including one meta-analysis [70], have usually found that parity is associated with reduced risk of ER+/PR+, but not ER-/PR- cancer (*See Table 1-3*). In three studies, higher parity was associated with an elevated risk of ER-PR- cancer, when compared with controls [71] and compared with ER+PR+ cases [65, 72], while a few studies indicated that parity did not differ by joint ER/PR status [41, 73]. One study examined a high risk population, women <50 years of age [27]. In this population, the protective effect of parity was confined to ER+/PR+ cancers (compared with controls), and increased with each additional pregnancy.

Molecular subtype: The first published paper to examine the association between parity and "molecular subtype", by Millikan *et al* in 2007, found that "basal-like" breast cancer, often used synonymously with "triple-negative" cancer, was positively associated with parity, in contrast to the findings for Luminal A cancer, where parity was protective [6]. Since Millikan's publication, many additional studies have confirmed or supported these findings [28, 59, 65, 74-80], while several studies have found no overall differential association with parity and molecular subtype [80-84]. A review article was published in 2016, which indicated that parity was protective

against developing the Luminal breast cancer subtypes, across 12 studies, of 15 evaluated [30]. For the HER2 subtype, one study indicated no association with parity [30]; however a case-only analysis indicated increased risk of HER2+ cancer, with increased parity, compared with Luminal A cancer [85]. While Kwan *et al* found no overall association between parity and molecular subtype, case-only analysis found that women who had three or more children and never breastfed were at increased risk for triple-negative or HER2+ cancers [81].

Only one study examined the association between parity and molecular subtype in a high risk population, although others limited their analyses to certain ethnicities that are at higher risk for triple-negative cancer specifically [6, 76, 79]. The study of high-risk women (younger than age 45) only examined triple-negative cancer compared to non-triple negative cancer; there was no association between parity and triple-negative cancer [68].

Grade: I found five studies that examined the association of parity and tumor grade [54, 86-88], all of which had slightly different findings. In Butt *et al*, nulliparity was more strongly associated with grade 3 tumors, compared with grade 1,2 tumors, while Albrektsen *et al* and Somasegar *et al* found no overall association between parity (vs. nulliparity) and tumor grade, but Albrektsen found that among parous women, higher parity was associated with more undifferentiated tumors, a component of tumor grade. In the two studies conducted in a high risk population, Largent *et al* and Jia *et al* found no association between parity and grade in women diagnosed with breast cancer prior to age 35 [54, 69].

1.C.2.b Risk Factor: Age at first birth

ER, PR, and joint ER/PR status: The results of the Althuis *et al* review found that a later age at first live birth was more consistently observed for ER-positive rather than ER-negative tumors,

with older ages in ER+ women associated with risk estimates ranging from 1.4 to 2.6 *(Table 1-3).* Another review in 2014 confirmed these findings, for HR+ tumors [50]. Of the studies published since the Althuis review, five studies found a positive association with late age at first birth and ER+, but not ER- breast cancer, while three studies found no such association. Althuis *et al* found no elevated risk specifically associated with PR expression [19]. Additional studies examining PR status have not found any association with PR status and age at first birth [65, 89].

In the review by Althuis, among studies that assessed joint ER/PR expression, there was a modest increase in hormone-receptor (HR) positive but not HR negative tumors among women with an older age at first birth. Most studies published since the review support this finding. A meta-analysis published in 2006, which used many of the same studies reviewed in the Althuis *et al* paper, found that women in the oldest age at first birth category were on average at 27% increased risk of ER+PR+ cancer, but age at first birth was not associated with ER-PR- cancer [70]. Five additional studies support this finding, including one in a case-only analysis of a high risk population [27]. However, other studies found positive associations with ER-PR- cancer [63, 71, 90] or with ER+ PR- cancer [73] and late age at first birth. Studies of high-risk populations have been inconsistent in regard to age at first birth and ER/PR status (*Table 1-4*).

Molecular subtype: Millikan *et al* in 2007, found that "basal-like" breast cancer was positively associated with a younger age at first full-term pregnancy, while Luminal A cancer was associated with older age at first full-term pregnancy [6]. Since Millikan's publication, three additional studies and a meta-analysis have confirmed these findings [30, 59, 74, 80], however four studies have either not found an association between age at first birth and molecular subtype [81-83] or found an association with late age at birth and HER2+, but not ER+, breast cancer [75]. Of two studies that examined the association between age at first birth and molecular

subtype in a high risk population, one found an inverse association between age at first birth and triple-negative breast cancer, and one found no association between age at first birth and subtype [68, 91]. Given the lack of agreement in findings, the relation between age at first birth and molecular subtype is unclear.

Grade: In Butt *et al*, there was a statistically significant positive association between late age at first live birth and grade 3 tumors. In Albrektsen *et al*, later age at first birth was associated with fewer high grade tumors, compared to earlier age at first birth. In the study of women <age 35, Largent *et al* found that early age (<20 years) at first full-term pregnancy was positively associated with tumors of higher grade, refuting the findings by Butt in an average risk population [54], while Jia *et al* found no association between grade and age at first birth [69].

1.C.2.c Risk Factor: Age at menarche

ER, PR, and joint ER/PR status: Older age at menarche was not associated with individual ER or PR status in the studies reviewed by Althuis in 2004, and age at menarche was not related to breast cancer risk at all, in a higher-risk population. However in case-only analysis, PR negativity was inversely associated with early age at menarche, compared to PR+ status [65]. Where ER and PR status were defined jointly, ER+/PR+ cancer was inversely associated with older age at menarche, with risk estimates ranging from 0.5 to 0.8, compared with menarche at younger ages, but was not associated with any hormone negative cancers (ER-PR+, ER+PR, ER-PR-)[19]. Since the Althuis review, a meta-analysis and 3 additional studies, including one that studied women under the age of 50, found that late age at menarche was not differentially associated in hormone-receptor positive cancers, although late age at menarche was associated with decreased breast cancer risk overall [27, 63, 70, 73, 92, 93]. In contrast, Cui *et al* found late

age at menarche was inversely associated with ER+, but not ER- tumors[57], Rosenberg *et al* found that earlier menarche (< age 12 years) was associated with ER-PR- cancer [41] and Setiawan *et al*, in the Multi-Ethnic Cohort study, found that late age at menarche (≥15) was associated with a protective effect against ER+PR+, but not ER-PR- cancers [40], as did Ritte *et al*, in their cohort study [61].

Molecular subtype: Millikan *et al* found no association between age at menarche and molecular subtype of breast cancer, in case-only analyses [6]. Additional studies have confirmed the Millikan findings [74], found a positive association between early age at menarche and the HER2 subtype [82] and a positive association between early age at menarche and Luminal subtype, but not other subtypes [72, 80]. A 2014 review indicated that younger age at menarche increased risk for HR+ breast cancer in about ½ of studies reviewed, but was rarely associated with HER2+ or Triple-negative breast cancer [50].

Grade: In the only study of an average-risk population, early age at menarche (≤11) was associated with a two-fold increased risk of medium or high grade tumors, compared to low grade tumors [94]. In the study of the high risk population of incident cases ≤ 35, age at menarche was not associated with grade [54].

1.C.2.d Risk Factor: Breastfeeding

ER, PR, and Joint ER/PR status: Althuis found that ER or PR expression was not differentially associated with breastfeeding [19], rather, in most studies breastfeeding has been associated with a decreased risk of all types of breast cancer. Since Althuis, a recent review and meta-analysis, by Islami *et al*, covering 27 studies, demonstrated that breastfeeding was inversely associated

with ER-PR- breast cancer, but not consistently inversely associated with ER+PR+ breast cancer [95].

Molecular subtype: The initial study of risk factors in relation to molecular subtypes, by Millikan *et al* found an important interaction between breastfeeding and parity in relation to molecular subtypes, namely, that women who were multiparous but did not breastfeed were at increased risk of triple-negative cancer [6]. Probably as a result of this finding, most studies that followed Millikan's have included breastfeeding as a risk factor of interest, and have sometimes included an interaction term that pairs breastfeeding and parity. A cohort study confirmed Millikan's findings [81], as did a case-control study in a high-risk population [96], and the review and meta-analysis by Islami *et al* demonstrated an inverse association between breastfeeding and triple-negative cancer [95]. However other studies have failed to find a differential association between breastfeeding and molecular subtypes (some did find an overall inverse association between breastfeeding and breast cancer) although these studies did not examine breastfeeding and parity jointly [29, 59, 74, 83]. In a pooled analysis, one study found that breastfeeding reduced risk of both Triple-negative and Luminal A subtypes, but only in African-American women [79], while a case-only study among a racially diverse group of women found that longer breastfeeding duration was positively associated with Luminal B, compared to Luminal A, breast cancer, and not associated with HER2+ or Triple-negative breast cancer, compared to Luminal A cancer [85]. A recent meta-analysis found that breastfeeding was strongly inversely associated with luminal breast cancer, not associated with the HER2+ subtype, and significantly inversely associated with triple-negative breast cancer [30]. A study in high-risk women, age 20-44, also indicated that breastfeeding was associated with inverse risk of luminal and triple-negative breast cancer, but not HER2+ breast cancer [91].

Grade: Only two studies have examined breastfeeding in relation to tumor grade. In these studies breastfeeding was not associated with tumor grade [54, 97].

1.C.2.e Risk factor: Oral contraceptive use

ER, PR and Joint ER/PR status: The Althuis review found modest evidence of a positive association with oral contraceptive use and ER- tumor subtypes, while a more recent cohort study found an inverse association of OC use and ER+ cancer [74]. However, studies examining OC and joint expression of ER and PR were inconclusive in the Althuis review [19]. In studies published more recently: OC use has not been associated with estrogen receptor status or joint estrogen/progesterone receptor status in two studies published since Althuis [27, 41] while other studies have demonstrated a borderline protective effect against ER+PR+ cancer in an Asian population [98], and a positive association for OC use on ER-PR- cancer in African American women [99]. Among high risk populations, Beaber *et al* found that recent OC use was positively associated with ER+ breast cancer [100] , while Bertrand *et al* found no association between OC use and ER+ or ER- cancer, among young African American women [67].

Molecular subtype: Millikan found no association with oral contraceptive use and molecular subtype, a finding confirmed in more recent analyses [74, 96]. In a detailed examination of OCs, that examined not only use but duration, time since use, and age at first use, Ma *et al* also found no associations between OCs and molecular subtype [59]. Studies of OC use and molecular subtype that yielded findings, demonstrated that ever use was protective against Luminal B, compared with Luminal A, cancer [81], and among young women age 20-44, that long duration of OC use (≥15 years) was associated with increased risk of triple-negative, but not HER2+, breast cancer [101].

Grade: In the only two studies known to examine OC use and tumor grade, one found that "never-users" of OCs had higher grade tumors than "ever-users" but also that each additional year of OC use conferred increased risk [102], while the in the other, which was conducted among women <35 years old, OC use was not associated with tumor grade [54].

1.C.2.f Risk factor: Hormone replacement therapy

ER, PR and Joint ER/PR status: The Althuis review found among older studies included in the review, there was no association with hormone replacement therapy and breast cancer risk [19]. However, among more recent studies in the review, including the Nurses' Health study, previous, but not current use of combined HRT was associated with increased risk of ER+, but not ER- tumors [42], and increased risk of ER+PR+ tumors, but not hormone negative tumors [103]. Among studies published since the Althuis *et al* review, hormone replacement therapy has been almost exclusively associated with ER+, or ER+PR+ tumors, but not ER- or ER-PR- tumors [40, 41, 104-106].

Molecular subtype: Few studies have examined HRT use in relation to molecular subtype. In two studies [82, 106], combined HRT therapy was associated with Luminal A and B types, as would be expected given these types are also ER/PR positive. Two studies using case-only analyses where Luminal A was the reference population showed differing results, with one having null findings [6] and the other demonstrating an inverse association between HRT use and Luminal B and HER2+ cancer types, compared to Luminal A types [81].

Grade: In one study, current, but not former use of HRT, was positively associated with low grade tumors; findings did not vary by regimen [105].

There are few studies of high risk populations and HRT use, primarily because HRT use generally occurs among a postmenopausal (and thus older and less high-risk) population.

1.C.3 Summary of Literature on other Factors: Age, Race, Menopausal Status, Body Mass Index, Smoking and Alcohol Use

For this dissertation, I will primarily concentrate on hormonal and reproductive risk factors. However, other factors that are known or suspected to be associated with breast cancer, and which differ in prevalence according to breast cancer subtype, will need to be examined and/or adjusted for.

The following is a brief summary of the literature to date on the associations between age, race, menopausal status, smoking, and alcohol use, and breast cancer tumor subtypes.

1.C.3.a Age at Breast Cancer Diagnosis and Tumor Subtype

ER- and PR- tumors, and higher grade tumors, are associated with a younger age at diagnosis. This association can somewhat be explained by the stronger association of ER-, PR- and high grade with *BRCA1* or *2* positivity, which is associated with early age at cancer onset, as well as the fact that, even without *BRCA1* or *2* positivity, breast cancers that occur in younger women are often of a more aggressive type, which corresponds to ER and PR negativity, HER2 positivity, and high grade [107]. Additionally, women in younger ages may have had less exposure to factors positively associated with ER+PR+ cancer, such as hormone replacement therapy, which is typically not prescribed to premenopausal (younger) women, thus a greater proportion of their cancers may not be hormone-sensitive.

1.C.3.b Race and Tumor Subtype

Many studies have examined racial differences in incidence of tumor subtypes, and this topic in itself would qualify for a systematic review. Most such studies have demonstrated that African-American women tend to have a higher incidence of ER-PR- breast cancer, and the first studies that elucidated triple-negative breast cancer demonstrated that African-Americans experience higher incidence of triple-negative breast cancer that do white women [5-7, 108, 109]. However, it is less clear whether this is an intrinsic association with race, or rather reflects different distributions of risk factors that are associated with triple-negative breast cancer, among African-Americans compared to other races [56, 110]. Among other races and ethnicities examined for this review, there does not appear to be differential incidence of any hormone-defined tumor subtype by race.*1.C.3.c Menopausal Status and Tumor Subtype*

Certain risk factors for breast cancer are differentially important depending on a woman's menopausal status. For example, hormone replacement therapy, which is almost exclusively used among women in peri- and post-menopause, can typically only be examined among a postmenopausal population. HRT use is associated with ER+ and PR+ tumors, and with low grade, and postmenopausal women are also more likely to have ER+, PR+ and lower grade tumors than premenopausal women. BMI also acts differently depending on a woman's menopausal status. Postmenopausal obesity is positively associated with breast cancer, and appears to be most associated with ER+/PR+ cancer, while premenopausal obesity does not appear to be associated with breast cancer at all [19, 111].

1.C.3.d BMI and Associated Characteristics and Tumor Subtype

As just noted, the role of obesity in breast cancer etiology differs depending on whether the cancer occurs pre- or post-menopause. Obesity measured at the time of diagnosis (i.e. current BMI) may also not reflect the role of excess weight in the development of cancer. Various studies have examined not only current BMI (BMI at the time of cancer diagnosis) but also weight at age 20, weight gain in adulthood, and factors with which obesity is associated, such as metabolic syndrome [40-42, 112-115]. Studies reviewed by Althuis *et al* did not find any association with premenopausal obesity and cancer defined by hormone receptor status [19]. High body mass index has been associated with ER+/PR+ tumors, but not ER-/PR- tumors [40-42, 116], particularly among women who gained a significant amount of weight in adulthood compared with women who gained little weight [41, 113]. High BMI was found to be associated with triple-negative breast cancer in one population [114], but not another [109], and metabolic syndrome, but not obesity alone (which is a component of metabolic syndrome) was associated with triple-negative breast cancer in another study [115]. One study that examined the association between BMI and grade found that BMI is positively associated with higher grade tumors in a premenopausal population [117]. High BMI and obesity has also been described consistently as a risk factor in male breast cancer, which is predominantly of the "Luminal" subtype [118].

1.C.3.e Smoking and Breast Cancer Tumor Subtypes

Smoking is not consistently associated with breast cancer, and has been looked at in a very limited fashion in regards to breast cancer defined by tumor characteristics. In such studies, smoking has not been differentially associated with any specific subtype defined by tumor

characteristic. Studies reviewed by Althuis *et al* did not find any differential association with smoking and cancer defined by ER or PR expression [19]. One other recent study that examined this association also did not find any differential relation between smoking and breast cancer defined by ER or PR subtype [116]. A study of postmenopausal women found no association between smoking and triple-negative breast cancer [119]. Only one study has examined the relation between smoking and grade, and found that smoking was associated with tumors of higher grade in a postmenopausal population [120].

1.C.3.f Alcohol and Breast Cancer Tumor Subtypes

It is acknowledged that there is an association between alcohol and overall breast cancer risk, and many, but not all, papers support an association between this risk and ER+ status. Papers differ in regard the importance of PR status. Althuis *et al* found that there was no consistent association between alcohol use and hormone-defined subtypes of breast cancer [19]. Since the Althuis review, additional publications have not clarified the relationship. A 2005 cohort study demonstrated a positive association between alcohol use and ER, but not PR, status [121]. A meta-analysis published in 2008 demonstrated an association between alcohol consumption and all ER/PR defined subtypes except ER-PR- [122]. In postmenopausal populations, alcohol was positively associated with ER+PR+ cancer, but not ER+PR- or ER-PR- cancer [123, 124]. A study of women with triple negative breast cancer showed a reduced risk for drinkers compared with non-drinkers [119]. No known studies have examined the association between alcohol use and tumor grade.

1.C.4 Literature regarding Risk Factors and Correlation of Tumor Characteristics

Most previous research regarding risk factor and breast cancer tumor characteristics has been via case-control design, and the examination of the association between the risk factor(s) of interest and the outcome of breast cancer performed using polytomous logistic regression, where the outcome of breast cancer is divided into several sub-outcomes defined, for example, by ER or PR status. However, because ER status is correlated with PR status, along with other characteristics of the tumor, such as tumor grade, it may be unclear whether the risk factor associations found with ER or PR status are etiologically related to development of ER+ or ER- cancers, PR+ or PR- cancers, or whether these risk factors are etiologically related to a factor correlated to ER or PR status, such as tumor grade.

Therefore, an alternative analytic technique has emerged for examining the association of multiple tumor subtypes by specific risk factors. This technique is known as the pseudo-conditional likelihood method, and it is an offshoot of polytomous logistic regression [125, 126]. This type of regression allows for the adjustment of correlated tumor characteristics, when examining a risk factor's association with a cancer outcome more specifically defined by the presence or absence of a tumor marker. Additional details on this method are described in Appendix 1. To date, few publications have utilized this method in examining breast cancer [14, 127], however a number of publications have used the method as a platform for examining colorectal cancer [128-130].

Among the breast cancer studies, in a study published in 2006, Garcia-Closas and colleagues examined the relation among various tumor characteristics (though not defined by ER/PR status) and several risk factors of interest. The relation between the risk factors and tumor characteristics

such as grade, tumor size, and nodal status was more clearly elucidated when the correlated tumor characteristics were adjusted for using the novel extension of polytomous logistic regression that can account for multiple disease outcomes [125]. These case-only analyses demonstrated, for example, that late age at first birth was associated with larger tumor size (> 2 cm) vs. small tumor size, and this finding remained significant even after adjustment for tumor grade and nodal status. While late age at first birth was also associated with positive (compared with negative) nodal status, the association did not hold after accounting for tumor grade and tumor size, indicating that the unadjusted findings were likely due to the correlation between large tumor size and positive nodes [14]. In another previous study that utilized the pseudo-conditional likelihood method, the investigators determined that ER-α levels (e.g., ER positivity) were inversely associated with BMI among premenopausal women, but this relationship was not maintained when adjusting for ER-β levels, PR levels, and HER2 levels; however, high PR levels (i.e., PR positivity) were positively associated with high BMI among post-menopausal women, and this relationship was maintained when adjusting for the other marker levels [127]. High HER2 levels were associated with high BMI among premenopausal women, however this trend became significant only after the other markers (ER-α, ER-β, PR) were adjusted for [127]. These studies indicate that risk factors may have an apparent association with a specific tumor characteristic, such as ER status, but in reality are associated with a different, but correlated characteristic, such as tumor grade or HER2 status.

1.C.5. Literature Review: Summary and Conclusion

While we have made great strides improving treatment of and reducing mortality from breast cancer, there is still much that is not understood about the etiology of breast cancer, how different subtypes of breast cancer develop and what patterns of biomarker expression exist.

Understanding more about why some cancers develop certain characteristics or express certain biomarkers, which have major implications for breast cancer treatment and prognosis, is important in quantifying breast cancer risk, especially among family members of those with breast cancer, and modifying behaviors that may increase a person's risk of developing a high-mortality or difficult to treat tumor. Among familial as well as sporadic breast cancer, better understanding of risk factors that lead to certain cancer subtypes may allow for behavior modifications that reduce risks, or for enhanced preventive behaviors.

1.D. RESEARCH GAPS AND DESCRIPTION OF AIMS

While the literature on risk factor associations with breast cancer tumors defined by ER- and PR-status is abundant, several gaps in research remain. First, few of these studies have been performed in a higher breast-cancer risk population, such as among younger women, women with a family history of breast cancer or women positive for *BRCA1* or *BRCA2*. Second, despite a preponderance of literature on risk factors and breast cancer hormone receptor status, some risk factors remain under-examined, and do not account for how various risk factors, such as oral contraceptive use or breastfeeding prevalence, may have changed over time. Third, almost no studies have accounted for the fact that hormone-receptor status correlates with other tumor characteristics, such as tumor grade, obfuscating the causal pathway through which risk factors may function to cause specific subtypes of disease. Finally, no studies to date have explored the relationship between risk factors and tumor characteristics in the context of a family-based design.

Analyses as part of this dissertation examine the following specific Aims, intended to address the current gaps in knowledge. This dissertation addresses these gaps by analyzing data by subtype

in a large high-risk breast cancer population, the Breast Cancer Family Registry, that includes both population-based and sibling controls. The analysis will primarily concentrate on the hormonal and reproductive factors of parity, breastfeeding, and oral contraceptive use, and will explore potential cohort effects for OC use, parity, and breastfeeding behavior, and cancer subtype. Analysis will also be performed in both a traditional case-control as well as a family-based design, using women who have personal or familial experience with breast cancer, to examine within family differences. Risk factors will be examined by both joint ER/PR status and molecular subtype, and by the important prognostic factor of tumor grade, and tumor characteristics will be adjusted for one another via a two-stage regression approach, to account for the correlation among ER status, PR status, and grade, or ER status, PR status, and HER2 status.

Specific Aim 1: *To understand the role of select reproductive and hormonal risk factors in a high-risk breast cancer population with significant family history of breast cancer, using population-based data from the BCFR, by evaluating the associations among selected breast cancer risk factors, and estrogen and progesterone receptor status, in breast cancer cases versus unrelated controls, using polytomous logistic regression, and considering role of family history and age cohort effects.*

Main analyses and findings for Specific Aim 1 are included in Chapter 2.

Specific Aim 2: To evaluate the associations among select reproductive and hormonal breast cancer risk factors and breast cancer estrogen and progesterone receptor status, using clinic-based data from the BCFR, by comparing the risk factor proportions in breast cancer cases versus related controls, utilizing the methods of polytomous logistic regression, as well as

generalized estimating equations that account for the correlation between cases and familial controls; while considering the role of family history and BRCA1/2 status, in terms of risk factor effect on outcome.

Main analyses and findings for Specific Aim 1 are included in Chapter 3.

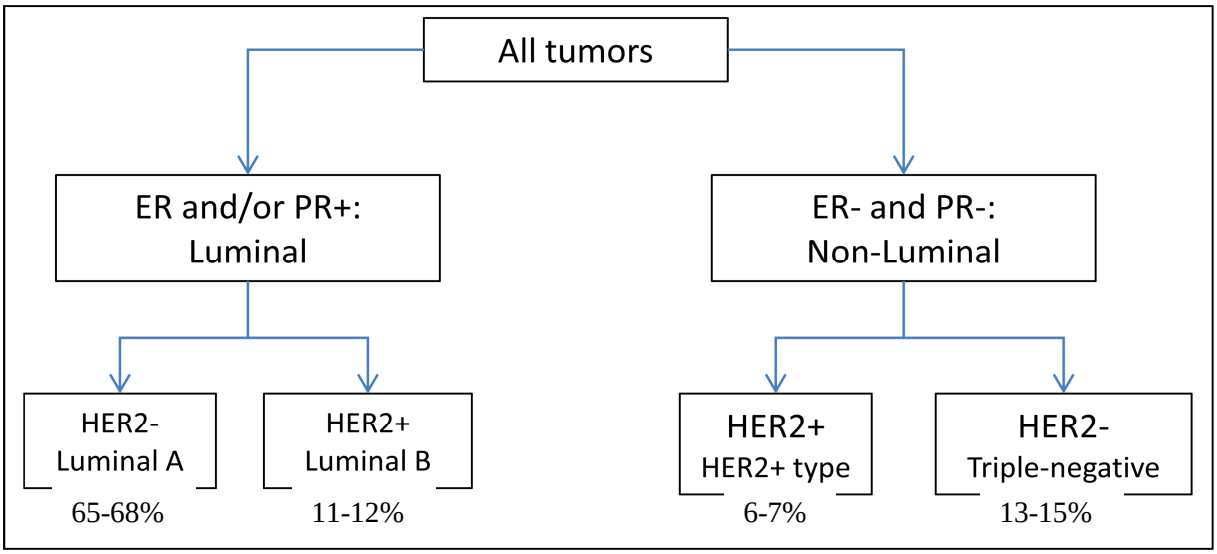
Specific Aim 3 *To evaluate the associations among breast cancer risk factors and breast cancer tumor characteristics, including ER and PR status, HER2 status and tumor grade, using population-based and clinic-based data from the BCFR, by comparing risk factor proportions among cases who do and do not exhibit various tumor characteristics (case-only analysis), using binomial logistic regression as well as a regression approach (pseudo-conditional likelihood approach) that accounts for the correlation among tumor characteristics.*

Main analyses and findings for Specific Aim 3 are included in Chapters 2 and 3.

A comparison of the findings in the population-based and clinic-based data sets is included in Chapter 4.

Additional methodology information and tables are included in Appendix 1.

Figure 1-1: Classification of invasive breast cancer subtypes according to immunohistochemistry marker profile, using ER, PR and HER2 status



(Adapted from: Blows F, et al, 2010)[131]

Table 1-1: Components of Tumor Grade

Total Feature Score*	Tumor Grade	Features of Cells
3-5	Grade 1	Well-differentiated (appear normal, growing slowly, not aggressive)
6-7	Grade 2	Moderately-differentiated (semi-normal, growing moderately fast)
8-9	Grade 3	Poorly-differentiated (abnormal, growing quickly, aggressive)

**The total feature score is summed from 3 components: (a) Tubule Formation (score range 1-3), (b) Tumor Mitotic Activity—rate of cell division (score range 1-3), (c) Nuclear Grade—Cell size and uniformity (score range 1-3)*

Table 1-2: Breast Cancer Subtypes among those with Family History of Breast Cancer: Summary of Literature

Study	*Topic*	*Findings*
Allen-Brady K, et al, *IJC* 2005	Ductal or lobular breast cancer and risk of BC in relatives	Morphology-specific relative risks showed that relatives of probands with lobular breast cancer had an increased risk of lobular cancer (FRR=4.51), as well as an increased risk of any breast cancer (FRR=2.47), compared to first degree relatives of cases with any histology (FRR=1.83) [132]
Melchor, L et al, *Oncogene* 2008	*BRCA* status and molecular subtype among those with familial BC	Patients with *BRCA1* positivity most often present with a "basal-like" pathology (associated with ER, PR and HER2 negativity); familial patients that are not *BRCA1* or *2* positive most often present with a "Luminal A" pathology (associated with ER or PR positivity and HER2 negativity)[45]
Welsh ML et al, *Breast Can Res Treat*, 2009	Degree of family history and ER/PR status	Women with a first-degree family history of breast cancer are more likely to have ER+/PR+ and ER+/PR- tumors (but not ER-/PR-) tumors, compared with women with no family history, while women with a 2nd degree family history only are not at increased risk for any ER/PR -defined tumor subtype.[47]
Mavaddat N, et al, *Breast Cancer Res*, 2010	ER status and familial relative risk (FRR) in relatives	In a study that computed familial relative risk (FRR) for breast cancer by subtypes (defined by ER, PR, and HER2 status), there was no difference between breast cancer FRR for relatives of patients with ER- breast cancer or ER+ breast cancer [133].
Phipps, et al, *Breast Cancer Res Treat* 2011	Family history of breast cancer in first degree relative and risk of triple negative BC	Having a first-degree family history of breast cancer was associated with an increased risk of triple-negative breast cancer [46]
Jiang X, et al, *PloS One* 2012	Family history and breast cancer hormone receptor status	In a Spanish cohort, women with a family history of breast cancer were non-significantly more likely to have ER-PR- tumors than women without family history, but only if diagnosed prior to age 50 [44]
Ricks L, et al, *J. Community Genet* 2014	Family history of cancer and BC clinicopathological features	Self-reported family history of any cancer was associated with high grade, and ER-PR- breast cancers [15]

Table 1-3. Reproductive and Hormonal Risk Factor Associations with Breast Cancer Tumor Characteristics in Average risk populations: Summary of Studies by Risk Factor

Outcome Studied	Publication Type	Publication Date(s)	Summary of Findings	References
Parity				
ER Status	Review	2004	Parity vs. nulliparity reduced risk of ER+, OR 0.5-0.8	[19]
	Original research	2005-2014	Parity vs. nulliparity reduced risk of ER+, but not ER- BC	[56, 57, 59, 60, 62, 63, 65]
		2006, 2007	Risks associated with parity and number of births did not significantly differ by hormone receptor status	[41, 66]
PR Status	Review	2004	PR+ women more likely than PR- to be nulliparous	[19]
	Original Research	2007	PR status not associated with parity	[66]
Any HR Status	Review	2014	Increased parity associated with decreased HR+ cancer	[50]
Joint ER/PR Status	Review	2004	Consensus finding not reached	[19]
	Meta-analysis	2006	Each additional live birth further reduced risk of ER+ PR+, but not ER- PR- cancer	[70]
	Original Research	2005-2016	Parity (vs. nulliparity) inversely associated with ER+/PR+, but not ER-/PR- cancer	[40, 58, 63, 65, 71, 90, 98]
		2011, 2013	Higher parity associated with elevated risk of ER-PR- cancer	[65, 71, 72]
		2005, 2011	Parity did not differ by joint ER/PR status	[41, 73]
Molecular Subtype	Original research	2007-2011	Increasing parity positively associated with triple-negative breast cancer, protective against Luminal A cancer	[5,40,45-48]
		2008-2010	No differential association between parity and molecular subtype	[21,49,50]
	Meta-analyses	2016	Nulliparity associated with increased risk of Luminal subtype in Asian populations; parity protective against Luminal subtype	[30, 80]
	Review	2014, 2016	Increased parity positively associated with TNBC in 3 of 13 studies	[50]
Grade	Original research	2009	Nulliparity more strongly associated with Grade 3 vs. Grade 1, 2 tumors	[86]
		2010, 2016	Parity not associated with tumor grade	[87, 88]

Age at First Birth				
ER Status	Review	2004, 2014	Later age at first live birth more consistently observed for ER-positive than ER-negative tumors; with risk estimates ranging from 1.4 to 2.6	[19, 50]
	Original research	2007-2013	Later age at first birth positively associated with ER+, but not ER-, breast cancer	[58, 62, 64, 65, 134]
ER Status	Original research	2005-2007	Later age at first birth not differentially associated with ER+ vs. ER- breast cancer	[55, 63, 89]
PR Status	Review, Original Research	2004-2011	PR status not associated with age at first birth	[19, 65, 89]
Joint ER/PR Status	Review	2004	Modest increase in HR positive but not HR negative tumors among women with an older age at first birth	[19]
	Meta-analysis, original research	2005-2011	Women with older age at first birth were at increased risk of ER+PR+ cancer, but age at first birth was not associated with ER-PR- cancer	[40, 41, 58, 70, 73, 98]
	Original research	2008	Positive association between late age at first birth and ER-PR- cancer	[90]
	Original research	2013	No association between age at first birth and ER/PR defined subtypes	[72]
Molecular subtype	Original research	2007-2011	Positive association between late age at first birth and Luminal A breast cancer, positive association between early age at first birth and triple-negative breast cancer	[6, 59, 74]
	Original research	2008-2011	No differential association between molecular subtype and age at first birth	[75, 81-83]
	Meta-analysis	2016	Positive association between late age at first birth and Luminal A breast cancer, no association with Triple-negative cancer	[30]
Grade	Original research	2009	Late age at first birth associated with higher grade tumors	[86]
	Original research	2010	Earlier age at first birth associated with higher grade tumors	[87]
Age at Menarche				
ER Status	Review	2004	Age at menarche not differentially associated with ER status	[19]
	Original research	2008, 2011	Age at menarche not associated with ER status	[65, 94]
	Original research	2014	Early age at menarche positively associated with ER+, but not ER- cancer	[57]
PR Status	Review	2004	Age at menarche not differentially associated with PR status	[19]

	Original research	2007, 2011	Late age at menarche inversely associated with PR+ breast cancer	[65, 89]
Joint ER/PR status	Review	2004	ER+/PR+ cancer inversely associated with older age at menarche, with risk estimates ranging from 0.5 to 0.8, compared with menarche at younger ages, but not associated with HR negative cancers	[19]
	Meta-analysis	2006	Late age at menarche decreased risk of all subtypes of breast cancer, but protective effect statistically significantly greater for ER+PR+ cancer	[70]
	Original research	2005-2015	Late age at menarche not differentially associated with ER/PR status	[63, 73, 92, 93, 98]
		2009, 2013	Late age at menarche inversely associated with ER+/PR+ cancer only	[40, 61]
Molecular subtype	Original research	2007, 2011	No relation between molecular subtype and age at menarche	[6, 74]
		2008	Early age at menarche was positively associated with HER2+ disease	[82]
Grade	Original research	2008	Early age at menarche was associated with a two-fold increased risk of medium/high grade cancer	[94]
Breastfeeding				
ER Status	Review	2004	Breastfeeding associated with reduced risks of all types of breast cancer	[11]
	Meta-analysis	2005	Breastfeeding associated with reduced risks of all types of breast cancer	[70]
ER Status	Original research	2008	Breastfeeding inversely associated with ER+, but not ER- cancer	[62]
PR Status	Review	2004	Breastfeeding not differentially associated with PR status	[11]
Joint ER/PR status	Review	2015	Breastfeeding inversely associated with ER-PR- breast cancer	[95]
	Original research	2005-2011	Breastfeeding associated with reduced risk of all cancer subtypes, no differential association	[58, 63, 90, 98]
	Original research	2005, 2011	No association between breastfeeding and cancer subtype	[71, 73]
Molecular Subtype	Original research	2010	Breastfeeding inversely associated with multiple molecular subtypes	[59, 83]
	Original research	2007, 2009	Having multiple children and not breastfeeding associated with increased risk of triple-negative cancer	[6, 81]
	Pooled analysis, Meta-analysis	2016, 2017	Breastfeeding reduced risk of both Luminal A and triple-negative cancer	[30, 79]
	Review	2015	Breastfeeding inversely associated with triple-negative subtype	[95]

Grade	Original Research	2014	Breastfeeding not associated with grade	[97]
Oral Contraceptive Use				
ER Status	Review	2004	OCs positively associated with ER- cancer in earlier studies, no association in more recent studies	[19]
	Original research	2011	OC use of >10 years associated with reduced risk of ER+ cancer	[74]
PR Status	No studies of average risk populations			
Joint ER/PR status	Review	2004	OCs not differentially associated with joint ER/PR status	[19]
	Original research	2010	OC use positively associated with ER- PR- cancer	[99]
Molecular subtype	Original research	2007-2011	OC use not differentially associated with molecular subtype	[6, 59, 74]
	Original research	2009	OC use associated with reduced incidence of Luminal B compared with Luminal A cancer	[81]
Grade	Original research	2008	Never OC use positively associated with higher grade tumors (case-only analysis); long duration of use also associated	[102]
Hormone Replacement Therapy				
ER Status	Review	2004	Past use of combined HRT associated with increased risk of ER+, but not ER- tumors	[19]
PR Status	Original research	2009	Current HRT use associated with PR+ ductal tumors	[104]
Joint ER/PR status	Review	2004	Past use of combined HRT associated with increased risk of ER+PR+ tumors, but not hormone negative tumors	[19]
	Original research	2006-2011	Use of combined HRT associated more strongly with ER+PR+ subtypes	[40, 98, 104, 105]
Molecular Subtype	Original research	2007, 2008	Combined HRT associated with Luminal A and B subtypes	[6, 82]
	Original research	2009	Combined HRT associated with reduced risk of Luminal B cancer compared with Luminal A	[81]
Grade	Original research	2008	Current use of HRT positively associated with low grade tumors	[105]

Table 1-4. Reproductive and Hormonal Risk Factor Associations with Breast Cancer Tumor Characteristics in high risk populations: Summary of Studies by Risk Factor

Parity				
Study	Outcome Studied	Population Studied	Publication Date(s)	Summary of Findings
Nichols, HB et al [55]	ER Status	Premenopausal, Chinese and Vietnamese	2005	Parity vs. nulliparity reduced risk of ER+, but not ER- BC
Largent, JA et al [54]	ER Status	Women <35 years old	2005	Parity not associated with ER+ or ER- cancer
Jia, X et al [69]	ER Status	Women ≤35 years old	2015	Parity positively associated with ER+ cancer
Bertrand, KA et al[67]	ER Status	African-American women <45 years old	2016	Nonsignificant (NS) trend for inverse association between high parity and ER+, NS trend for positive association between high parity and ER-
Ma, H et al [27]	Joint ER/PR Status	Women <50 years old	2006	parity association was confined to ER+/PR+ cancers (compared with controls) , and increased with each additional pregnancy
Dolle JM et al [68]	Molecular s\Subtype	Women <45 years old	2009	No association between parity and TNBC; inverse association between high parity and non-TNBC
Gaudet, MM et al [96]	Molecular Subtype	Women ≤56 years old	2012	Nulliparous women at 3-fold risk of Luminal A and B cancer, no increased risk of triple-negative or HER2
Li, CI et al [91]	Molecular Subtype	Women age 20-44	2013	Parity similarly associated with ER+, HER2+, and triple-negative breast cancer
Largent, JA et al [54]	Grade	Women <35 years old	2005	Parity not associated with tumor grade
Nagatsuma AK et al [135]	Grade	Premenopausal women	2013	Recent parity (but not overall parity) associated with higher tumor grade
Jia, X et al [69]	Grade	Women ≤35 years old	2015	Parity not associated with tumor grade
Age at first Birth				
Nichols, HB et al [29]	ER Status	Premenopausal, Chinese and Vietnamese	2005	Late age at first birth associated with both ER+ and ER- tumors
Largent, JA et al [54]	ER Status	Women <35 years old	2005	Age at first birth not associated with ER+ or ER- tumors
Bertrand, KA et al [67]	ER Status	African-American women <45 years old	2016	Positive association between later age at first birth and ER- cancer; NS inverse association between late age at first birth and ER- tumors

Ma H et al [27]	Joint ER/PR status	Women <50 years old	2006	Age at first birth only associated with ER+PR+ tumors
Dolle, JM et al [68]	Molecular Subtype	Women <45 years old	2009	No association between age at first birth and TNBC or non-TNBC
Li, CI et al [91]	Molecular Subtype	Women age 20-44	2013	Age at first birth inversely associated with triple-negative breast cancer, not associated with HER2+
Largent, JA et al [54]	Grade	Women <35 years old	2005	Early age at first birth associated with higher grade tumors
Age at Menarche				
Nichols, HB et al [55]	ER Status	Premenopausal Chinese and Vietnamese	2005	Age at menarche not associated with breast cancer
Bertrand, KA et al, [67]	ER Status	African-American women <45 years old	2016	NS trend to inverse association between later age at menarche and both ER- and ER+ cancer.
Ma H, et al [27]	Joint ER/PR Status	Women <50 years old	2006	Late age at menarche not differentially associated with hormone-receptor positive cancers
Gaudet, MM et al [96]	Molecular Subtype	Women ≤56 years old	2012	Age at menarche associated with reduced risk of Luminal B cancer
Largent, JA et al [54]	Grade	Women <35 years old	2005	Age at menarche not associated with tumor grade
Breastfeeding				
Largent, JA et al [54]	ER Status	Women <35 years old	2005	Breastfeeding associated with ER- cancer only
Bertrand, KA et al, [67]	ER Status	African-American women <45 years old	2016	Ever breastfeeding inversely associated with ER-, but not ER+, breast cancer
Nichols HB et al [55]	ER Status, PR Status, HER2 Status	Premenopausal Chinese and Vietnamese	2005	Breastfeeding not associated with ER-, PR-, or HER2- cancer
Ma H, et al [27]	Joint ER/PR Status	Women <50 years old	2006	Breastfeeding inversely associated with both ER+PR+ and ER-PR- cancer
Gaudet, MM et al [96]	Molecular Subtype	Women ≤56 years old	2012	Breastfeeding inversely associated with Luminal B, TNBC, HER2+ subtypes
Li, CI et al [91]	Molecular Subtype	Women age 20-44	2013	Breastfeeding inversely associated with Luminal and TNBC, but not HER2 cancer
Largent, JA et al [54]	Grade	Women <35 years old	2005	Breastfeeding not associated with grade
Oral Contraceptive Use				
Bertrand, KA et al, [67]	ER Status	African-American women <45 years old	2016	Ever use of OCs and recency of use not associated with ER+ or ER- cancer

Beaber ER, et al [100]	ER Status	Women aged 20-49	2014	Recent OC use positively associated with ER+ risk
Ma H, et al [27]	Joint ER/PR Status	Women <50 years old	2006	OC use not associated with ER+PR+ or ER-PR- cancer
Gaudet, MM et al [96]	Molecular Subtype	Women ≤56 years old	2012	Oral contraceptive use not associated with molecular subtype
Beaber, EF et al [101]	Molecular Subtype	Women aged 20-44	2014	Lifetime duration of OC use for ≥15 years associated with increased risk of all BC, risk magnitude greater in TNBC subtype
Largent, JA et al [54]	Grade	Women <35 years old	2005	Oral contraceptive use not associated with grade
HRT Use				
No high risk populations studied				

Chapter 2

Select Reproductive Risk Factors and Risk of Estrogen and Progesterone Receptor-Defined Breast Cancer in Population-Based sites of the Breast Cancer Family Registry

2.A. INTRODUCTION

Breast cancer is a heterogeneous disease**,** with different etiologic factors contributing to disease causation to a greater or lesser degree depending upon breast cancer type. In the past 20 years, an increasingly abundant literature has surfaced to describe how known risk factors for breast cancer may differ among the different subtypes of breast cancer defined by estrogen-receptor (ER) and progesterone receptor (PR) expression [14, 19, 40-43, 54, 63, 65, 70, 73, 81, 90, 121-123, 127, 136-149]. The bulk of literature has associated most reproductive factors and hormonal risk factors, such as parity, age at first birth, and exogenous hormone use, with hormone positive (ER+ and/or PR+) cancers. For example high parity, lower age at first birth, and higher age at menarche have been associated with reduced risk of ER+ and PR+ cancers [19, 27, 40, 55, 58, 63, 65, 70, 71, 90, 98], and postmenopausal exogenous hormone use (hormone replacement therapy, or HRT) is positively associated with ER+ and PR+ breast cancer [19, 40, 98, 104, 105]. By contrast, ER and PR negative breast cancer (ER-PR-), which is positively associated with African American race, younger age at onset, high tumor grade, and poor prognosis compared with ER+PR+ cancer [3, 5, 108, 109, 146, 150], has not demonstrated the same associations with reproductive and hormonal risk factors. For example, age at first birth appears to be unrelated to ER-PR- cancer, and high parity has been associated with increased, rather than decreased risk, in many studies [6, 27, 40, 41, 65, 70, 71, 73, 81, 98]. Breastfeeding is one of the few factors consistently associated with a reduction in both hormone receptor positive and negative breast cancer by a majority of studies [19, 27, 58, 70, 90, 98]. For ER-PR- or triple-negative (ER-PR-

HER2-) cancer, in particular, breastfeeding may mitigate the increased risk of ER-PR- cancer associated with multiparity [6, 71, 81, 151].

Many studies that have previously examined the relation between reproductive and hormonal risk factors have failed to consider secular trends in these factors, and how changes over time may subsequently affect assessment of risk. For example, usage of oral contraceptives (OCs) has changed dramatically over time due to both historical events and changes in women's reproductive practices. Because OCs did not become widely available until 1961, women in older cohorts likely did not have access to OCs either at all, or prior to childbearing, whereas younger cohorts had access upon reaching reproductive age, and were more likely to use OCs prior to first full-term pregnancy. Prior to 1975, OCs contained a higher dosage of both estrogens and progestins, leading to concerns about increased risk for female cancers [152, 153]. In 1975, estrogen and progestin content in OCs was reduced in most industrialized countries, including the United States, Canada, and Australia. Therefore, older cohorts using OCs would primarily have received higher doses of exogenous hormones, while later cohorts received primarily the low-estrogen/progestin doses. The overall proportion of women using oral contraception, and average duration of use, has also increased over time.

Previous studies have supported that year of OC use (before or after 1975) is an important variable in assessing breast cancer risk, however these studies have not examined year of use by ER or PR status, or whether changes in duration or timing of use before or after pregnancy by successive cohorts affect risk [153, 154].

While literature examining reproductive risk factors and breast cancer subtypes defined by ER and PR status is abundant, relatively few studies have reported risk of oral contraceptives in a high breast cancer risk population. Potential risk factors may be different for younger, higher risk

populations, and understanding how risk factors interact in these populations has potential for risk prediction. Whether risk factor associations found in average-risk populations extend to women to women at high risk of breast cancer is also critical for prevention, as there are few prevention options available to these women apart from risk-reducing surgeries and chemoprevention; options that are particularly difficult to consider during childbearing age. This is especially true in the case of ER-PR- cancer, where few modifiable risk factors are known.

Additionally, few studies have examined whether reproductive and hormonal risk factors differ according to tumor grade [54, 86-88, 94]. Tumor grade classifies cancer cells according to their appearance under a microscope (how abnormal they look compared to normal breast tissue) and how quickly the tumor is likely to grow and spread. Poorly differentiated tumors are more aggressive, are often less amenable to treatment and have poorer prognosis compared with well differentiated tumors, even after accounting for stage and nodal status, thus it is of value to understand if modifiable risk factors are associated with high grade tumors. One study has found nulliparity to be more strongly associated with higher grade tumors [86]. In the only study conducted in a high risk population, Largent *et al* found no association between parity, breastfeeding, or oral contraceptive use and grade in women diagnosed with breast cancer prior to age 35 [54].

In this chapter, I evaluate associations between known and suspected reproductive and hormonal breast cancer risk factors and breast cancer, categorized by ER and PR status, using population-based data from the Breast Cancer Family Registry, specifically concentrating on parity, breastfeeding, and oral contraceptive use, to evaluate whether the reduction in risk from breastfeeding in the presence of high parity, which has been described in the literature, extends to higher risk women, and to potentially clarify the role of OCs on breast cancer risk, where the

literature to date has been ambivalent. I consider possible age-cohort effects by examining year of birth in relation to OC use, parity, and breastfeeding, to understand whether possible age cohort effects can explain previous equivocal findings. I also evaluate the data by race, to evaluate whether higher prevalence of ER-PR- cancers in specific races (particularly among African American women) could be partially explained by environmental factors that may differ by race (such as breastfeeding). I also examine the association of these factors with breast cancer risk in women with a first-degree family history, to determine how risk factors may change in the face of a strong genetic predetermination for breast cancer. Finally, I use a statistical method that accounts for correlated outcomes, to assess the effect of risk factors on individual breast cancer tumor characteristics that are correlated with one another, in order to better understand the possible etiology of risk factors on ER- and PR-defined subtypes, and their relation to correlated tumor characteristics such as grade.

2.B. MATERIALS AND METHODS

Study Population

In 1995, the National Cancer Institute funded six international sites establishing the Breast Cancer Family Registry (BCFR), a resource for genetic studies of breast cancer. Six participating sites from the United States, Canada, and Australia ascertained families either from population-based cancer registries (in the San Francisco Bay, CA area, Ontario, Canada, and Melbourne and Sydney, Australia) or from clinical and community settings (producing clinic-based families in New York, NY, Philadelphia, PA, and Salt Lake City, UT) [155, 156].

Most families were enrolled in the BCFR from 1996-2000. During the period 2001-2005, several sites continued to recruit (1) families known to segregate *BRCA1* or *BRCA2* mutations, (2) families with multiple cases of breast or ovarian cancer, (3) selected relatives of previously

enrolled families, (4) families of Ashkenazi Jewish ancestry and (5) families from specific racial and ethnic groups [155]. The recruiting criteria for each population-based site are detailed in a previous publication, [155], and summarized in *Appendix Table A1-1*.

A total of 5,732 women with a first primary invasive breast cancer (2,038 from San Francisco, 2,553 from Ontario, and 1,141 from Australia), have been enrolled in the population-based sites, 5,087 of whom completed the epidemiology questionnaire. Of those recruited, questionnaire data were obtained for 76%, 72%, and 75% of eligible cases from Northern California, Ontario, and Australia, respectively. In addition, 3,013 population-based controls (634 in San Francisco, 1711 in Ontario, and 668 in Australia), have been enrolled, and 2,997 have completed the epidemiology questionnaire. The overall population-based study sample size is 5,087 female cases with a first primary breast cancer, and 2,997 unrelated controls.

Of the 5,087 cases, 4,011 (79%) of women have ER/PR information available and were therefore eligible for analysis, including 1,994 cases from Northern California, 1,088 from Ontario, and 929 from Australia. I used data from all 2,997 population-based controls that completed the epidemiology questionnaire. For the majority of the data collection period, pathologists did not ascertain HER2 status of breast tumors, because HER2 was not recognized as an important prognostic marker, nor was there targeted therapy available for HER2+ patients. As a result, only a subsample of the BCFR population had HER2 data available for analysis. Minority cases were targeted for HER2 examination in California, and a subset of women (both white and minority) had HER2 data available from the Ontario site. HER2 status was not available for cases from the Australia site of the BCFR. Data are thus available on HER2 status for a subgroup of women from Northern California and Ontario (N=798). *Appendix Figure A1-2* depicts cases who did and did not have ER/PR or HER2 data available.

Case Ascertainment

At each of the three study sites, incident breast cancer cases were identified through population-based cancer registries. In San Francisco area and Ontario, women likely to be at increased genetic risk of breast cancer were oversampled; all cases from local population-based cancer registries with specific indicators of genetic risk and a random sample of cases without these indicators were invited to participate. In Northern California, cases aged 18-64 years between January 1, 1995 and December 30, 2000 were recruited if they met one or more of the following criteria: diagnosis before age 35, bilateral breast cancer with diagnosis before age 50, prior ovarian or childhood cancer, or a history of breast, ovarian, or childhood cancer in ≥ 1 first degree relative. Cases not meeting these criteria were randomly sampled at 5% for non-Hispanic whites and 20% for cases of other race/ethnicity. Enrollment of cases diagnosed between October 1, 1998 and December 30, 2000 was limited to those who self-identified as African-American, Hispanic, Chinese, Japanese or Filipina.

In the Ontario site, cases aged 18-69 years between January 1, 1996 and December 31, 1998 were included if they met one or more of the following criteria: Ashkenazi Jewish heritage, diagnosis before age 36, prior breast or ovarian cancer, first- or second- degree relative(s) with breast or ovarian cancer, first- second- or third-degree relative(s) with early age at diagnosis for breast or ovarian cancer (before age 36), multiple primary breast cancers, or male breast cancer; or 3 or more first degree relatives with any combination of breast, ovarian, colon, prostate, pancreatic cancers and/or sarcoma. Cases not meeting these criteria were randomly sampled at 25%.

In the Australia sites, all women diagnosed with invasive breast cancer from 1996-1999, aged 18-39 years, and residing in Melbourne or Sydney at the time of diagnosis, were identified. In

Melbourne, women aged 40-49 years and 50-59 years were randomly sampled at 41% and 25%, respectively, while in Sydney, both age groups were randomly sampled at 28%.

Control Ascertainment

The study investigators randomly sampled unrelated controls from the general population living in the catchment area of each of the regional cancer registries, and controls were frequency matched to cases by 5-year age groups; in Northern California, they were additionally frequency matched by race/ethnicity. In Ontario and Northern California, controls were identified through lists of randomly selected residential telephone numbers. In Ontario, of 2,688 eligible population controls, 1,706 (64%) completed the risk factor and family history questionnaires and comprise the Ontario control group. In Northern California, 67% (623) of 930 eligible controls completed the family history and risk factor questionnaires, and comprise the Northern California control group. In Melbourne and Sydney the population controls were randomly selected from electoral rolls (for which registration is compulsory for adults in Australia) by use of proportional random sampling based on expected age distribution of the cases. Of 898 controls selected, 668 (74%) completed the family history and risk factor questionnaires and comprise the Australia control group.

Risk Factor Data Collection

Cases and controls completed structured questionnaires assessing breast cancer risk factors and family history of cancer (family history was assessed through a telephone questionnaire, and breast cancer risk factor information was ascertained through an in-person interview for the cases, while breast cancer risk factors and family history were ascertained through a mailed questionnaire for the controls). In addition to detailed family history of cancer, information was collected on established and suspected breast cancer risk factors, including oral contraceptive

use, menopausal hormone therapy use, age at menarche, parity, age at first childbirth, breastfeeding history, smoking history, alcohol use, education, body mass index (BMI), and menopausal status.

Tumor Marker Data Collection

For 2351 cases, BCFR study pathologists ascertained ER and PR status from patient tumor tissue using immunohistochemistry and/or pathology reports using a standardized protocol and pathology reporting forms. For the remaining cases (N=1660), ER and PR status were provided by the relevant Cancer Registry for that population, or through patient medical records. For California cases with HER2 status available (N=798), the information on HER2 status was provided by the California Cancer Registry (N=639), or patient medical records (N=159). The distribution of risk factors did not differ between cases that did or did not have ER/PR data available for review (data not shown).

Where tissue samples were available, BCFR pathologists examined sections from histologic slides and/or paraffin tumor blocks and categorized tumors as ER or PR positive if ≥10% of tumor cells stained positive. This cutoff for positivity was typical for samples collected and examined at the time of data collection, although current practice classifies positivity at greater than 1% tumor cells stained positive. Where tissue samples were not obtained, pathologists reviewed pathology reports and medical records and recorded the ER and PR status listed on the report, or, if information existed on the percent of cells staining positive, employed the same requirements that ≥10% of cells staining positive resulted in a definition of ER or PR positive. For ascertaining HER2 status, pathology reports or medical records were used to ascertain status, and when tissue was available, study pathologists also examined histologic slides or paraffin tumor blocks and characterized tumors as HER2 positive or negative. Immunostaining for HER2

was considered positive when strong intensity of membranous staining was present in the majority (>50%) of cancer cells. Current procedure more commonly defines breast cancer as HER2+ when strong intensity of membranous staining is positive in >30% of cancer cells.

Of the cases, 2486 were ER+PR+, 920 were ER-PR-, 397 were ER+PR-, and 208 were ER-PR+, using the classification methods described above. Of the sub-population for whom HER2 data are available, 470 are classified as Luminal A (ER+ and/or PR+, HER2-), 119 as Luminal B (ER+ and/or PR+, HER2+), 67 as HER2+ (ER- and PR-, HER2+) and 142 as Triple-negative (ER-, PR-, and HER2-).

Statistical Analysis

I conducted a case-control analysis using ER and PR status to define the cases, using unordered polytomous logistic regression, resulting in 4 ER- and PR-defined groups (ER+PR+, ER+PR-, ER-PR+, ER-PR-) which were each compared to the control group. I additionally used binary logistic regression to examine case/case differences, comparing ER-PR- cases to ER+PR+ cases. To determine whether risk factor associations differed by age cohort, for risk factors of interest (parity, breastfeeding, oral contraceptive use) I stratified women on the basis of birth year into four age cohorts: birth year 1926-1939, 1940-49, 1950-59, and 1960-1981. To examine role of family history, I conducted stratified analyses based on first-degree family history. For the sub-population where HER2 status was available, I conducted case-only analysis comparing Luminal B, HER2+ and Triple-negative cases to the referent of Luminal A cases.

Using multivariable unordered polytomous regression, adjusted for age, race/ethnicity, and study site, I compared known or suspected breast cancer risk factors, including OC use (never, ≤ 5 years, > 5 years), starting date of OC use (never, any use prior to 1975, all use in 1975 or later);

age at menarche (≤11, 12, ≥ 13 years); parity (nulliparous, 1-2 live births, ≥3 live births); age at first birth (continuous); lifetime breastfeeding duration (never, <12 months, ≥ 12 months); combined parity and breastfeeding (nulliparous, 1-2 children never breastfed, 1-2 children ever breastfed, ≥ 3 children never breastfed, ≥ 3 children ever breastfed); smoking history (never smoker, former smoker, current smoker), BMI (continuous), education (< high school, high school or higher), alcohol consumption (< 7 drinks per week, ≥ 7 drinks per week, current non-drinker), history of ≥ 1 first-degree relative with breast cancer (yes, no), and menopausal status (pre- or postmenopausal).

The key variables of interest were parity, oral contraceptive use, breastfeeding, and a combined breastfeeding/parity variable. The multivariable model included these variables and was adjusted for age, site, race, and any of the variables noted in the previous paragraph that was found to be significant in at least one ER/PR grouping (ER+PR+, ER+PR-, ER-PR+, ER-PR-) compared with a control group, was strongly associated with a risk factor of interest, such that it confounded the association between that factor and the outcome when added to a regression, or for which there was strong evidence in previous literature of an association between the risk factor and breast cancer. I considered a risk factor to be a confounder if the suspected confounder changed the effect measure [β] of the risk factor of interest on the outcome by 10% or greater, or if a preponderance of previous literature supported an association. A risk factor was considered significantly related to the outcome if the 95% confidence interval did not include the value of "1". All statistical tests were two-sided.

Because the nature of various reproductive and hormonal risk factors has changed over time, I examined effects using four "cohorts" defined by birth year, to identify whether associations with oral contraceptive use, parity, and breastfeeding behavior, demonstrated in the overall

sample remained when examined within birth cohort. To examine the significance of this change, I created in interaction term between year of birth and the risk factor of interest (i.e. oral contraceptives) and tested whether the interaction terms was significant (p-value 0.05). For all cohorts, the interaction term for oral contraceptive use was significant in a polytomous logistic regression (data not shown). I then conducted polytomous logistic regression with two case groups (Any ER or PR positivity, designated HR+, and ER-PR-) and one control group, stratified by age cohort.

To examine these tumor characteristics after accounting for their correlation with one another, I examined ER, PR, and the additional tumor characteristic of grade in case-only analyses using binary logistic regression, comparing ER- tumors to ER+ tumors, PR- tumors to PR+ tumors, and high-grade tumors to low-grade tumors (where high grade was considered grade 3, and low-grade was considered grade 1,2). I then accounted for the correlation between these factors by simultaneously adjusting for the correlation of ER status to PR status, for example, to see how findings changed.

All statistical analyses used SAS Version 9.2 Software (SAS Institute, Cary, NC) or MATLAB.

2.C. RESULTS

Table 2-1 summarizes frequencies of demographic characteristics, risk factors and (for the cases) tumor characteristics, for controls and breast cancer cases categorized by joint ER/PR status. The distribution of ER and PR status was very similar across sites (ER+PR+: 64%, 60% and 61%, ER+PR-: 9%, 10%, and 11%, ER-PR+: 5%, 8%, and 4%, and ER-PR-: 22%, 21%, and 24% for Ontario, Australia, and California, respectively. Due primarily to oversampling for non-white race and positive family history among the cases, cases consist of a higher proportion of non-

whites, and are much more likely to have a family history of breast cancer, than controls. Cases regardless of hormone status had a higher rate of nulliparity and were less likely to breastfeed than controls, reflecting differences in known breast cancer risk factors.

Appendix Table A1-2 summarizes demographic, risk factor and tumor characteristic frequencies, for cases categorized separately according to independent ER status, PR status, and grade. ER-, PR-, and high grade cases were more often younger and premenopausal, and were much for likely to be *BRCA1+*. ER- and high grade cases were more likely to have used OCs for more than 5 years, and less likely to have used HRT.

Parity & Breastfeeding

Table 2-2 presents the multivariable adjusted ORs for the association of parity and breastfeeding, and a combined parity/breastfeeding variable on each breast cancer subtype, categorized as either ER+PR+, ER+PR-, ER-PR+, or ER-PR-, compared with the control group, and also includes the findings for parity and breastfeeding from case-only analyses comparing ER-PR- cases to ER+PR+ cases.

High parity (≥3 live births) was associated with an increased risk of ER-PR- cancer (OR=1.59, 95% CI 1.15-2.18, vs. nulliparity) and 1-2 live births was associated with a borderline increased risk (OR=1.33, 95% CI 1.00-1.76). When stratified by menopausal status, high parity was associated with increased risk of ER-PR- cancer in premenopausal women only (OR=1.50, 95% CI 1.04-2.17, 1-2 live births; OR=1.68, 95% CI 1.10-2.56, ≥3 live births, vs. nulliparity).

Breastfeeding was associated with a reduced risk of all breast cancer subtypes, but most strongly with ER-PR- cancer (OR=0.72, 95% CI 0.57-0.91 <12 months of breastfeeding vs. never; OR=0.52, 95% CI 0.40-0.68, ≥12 months of breastfeeding vs. never), with even greater risk

reduction found in postmenopausal women (OR=0.34, 95% CI 0.21-0.54, ≥12 months of breastfeeding vs. never). Breastfeeding >3 months was non-significantly inversely associated with ER-PR- cancer, and a breastfeeding variable defined continuously was significant, indicating that additional months of breastfeeding may confer additional benefit (data not shown).

When combined with breastfeeding behavior, the increased risk of ER-PR- breast cancer associated with high parity was only found in women who had children but did not breastfeed (OR=1.57, 95% CI 1.10-2.24, ≥3 live births, no breastfeeding, vs. nulliparity). Case-only comparisons (with ER+PR+ tumors as the referent) also showed increased risk of ER-PR- tumors for parity combined with a lack of breastfeeding (OR=1.59, 95% CI 1.19-2.13, 1-2 live births, and OR=1.69, 95% CI 1.20-2.38, ≥3 live births vs. nulliparity).

Oral Contraceptive Use

Table 2-3 presents the multivariable adjusted ORs for each breast cancer subtype, compared with the control group, for OC use and OC start date, and also includes the findings for OC use for the case-only comparisons comparing ER-PR- cases to ER+PR+ cases.

Oral contraceptive use was not associated with ER-PR- breast cancer (OR=1.13, 95% CI 0.89-1.44 for use >5 years vs. none). However, first use of OCs prior to 1975 was positively associated with ER-PR- cancer (OR=1.32, 95% CI 1.04-1.67), but not with hormone receptor positive cancers. First use in 1975 or later was not associated with ER-PR- cancer.

OC use was inversely associated with ER+PR+, ER+PR-, and ER-PR+ breast cancer, with OR estimates statistically significant for ER+PR+ cancer (OC use >5 yrs vs. none: OR=0.83, 95% CI=0.69-0.98). Inverse associations with hormone receptor positive subtypes were stronger when

OC use began in 1975 or later (OR=0.59, 95% CI 0.48-0.73, ER+PR+; OR=0.52, 95% CI, 0.36-0.76, ER+PR-, OR=0.34, 95% CI, 0.21-0.56, ER-PR+). Findings for OC use pre or post-1975 did not differ for cancer diagnosed pre- or post-menopausally (data not shown).

In case-only analysis, there was a stronger association between OC use and ER-PR- cancer compared to ER+PR+ cancer (OR=1.35, 95% CI=1.07-1.70, OC use >5 years vs. none). Case-case differences also existed for OC use pre- or post-1975, with statistically significant positive associations for ER-PR- cancer compared with ER+PR+ cancer.

Role of Family History

Breastfeeding remained significantly associated with ER-PR- cancer, compared with controls, in cases with a first-degree family history of breast cancer (OR=0.51, 95% CI 0.28-0.94), and the inverse association between breastfeeding and breast cancer, did not differ among those with and without a family history of breast cancer, for all ER/PR-defined subtypes. By contrast, parity was positively associated with ER-PR- cancer, compared with controls, only among ER-PR- cases *without* a first-degree family history of breast cancer (OR=1.64, 95% CI 1.16-2.33, ≥3 live births vs. nulliparous). There was no such association for ER-PR- cases with a family history (OR=1.11, 95% CI 0.54-2.27, ≥3 live births vs. nulliparous). Oral contraceptive use was also not associated with ER-PR- cancer in cases *with* a family history of breast cancer (OR=0.68, 95% CI 0.40-1.15, >5 yrs of OC use, vs. none), but was associated in those *without* family history (OR=1.33, 95% CI 1.01-1.75, >5 yrs of OC use, vs. none).

Differences by Race/Ethnicity

African-American women (OR=1.71, 95% CI 1.22-2.40) and Hispanic women (OR=1.43, 95% CI 1.02-2.00) were more likely to be ER-PR- than ER+PR+, compared with non-Hispanic White

women. I found that the trend for the combined parity-breastfeeding measure held across race/ethnicities, with associations for ER-PR- cancer higher among parous women who did not breastfeed than among women who did, for non-Hispanic Whites, African Americans, Hispanics, and Asian Americans (*Figure 2-1*).

In-depth Exploration of Selected Risk Factors by Birth Cohort

Appendix Table A1-3 describes the frequency and prevalence of risk factors within cases and controls in four cohorts defined by birth year: birth year 1926-1939, birth year 1940-49, birth year 1950-59 and birth year 1960-81. Cases from the oldest cohort (birth year 1926-1939) had the lowest mean duration of OC use, and the highest proportion of OC users who commenced using OCs after their first live birth. The also had the lowest mean age at first live birth, and the highest parity, of all age cohorts.

Figure 2-2 depicts the changing nature of oral contraceptive use over time, and shows in a forest plot the association of OC use with breast cancer risk, by hormone receptor status, within each birth-year-defined cohort. Because findings were not different based on categorical duration of OC use (≤5 year, > 5 years), these measures were combined into an oral contraceptive "Never/Ever" dichotomous variable. Additionally, ER+PR+, ER+PR-, and ER-PR+ cases were combined into a single "hormone positive" (HR+) subgroup, for comparison to the control group and the ER-PR- group. I found that among those in the oldest cohort (Cohort 1), who had the least experience with OCs, OC use was not associated with either HR+ or ER-PR- cancer, and ORs were similar between the case groups. In the 2nd oldest cohort (Cohort 2), for birth years 1940-1949, OC use was associated with ER-PR- cancer, compared with controls (OR=1.53, 95% CI 1.04-2.24), but was not associated with HR+ cancer. In the cohort encompassing birth years 1950-1959 (Cohort 3), OC use was not associated with ER-PR- cancer, but was inversely

associated with HR+ cancer (OR=0.66, 0.48-0.91). Cohort 2 mostly commenced use of OCs prior to 1975, whereas Cohort 3 commenced using OCs both before and after 1975. Among ER-PR- patients in Cohort 2 who used OCs prior to 1975, the OR was 1.55 (95% CI, 1.04-2.29), while among those in Cohort 3 who used OCs prior to 1975 the OR was not statistically significantly elevated, but for those whose first used OCs in 1975 or later, both for HR+ and ER-PR- cases there was an inverse association between OC use and risk, compared with controls (*Appendix Table A1-4; Figure 2-2*).

Because the oldest cohort tended to use OCs after first live birth, while younger cohorts tended to begin use prior to first live birth, I examined whether OC use differed according to whether use had begun before or after first live birth (women who had used OCs but were nulliparous were excluded from analysis). Not accounting for age cohort, first use of OCs after first live birth was associated with ER-PR- cancer (OR=1.35, 95% 1.05-1.72), compared to never use, but was not associated with HR+ cancer. This estimate was stronger among women in age Cohort 2 (birth year 1940-49) and significant regardless of hormone receptor status (OR=2.12, 95% CI 1.35-3.33 for ER-PR- cancer, OR=1.45, 1.06-1.99, HR+ cancer), but not significantly associated with other cohorts. I also examined whether first use after first live birth was associated with breast cancer risk, in women who began using OCs prior to 1975. Commencement of OCs after first live birth, rather than prior to first live birth, was associated with an increased risk of ER-PR- cancer (OR=1.71, 95% CI 1.28-2.28) in women who commenced use prior to 1975, which includes a large proportion of the women in the 1940-1949 birth cohort. In women who commenced use in 1975 or later, the manner in which they used OCs (before or after first live birth) was not associated with breast cancer risk for either HR+ or ER-PR- women.

Parity and breastfeeding association with ER and PR status were also examined by age cohort. These findings are contained in *Appendix Table A1-4.* I found that parity is protective against ER+PR+ cancer among older women only, but this is primarily an age/menopausal status effect rather than an age cohort effect. In addition, there does not appear to be a cohort effect for breastfeeding. Rather, breastfeeding appears to be protective for all cohorts, and more strongly inversely associated with ER-PR- cancer in all women.

Differences by Molecular Subtype

Because only a small sample of cases had HER2 data available, findings by molecular subtype are shown in *Appendix Table A1-5.* In case-only analysis, high parity was associated with an increased risk of HER2+ and triple-negative breast cancer (OR=2.85, 95% CI 0.97-8.52, for HER2 vs. Luminal A cancer; OR=2.72, 95% CI 1.33-5.55, for triple-negative vs. Luminal A cancer), whereas breastfeeding was inversely associated with triple-negative cancer (OR=0.48, 95% CI 0.28-0.81, < 12 months of breastfeeding vs. none; OR=0.56, 95% CI 0.31-1.01, ≥ 12 months of breastfeeding vs. none). Parous women who did not breastfeed were more likely to have HER2+ (OR=3.37, 95% CI 1.21-9.40, HER2+ vs. Luminal A, for 1-2 live births, no breastfeeding; OR=3.01, 95% CI 0.92-9.86, ≥ 3 live births, no breastfeeding) or triple-negative cancer (OR=2.44, 95% CI 1.24-4.82, triple negative vs. Luminal A, for 1-2 live births, no breastfeeding; OR=2.10, 95% CI 0.93-4.76, for ≥ 3 live births, no breastfeeding) compared with nulliparous women. OC use of > 5 years, compared to never, was positively associated with triple-negative cancer (OR=1.73, 95% CI 1.03-2.90), as was OC use that began prior to 1975 (OR=2.17, 95% CI 1.10-3.94).

Pseudo-conditional likelihood findings

Tables 2-4A and 2-4B present the multivariable ORs for ER+ vs. ER- tumors, PR+ vs. PR- tumors, and Grade 3 tumors vs. Grade 1,2 tumors, for the key risk factors of interest: oral contraceptive use, parity, and breastfeeding. For each tumor characteristic, the first (left-hand) column represents the multivariable OR unadjusted for correlation with the other tumor characteristics, and the 2nd (right-hand) column represents the multivariable OR, adjusted using the pseudo-conditional likelihood approach, to account for the correlation among tumor characteristics (i.e., ER status is adjusted for PR status and grade, PR status is adjusted for ER status and grade, and grade is adjusted for ER status and PR status.)

Parity: Parity was positively associated with ER- status, compared to ER+ status, in a multivariable model unadjusted for PR status and grade (OR=1.45, 95% CI 1.11-1.89, 1-2 live births vs. nulliparous; OR=1.61, 95% CI 1.19-2.17, ≥ 3 live births vs. nulliparous); and in a pseudo-conditional likelihood model adjusted for PR status and grade (OR=1.43, 95% CI 0.98-2.09, 1-2 live births vs. nulliparous; OR=1.53, 95% CI 1.00-2.33, ≥ 3 live births vs. nulliparous). Parity was not associated with PR status or grade.

Breastfeeding: Breastfeeding for 12 months or longer was associated with a reduced risk of ER- cancer, compared to ER+ cancer, and PR- cancer compared to PR+ cancer (OR=0.65, 95% CI 0.49-0.85, ER- vs. ER+, OR=0.72, 95% CI 0.55-0.92, PR- vs. PR+). However, in models adjusted for ER status and grade, breastfeeding history was no longer associated with PR status (OR=0.90, 95% CI 0.64-1.26, for ≥ 12 months of breast feeding vs. never), indicating apparent inverse associations between breastfeeding with PR-negativity are likely due to this factor's correlation with ER-negativity.

Oral contraceptive use: Those who used oral contraceptives for greater than 5 years were more likely to be ER- compared with never users (OR=1.26, 95% CI 1.00-1.59, *Table 2-4B*). Once the model was additionally adjusted for PR status and grade using the pseudo-conditional likelihood approach, ER status was no longer associated with OC use greater than 5 years (OR=1.07, 95% CI 0.77-1.50). OC use greater than 5 years was associated with high grade (OR=1.41, 95% CI 1.15-1.86) and remained associated with high grade, compared to low grade, cancers after adjustment for ER and PR status (OR=1.37, 95% CI 1.08-1.73). OC use was also positively associated with grade, when OC use began in 1975 or later, in models both unadjusted and adjusted for ER and PR status. PR status alone was not associated with oral contraceptive use in any model.

2.D. DISCUSSION

Effects of Reproductive and Hormonal Risk Factors

In this population-based study, which was enriched with women at higher than average population risk for breast cancer (due to oversampling of cases with early-onset breast cancer and/or a family history of breast cancer), I found that the factors of oral contraceptive use, parity, and breastfeeding differ for different subtypes of breast cancer defined by ER/PR status, that the effects of these risk factors can change over time, and that they can differ depending on whether cases have a family history of breast cancer

I found that high parity was associated with an increased risk of ER-PR- cancer, compared with controls, and that breastfeeding for a total duration of ≥12 months reduced this risk. Breastfeeding for a total duration of 12+ months was protective against cancer regardless of hormone status, however findings differed when comparing the case groups to one another, such that breastfeeding was more strongly protective against ER-PR- cancer than ER+PR+ cancer.

Previous studies have found that duration of breastfeeding, coupled with parity level, is an important factor for risk of triple-negative (ER-PR-HER2-) breast cancer [81, 108, 151]. When I examined this combined variable for ER-PR- cancer, (the majority of which is likely to be triple-negative) I also observed that multiparity, combined with no breastfeeding, was associated with an increased risk of ER-PR- cancer, and triple-negative cancer, but not with hormone receptor positive cancer. Thus the positive association I found between high parity and ER-PR- cancer, is mitigated by breastfeeding, such that women who are multiparous are no longer at increased risk of ER-PR- cancer if they breastfeed. I found that this association for ER-PR- cancer was similar across race/ethnicity, and that breastfeeding was inversely associated with ER-PR- cancer among those with and without first degree family history of breast cancer.

Studies that examine joint ER/PR status have largely demonstrated an inverse association between parity and ER+/PR+ cancer risk, but not ER-PR- risk [40, 59, 70, 71, 90, 98]. A minority of studies has found no differential effect of parity on cancer risk by hormone status [41, 73, 89]. In studies examining higher risk women, a 2006 publication by Ma *et al* in women under age 50 found that the protective effect of parity was confined to ER+/PR+ cancers, and that the protective effect increased with each additional pregnancy [27], while another study, of premenopausal women in China and Vietnam, found that increasing parity (compared with nulliparity) was protective against ER+ cancer, but not significantly associated with ER- cancer [55]. In a study of very young women, 35 years and under, ER status was not associated with parity. Thus, the bulk of research in this area has suggested that any parity versus nulliparity, and multiparity compared with uniparity, offer protection against ER+ and PR+, but not ER-PR-, breast cancer. While my analysis did not find an association between parity and reduced cancer risk for hormone receptor positive breast cancer, I did find this to be true among postmenopausal

women with 1-2 births. I also found a positive association between parity and ER-PR- cancer, similar to the findings of Yang et al, in their case-only analysis [65], and reflecting similarities to findings among studies that examined triple-negative breast cancer [6, 74]. Research has shown that following pregnancy, women experience a transient increase in breast cancer risk that peaks approximately 5 to 6 years postpartum, and that over time, the increased risk following pregnancy diminishes, such that a crossover in risk occurs and women who have had their first birth below age 35, have a lower breast cancer risk than age-matched women who have never given birth [157, 158]. One potential mediator underlying the poor prognosis of breast cancer diagnoses following pregnancy is postpartum mammary gland involution [157]. Upon pregnancy, the epithelium extensively proliferates to meet the demand of lactation. Following lactation, or pregnancy if lactation does not occur, the mammary gland undergoes the process of postpartum involution to return to a state morphologically resembling the relatively simple ductal network of the pre-pregnant gland. Support for postpartum involution eliminating lactationally-competent lobules in women is demonstrated by the observation that the epithelial content in the breast following pregnancy becomes indistinguishable from that of nulliparous women within 18 months postpartum [157]. Evidence shows that the involuting mammary gland has similar characteristics to tumor-promotional microenvironments, indicating that the process can result in tumor proliferation [159]. This may be one of the processes by which multiparity in the absence of breastfeeding increases risk of some subtypes of breast cancer.

This research confirms earlier findings that breastfeeding decreases risk of breast cancer, regardless of hormone receptor status. A recent review supported that ER or PR expression was not differentially associated with breastfeeding [19], and most other studies have confirmed this finding for both ER/PR-defined subtypes [27, 58, 62, 63, 90, 98], and subtypes defined by ER,

PR, and HER2 status [59, 83, 96]. Some studies have shown, as this one did, that the inverse association with breastfeeding is stronger for ER-, ER-PR-, or triple-negative breast cancer [6, 54, 81, 96]. When examined in tandem, this research confirms earlier research demonstrating that high parity, coupled with lack of breastfeeding, is associated with increased risk of ER-PR- cancer, while high parity in the presence of breastfeeding is not associated with risk [6, 81]. Thus, among parous women, breastfeeding can reduce risk of ER-PR- cancer and represents a modifiable factor. One mechanism by which breastfeeding may mediate the effect of recent parity on breast cancer is to delay and slow the process of mammary gland involution. Proliferation of epithelial cells occurs during pregnancy; in the absence of breastfeeding, the involution of these mammary cells can occur rapidly.

It has long been hypothesized that high- and low-risk breast tumors are distinct breast cancer subtypes with distinct risk factor profiles [6, 160, 161]. In this study, the increased risk found for ER-PR- cancer among women with high parity was primarily confined to women who did not breastfeed. Fifteen years ago, the Collaborative Group on Hormonal Risk Factors in Breast Cancer determined that breastfeeding is protective against breast cancer above and beyond the protection conferred by parity [162]. Hypothesized potential protective mechanisms include the removal of estrogens via breast fluid, excretion of carcinogenic agents through breast milk, delay in ovulation associated with breastfeeding, and induction of terminal differentiation of breast epithelial cells[163]. It has been shown that *BRCA1* mutation carriers, who are typically diagnosed with ER-PR- cancer, were less likely to develop breast cancer if they breastfed for at least one year, compared with BRCA1 mutation carriers who did not breastfeed; there was no association with breastfeeding among BRCA2 mutation carriers, who usually have ER+ tumors[164]. It is hypothesized that full-term pregnancy followed by failure to breastfeed or

short duration of breastfeeding could result in retention of initiated progenitor cells (that would have died or differentiated during lactation) and these retained cells could presumably develop into “basal-like” breast tumors, which are often characterized, and usually defined, by ER- and PR- negativity.

Oral contraceptive use

Overall, oral contraceptive use greater than 5 years was associated with a reduced risk of hormone receptor positive breast cancer, and was not associated with ER-PR- cancer. Additional analysis revealed that, In particular, oral contraceptive use was associated with a decreased risk of ER+ cancer *only* if date of first use occurs in year 1975 or later, while OC use prior to 1975 is associated with increased risk of ER-PR- cancer. When year of birth was considered, I found that OC use was positively associated with ER-PR- cancer in women from the 1940-49 age cohort only, most of whom began using oral contraceptives prior to 1975, and that OC use was inversely associated with women born from 1950-1959, but this association was only maintained among women who began using oral contraceptives in 1975 or later. In addition to changes in OC formulation, timing of use associated with parity seemed to be important in the association between OC use and HR+ or ER-PR- cancer. Overall, first use of OCs after first childbirth was associated with an increased risk of ER-PR- cancer, and an increased risk of both HR+ and ER-PR- cancer in the cohort born from 1940-1949. Earlier published studies reported positive associations between ER-PR- breast cancer and OC use (reviewed in [19], whereas most recent studies have demonstrated, like this study, no overall association between ER-PR- breast cancer and OC use [27, 98], (although some studies have reached different conclusions [99]). I found that OC use in 1975 or later was inversely associated with ER+PR+ breast cancer, and a positive association between OC use and ER-PR- breast cancer was limited to women who initiated use

prior to 1975. Analysis of year of initiation as an important variable in evaluating the association between OC use and breast cancer risk has become more common [12, 165, 166], but has not regularly been examined in previous research on OC use and breast cancer risk by hormone receptor status. Data on OC use and breast cancer risk in *BRCA1* mutation carriers, including some from the BCFR study sample[153, 154, 167], have demonstrated no increased risk with OC use initiated after 1974 (and *BRCA1* tumors are usually ER-PR-), and examination of OC use among women with family history of breast cancer found increased risk of breast cancer only among women who began OC use prior to 1975 [166]. Therefore, among these higher risk populations, findings were similar to those in my analysis, and may be an important factor in explaining why previous research regarding OC use and breast cancer risk has yielded conflicting results. In this study, findings were similar for any hormone-positive (ER+ and/or PR+) subtype, and only different for the ER-PR- type, indicating that any etiology related to OC use may be through both estrogen and progesterone-related mechanisms. It is unclear why OCs used prior to 1975 would be more strongly associated with ER-PR- cancer, and with high tumor grade. Studies of synthetic progestins used in oral contraceptives have generally found that the proliferative actions of progestins used in oral contraceptives are mediated through the estrogen receptor [168, 169] which does not explain why ER- cancer is more likely to be affected, unless the estrogen receptor is effectively "turned off" by such proliferation. Typical estrogen doses used in the 1960s were more than double the doses used in the 1980s, and progestin doses were also higher and included different types of progestins than current OCs[166]. Biologic and clinical evidence support a role for exogenous estrogen effects on carcinogenesis mediated through estrogen-receptor α (ERα) receptor, yet evidence supports that receptor-independent pathways may also exist [170]. This connection will require further investigation.

The inverse association between OC use and hormone positive breast cancers found in this study is inconsistent with most other published studies that have found either no association between OC use and hormone positive breast cancers or a positive association, although a recent study in a Chinese population found a protective effect in women who were hormone positive [98] as did a study using data from the Women's Health Initiative [74]. Most previous studies of OC use and breast cancer risk have not examined findings by subjects' birth years. A previous study of Icelandic women that examined OC use by birth year found an increased odds of breast cancer for longer duration of OC use among women with birth years after 1950, but did not separate the cancer by hormone status [171]. Women who were born between 1940-1949 and used OCs experienced increased risk of ER-PR- cancers, while women born earlier or later did not. The reason for the increased risk among this cohort appears to be explained by the fact that most of these women used OCs that were manufactured prior to 1975, which contained high doses of estrogen, and may also be due to the nature in which the women used OCs, with approximately half of this cohort initiating OC use after first full-term pregnancy, rather than before. Women born prior to 1940, in our study, would have used OCs in a similar manner, and been exposed to OCs that had the high estrogen content. However, these women did not experience an elevation in risk. This may be because OC use was less common and of shorter duration among these women, or could be due to the selection process of older women into the BCFR sample. Many older women were recruited into the study if they had family history of breast cancer, and it is possible that family history served as a competing risk in these women, rendering OC use less important as a causal factor. I also found that in the birth cohort of 1950-59, women who used OCs had reduced risk of breast cancer, and this was true in both HR+ and ER-PR- cancer. The reduced risk only occurred in women who initiated OC use in 1975 or later, indicating that

change in estrogen and/or progesterone content of OCs at that time likely explains the protective effect. The protective effect does not carry into the youngest cohort of women, whose average age at cancer diagnosis was only 32. In these women, competing risks, primarily genetic in nature, likely drive breast cancer diagnosis, such that OC use may be unimportant in risk of early breast cancer.

Pseudo-conditional likelihood findings

I found, similar to other studies, that high parity was positively associated with ER-, compared with ER+ cancer [55, 62, 65, 89], and that breastfeeding was inversely associated with ER-, compared with ER+ cancer [91], associations that were maintained after adjustment for PR status and grade. I found that there was no association with PR status and parity, after adjustment for ER status and grade, in contrast with the few studies that have examined PR status individually in relation to parity, which found that nulliparity was inversely associated with PR negativity [65, 89]. Like most previous studies, I found no association with parity or breastfeeding, and tumor grade. Most studies examining ER status have found that parity is associated with a reduced risk of ER+ cancers [19, 58, 63, 65], with a greater reduction found in multiparous women. Parity is postulated to confer protection against breast cancer through four mechanisms: it increases differentiation in mammary gland tissue, induces changes in circulating hormone levels, parity decreases mammary stem cell activity, and decreases levels of estrogen receptor in the breast [172]. Decreasing levels of estrogen receptors, and inducing changes in hormone levels, in particular, would represent plausible mechanisms by which parity would decrease ER+, but not ER-, breast cancer.

In comparisons of ER- vs. ER+ cases, PR- vs. PR+ cases, and low grade vs. high grade cases, after simultaneous adjustment for the other tumor characteristics, apparent associations between

ER- and PR-, and OC use, in fact seemed to be driven by these tumor characteristics' correlation with high grade. It appeared that OC use was associated with high grade, rather than low grade tumors, although after adjustment for ER- and PR- status, this was only the case when OC use was initiated in 1975 or later. Only two previous studies have examined OC use and grade. One study (which studied extremely young women) found no association between OC use and grade. However a study in a small sample of cases (N=215), found that while "never users" of oral contraceptives had higher grade cancers, long duration of use was also positively associated with high grade [102]. Other data on OC use and tumor characteristics have been ambivalent. The Collaborative Group on Hormonal Factors in Breast Cancer found that "ever users" of OCs had tumors that were less clinically advanced [12]; however other studies have demonstrated that OC users have poorer prognosis tumors [173, 174]. Because of conflicting literature, the biological process by which OC use might result in high tumor grade are unclear.

Methodologic Considerations

Comparison group: For this analysis, I used population-based controls as the common referent group. I did not observe some established associations between hormonal and reproductive factors and hormonal status. For example, while greater age at menarche is associated with a reduced risk of hormone positive breast cancer in most studies (See Review, Chapter 1). I did not find this to be true in this study. Similarly, I did not find high parity to be inversely associated with ER+PR+ cancer, except among postmenopausal women, while most studies have found parity to be protective against this subtype even among younger women (Review, Chapter 1). In the BCFR sample, differences have been observed between population controls and sister controls in some risk factors that are possibly associated with participation in research [175]. Specifically, BCFR population-based controls are more likely to have been highly educated, and

have fewer births and higher average age at first birth, than are sister controls. Because high education, low parity and older average age at first birth are established breast cancer risk factors, cases were not less likely to be nulliparous or have higher average age at first birth than population controls, however, they were more likely to be nulliparous and have higher average age at first birth than sister controls [175].

For all the analyses reported in this chapter, I used both an unordered polytomous regression model, and a binary logistic model for the purposes of case/case comparisons. For the case/case analysis, my inferences would not be affected by any differential participation in the control group. For example, when comparing ER-PR- cases to ER+PR+ cases in terms of parity, I found that ER-PR- cases were significantly more likely to have three or more live births than ER+PR+ cases, with ORs that were similar to those found when comparing ER-PR- cases to population controls.

Case Selection: The BCFR sample of cases is not representative of all women with breast cancer; they are younger, more often ethnic and racial minorities, and more often have a family history of breast cancer. As a result, the distribution of the different ER and PR-defined subtypes might not be representative of these subtypes in a sample of cases unselected for these characteristics. For example, among older cases (birth year before 1940), more than 40% had a first-degree family history of breast cancer, primarily because many women who were older when were diagnosed with breast cancer were required to have a family history of cancer in order to be included in the study. Regardless, the purpose of this analysis is to examine risk factor by tumor subtypes specifically in a high-risk population, therefore comparisons to "typical" populations are neither expected or of value versus comparisons to other high risk

populations. To this end, my findings do reflect findings that examine younger, more ethnically diverse populations.

For the analysis of molecular subtypes, the population differed from the overall study sample in that it comprised mostly racial/ethnic minority cases from Northern California and Ontario, as few non-Hispanic whites were enrolled in the BCFR after year 2000, when HER2 data became available in the cancer registries. Due to these limitations, I conducted a case-only analysis and acknowledge that findings are preliminary, although they are in agreement with those of other studies.

Selection Bias: For only a subgroup of cases was pathological data on hormonal status available for review. If these women were not representative of all eligible cases, one or more findings could be biased, with the direction of the bias differing depending on the differences between those who participated and had pathology for review and those who did not. Distributions of parity and other risk factors for my sample and the entire case sample were similar (data not shown), improving the likelihood that cases with ER and PR data available are representative of the distribution of these hormonal subtypes for the entire case sample.

Case definitions: BCFR pathologists used common laboratory procedures and conducted a centralized pathology review to categorize cases as ER+PR+, ER+PR- ER-PR+, and ER-PR-. Unlike many previous studies, investigators did not rely on data from cancer registries for ER/PR classification. A recent study has demonstrated that registry-provided data may undercount the rarer ER/PR combinations (ER-PR+ and ER+PR-), and that centralized pathology review should be considered a gold-standard when classifying tumors by hormone receptor [176]. Thus, a centralized review is considered a strength of this study. The criteria for defining women as ER+,

PR+, or HER2+ were more stringent for this analysis, than criteria typically used today. This may limit the ability to compare this study's findings, to similar analyses in patients who have been classified as HR+ or HER2+ under less stringent criteria for positivity.

Information bias: The possibility of recall bias exists because I relied on participants' recalls of their exposures. However, the purpose of this analysis was to determine whether risk factor associations differed by subtype, using controls as a common comparison group. Because it is unlikely that cases report exposures differently based on their ER, PR, HER2 status, or grade, it is unlikely that OR estimates would be affected by recall bias of exposures that are differential by subtype.

Multiple comparisons: I conducted multiple comparisons with different exposures and outcomes, and used detailed constructs for exposures of interest (creating multiple categories for constructs) making it likely that one or more findings are due to chance. However, I did restrict my comparison to selected breast cancer risk factors and specifically examined whether key findings were robust.

Summary

Overall, I found that multiparity is associated with an increased risk of ER-PR- cancer, but this risk is reduced by breastfeeding, such that multiparous women who breastfeed are no longer at increased risk, regardless of race. Breastfeeding had a protective effect on both hormone-positive and ER-PR- cancer, but was more strongly protective against ER-PR- cancer. Although childbearing practices and breastfeeding incidence and duration have changed over time, neither of these factors appears to be associated with a cohort effect. I also found that oral contraceptive use was positively associated with ER-PR- cancer only among women who had begun use prior

to 1975, and risk differed according to age cohort and timing of use (before or after first live birth). In this study, which examined a high risk population using centralized pathology review, I confirmed previous findings regarding the role of pregnancy and breastfeeding in ER-PR- cancer, found that these findings are maintained across different race/ethnicity, and shed new light on the role that oral contraceptive use may have played in both HR+ and hormone-negative cancer in both older and younger women. I also determined that parity and breastfeeding appear more specifically related to ER status, rather than PR status an grade, however OC associations with ER and PR negativity, may in fact reflect an association with high tumor grade. These findings add to increasing evidence that risk factors differ depending on hormone receptor, confirm that such cancers need to be considered as separate entities, and provide preventive opportunities, in the form of breastfeeding promotion, against ER-PR- breast cancer. In the United States, initiation of breastfeeding has increased steadily since the 1970's and the average duration of breastfeeding is also increasing (Source: Surgeon General's Call to Action to Support Breastfeeding, Dept. of Health and Human Services, 2011). Recent trends examining SEER incidence data suggest that rates of ER-PR- breast cancer are decreasing and will likely continue to decrease in the coming years[2]. African-American women have lower rates of breastfeeding than other racial/ethnic groups (McDowell, M.M., NCHS report, 2008), and also have higher rates of ER-PR- breast cancer, suggesting that improving breastfeeding rates across all populations is essential.

Table 2-1: Demographic and Tumor Characteristics by ER/PR status, Breast Cancer Family Registry Population-Based Sample

	Controls N=2997 N (%)	**ER+PR+ N=2486 N (%)**	**ER+PR- N=397 N (%)**	**ER-PR+ N=208 N (%)**	**ER-PR- N=920 N (%)**
Age (μ± s.d.)	47.6±10.3	47.1±9.3	48.6±9.8	43.8±8.0	44.5±9.8
Race					
White	2487 (86)	1542 (62)	222 (56)	158 (76)	506 (55)
Black	96 (3)	221 (9)	45 (11)	16 (8)	131 (14)
Hispanic	72 (2)	229 (9)	46 (11)	7 (3)	113 (12)
Asian	165 (6)	445 (18)	79 (20)	23 (11)	149 (16)
Other	82 (3)	35 (1)	5 (1)	4 (2)	14 (2)
Site					
Ontario	1706 (57)	705 (28)	95 (24)	50 (24)	238 (26)
Australia	668 (22)	562 (23)	75 (19)	93 (45)	199 (22)
California	623 (21)	1219 (49)	227 (57)	65 (31)	483 (53)
First degree family history					
No	2732 (91)	1761 (71)	291 (73)	161 (78)	673 (73)
Yes	263 (9)	714 (29)	106 (27)	45 (22)	244 (27)
Menopausal Status					
Pre	1566 (55)	1431 (60)	172 (46)	149 (76)	574 (65)
Post	1262 (45)	951 (40)	205 (54)	47 (24)	310 (35)
Education					
< High school	908 (30)	710 (29)	114 (29)	56 (27)	289 (32)
≥ High school	2082 (70)	1740 (71)	275 (71)	150 (73)	602 (68)
OC Use					
Never	646 (22)	648 (27)	124 (32)	49 (24)	198 (23)
≤ 5 years	1117 (37)	948 (39)	129 (34)	71 (34)	328 (37)
> 5 years	1216 (41)	847 (35)	131 (34)	86 (42)	353 (40)
Date of first OC use					
Never	646 (22)	648 (27)	124 (32)	49 (24)	198 (23)
Before 1975	1435(48)	1165 (48)	167 (43)	97 (47)	370 (42)
1975 or later	898 (30)	630 (26)	93 (24)	60 (29)	310 (35)
Time of last OC use					
Never user	646 (24)	648 (30)	124 (36)	49 (27)	198 (26)
≤10 years	457 (18)	340 (15)	42 (12)	42 (23)	151 (20)
>10, <20 years	704 (26)	613 (28)	80 (23)	52 (29)	199 (27)
≥ 20 years	926 (34)	604 (27)	98 (28)	39 (21)	202 (27)
HRT Use					
Never	2081 (70)	1756 (74)	264 (70)	175 (88)	699 (80)
Former	246 (8)	199 (8)	37 (10)	9 (5)	59 (7)
Current	663 (22)	424 (18)	74 (20)	16 (8)	111 (13)

Age at menarche					
≤ 11	406 (14)	528 (22)	64 (16)	43 (20)	183 (21)
12	711 (28)	590 (24)	100 (26)	44 (21)	215 (24)
≥ 13	1760 (68)	1317 (54)	225 (58)	125 (59)	482 (55)
Parity					
Nulliparous	531 (18)	565 (23)	95 (24)	51 (25)	191 (21)
1-2	1334 (45)	1015 (41)	166 (42)	71 (34)	391 (42)
≥ 3	1132 (38)	906 (36)	136 (34)	86 (41)	338 (37)
Age at first birth	24.8	25.1	25.0	24.7	24.6
Breastfeeding duration					
Never	1203 (40)	1105 (45)	194 (49)	95 (46)	448 (50)
<12 mos.	991 (33)	764 (31)	113 (29)	51 (25)	267 (30)
≥ 12 mos.	803 (27)	595 (24)	86 (22)	60 (29)	187 (21)
Smoking					
Never Smoker	1542 (52)	1474 (60)	230 (59)	120 (58)	555 (61)
Former Smoker	919 (31)	611 (25)	99 (25)	47 (23)	200 (22)
Current Smoker	533 (18)	387 (15)	63 (16)	39 (19)	156 (17)
BMI	25.9	26.0	26.0	24.7	26.6
Tumor Grade					
1, 2	NA	1546 (74)	220 (67)	60 (39)	154 (20)
3	NA	554 (26)	109 (33)	93 (61)	628 (80)
BRCA1 status					
Status missing	NA	801 (32)	117 (29)	72 (35)	274 (30)
BRCA1 positive	NA	16 (1)	4 (1)	6 (3)	69 (8)
BRCA1 negative	NA	1669 (67)	276 (70)	130 (62)	577 (63)

Table 2-2: Association Between Parity and Breastfeeding, and Breast Cancer Classified by Hormone Receptor Status and Menopausal Status, Breast Cancer Family Registry Population-Based Sites

	ER+PR+* N=2174	**ER+PR-* N=341**	**ER-PR+* N=179**	**ER-PR-* N=791**	**ER-PR- vs. ER+PR+**
	OR (95%CI)	**OR (95%CI)**	**OR (95%CI)**	**OR (95%CI)**	**OR (95%CI)**
Parity (number of live births)					
Nulliparous	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
1-2	**0.80 (0.65-0.99)**	0.93 (0.64-1.35)	1.20 (0.71-2.02)	**1.33 (1.00-1.76)**	**1.62 (1.24-2.13)**
≥ 3	0.93 (0.73-1.17)	0.97 (0.64-1.49)	1.50 (0.85-2.65)	**1.59 (1.15-2.18)**	**1.66 (1.23-2.25)**
Breastfeeding duration (months)					
Never	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
< 12	1.04 (0.87-1.23)	0.84 (0.61-1.16)	0.66 (0.41-1.05)	**0.72 (0.57-0.91)**	**0.70 (0.56-0.88)**
≥ 12	**0.80 (0.66-0.98)**	**0.69 (0.48-0.99)**	**0.57 (0.35-0.94)**	**0.52 (0.40-0.68)**	**0.64 (0.50-0.84)**
Parity and breastfeeding (BF)					
Nulliparous	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
1-2 live births, never BF	0.80(0.63-1.00)	0.92 (0.62-1.38)	1.49 (0.86-2.60)	1.30 (0.96-1.75)	**1.59 (1.19-2.13)**
≥ 3 live births, never BF	0.90 (0.68-1.19)	0.95 (0.58-1.54)	1.01(0.49-2.06)	**1.57 (1.10-2.24)**	**1.69 (1.20-2.38)**
1-2 live births, ever BF	**0.78 (0.64-0.93)**	0.73 (0.52-1.05)	0.63 (0.38-1.05)	0.88 (0.68-1.14)	1.12 (0.87-1.45)
≥ 3 live births, ever BF	**0.82 (0.67-0.99)**	0.72 (0.50-1.04)	1.00 (0.64-1.56)	0.93 (0.71-1.22)	1.09 (0.84-1.42)
PREMENOPAUSAL WOMEN					
Parity (number of live births)					
Nulliparous	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
1-2	0.86 (0.65-1.15)	1.14 (0.78-2.54)	1.27 (0.66-2.42)	**1.50 (1.04-2.17)**	**1.73 (1.21-2.48)**
≥ 3	0.96 (0.69-1.33)	1.12 (0.57-2.21)	1.62 (0.81-3.26)	**1.68 (1.10-2.56)**	**1.70 (1.14-2.55)**
Breastfeeding duration (months)					
Never	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
< 12	1.05 (0.81-1.35)	0.86 (0.51-1.46)	0.75 (0.42-1.35)	0.74 (0.54-1.02)	**0.70 (0.51-0.96)**
≥ 12	0.76 (0.58-1.01)	0.88(0.50-1.54)	0.68 (0.36-1.19)	**0.61 (0.43-0.87)**	0.80 (0.56-1.13)
Parity and breastfeeding (BF)					
Nulliparous	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
1-2 live births, never BF	0.80 (0.59-1.09)	1.43 (0.76-2.67)	1.62 (0.83-3.18)	**1.56 (1.06-2.32)**	**1.94 (1.32-2.85)**
≥ 3 live births, never BF	1.05 (0.70-1.58)	1.08 (0.45-2.62)	1.04 (0.41-2.62)	1.49 (0.87-2.55)	1.35 (0.89-2.29)
1-2 live births, ever BF	0.84 (0.67-1.06)	1.23 (0.75-2.01)	0.79 (0.44-1.41)	1.03 (0.75-1.40)	1.21 (0.89-1.64)
≥ 3 live births, ever BF	0.80 (0.63-1.01)	0.99 (0.58-1.68)	1.20 (0.72-2.00)	1.13 (0.81-1.56)	1.37 (0.99-1.89)
POSTMENOPAUSAL WOMEN					
Parity (number of live births)					
Nulliparous	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)

1-2	**0.66 (0.47-0.93)**	**0.54 (0.33-0.91)**	0.62 (0.25-1.54)	0.84 (0.52-1.33)	1.26 (0.81-1.97)
≥ 3	0.84 (0.58-1.21)	0.77 (0.44-1.34)	0.82 (0.30-2.28)	1.11 (0.68-1.85)	1.30 (0.80-2.11)
Breastfeeding duration (months)					
Never	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
< 12	1.08 (0.83-1.39)	0.83 (0.54-1.26)	0.56 (0.24-1.30)	0.75 (0.53-1.07)	**0.70 (0.49-0.99)**
≥ 12	0.91 (0.67-1.27)	**0.49 (0.29-0.83)**	0.37 (0.13-1.03)	**0.34 (0.21-0.54)**	**0.37 (0.23-0.58)**
Parity and breastfeeding (BF)					
Nulliparous	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
1-2 live births, never BF	0.70 (0.48-1.00)	**0.58 (0.34-0.98)**	0.73 (0.29-1.85)	0.80 (0.39-1.30)	1.13 (0.72-1.81)
≥ 3 live births, never BF	0.75 (0.50-1.14)	0.70 (0.38-1.28)	0.61 (0.19-1.67)	1.12 (0.66-1.92)	1.46 (0.88-2.44)
1-2 live births, ever BF	**0.65 (0.46-0.91)**	**0.38 (0.22-0.65)**	**0.24 (0.08-0.72)**	**0.57 (0.35-0.93)**	0.88 (0.55-1.41)
≥ 3 live births, ever BF	0.86 (0.62-1.20)	**0.51 (0.31-0.85)**	0.44 (0.17-1.10)	**0.54 (0.83-0.88)**	**0.60 (0.38-0.97)**

*Note: Odds ratios (OR) and 95% confidence interval (CI), adjusted for age, race/ethnicity, study site, OC use, HT use, BMI, menopausal status, age at menarche, and education. ORs in **bold** are statistically significant.*
**Compared with population-based controls*

Table 2-3: Association Between Oral Contraceptive Use and Breast Cancer Classified by Hormone Receptor Status and Menopausal Status, Breast Cancer Family Registry Population-Based Sites

	ER+PR+* N=2174	**ER+PR-* N=341**	**ER-PR+* N=179**	**ER-PR-* N=791**	**ER-PR- vs. ER+PR+**
	OR (95%CI)	**OR (95%CI)**	**OR (95%CI)**	**OR (95%CI)**	**OR (95%CI)**
OC use					
Never	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
≤ 5 years	0.97 (0.82-1.15)	**0.73 (0.54-0.99)**	0.67 (0.44-1.04)	1.16 (0.92-1.47)	1.18 (0.94-1.49)
> 5 years	**0.83 (0.69-0.98)**	0.74 (0.55-1.01)	0.79 (0.52-1.20)	1.13 (0.89-1.44)	**1.35 (1.07-1.70)**
Year of first OC use					
Never	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
Before 1975	1.06(0.91-1.25)	0.80 (0.59-1.07)	1.12(0.73-1.73)	**1.32 (1.04-1.67)**	**1.28 (1.03-1.60)**
1975 or later	**0.59 (0.48-0.73)**	**0.52 (0.36-0.76)**	**0.34(0.21-0.56)**	0.82 (0.63-1.08)	**1.36 (1.06-1.75)**
OC use (Premenopausal)					
Never	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
≤ 5 years	0.97 (0.76-1.22)	0.65 (0.41-1.05)	0.62 (0.37-1.04)	1.00 (0.73-1.38)	1.05 (0.78-1.41)
> 5 years	**0.75 (0.59-0.94)**	0.83 (0.52-1.31)	0.67 (0.41-1.11)	0.98 (0.72-1.33)	1.31 (0.97-1.77)
OC use (Postmenopausal)					
Never	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
≤ 5 years	0.89 (0.69-1.14)	0.77 (0.51-1.15)	0.58 (0.25-1.32)	1.38 (0.95-1.99)	**1.50 (1.05-2.15)**
> 5 years	0.89 (0.68-1.16)	**0.63 (0.41-0.98)**	0.76 (0.35-1.67)	1.23 (0.83-1.81)	1.36 (0.96-1.98)

*Note: Odds ratios (OR) and 95% confidence interval (CI) adjusted for age, race/ethnicity, study site, parity, breastfeeding, HT use, BMI, menopausal status, age at menarche, and education. ORs in **bold** are statistically significant.*

**Compared with population-based controls*

Figure 2-1: Comparison of Odds Ratios by Race for Breastfeeding and Parity, Breast Cancer Family Registry Population-Based Sites, ER-PR- Cases vs. Controls

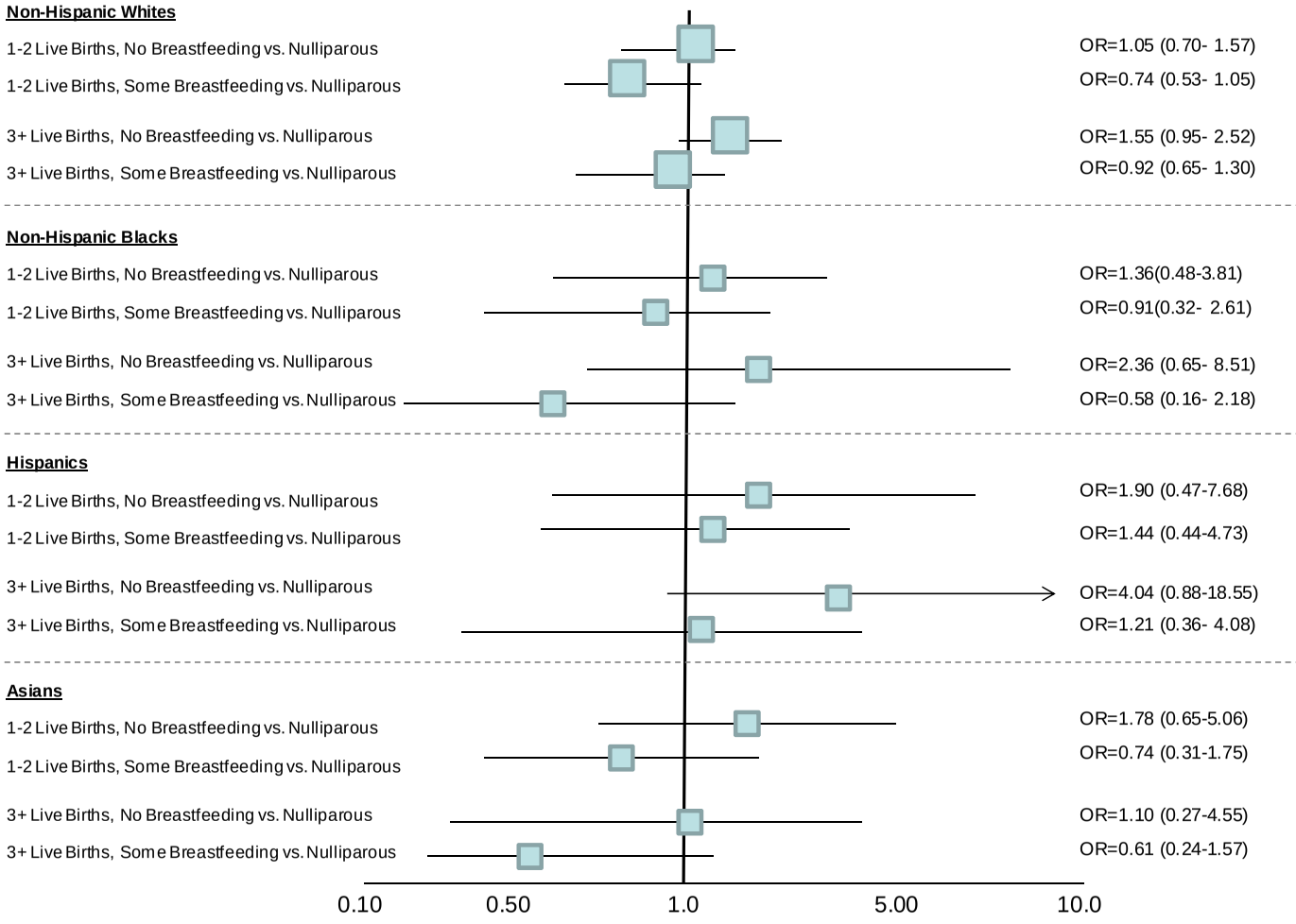


Figure 2-2: Nature of Oral Contraceptive use by Age Cohort and Odds Ratio by Cohort, Breast Cancer Family Registry Population-Based Sites

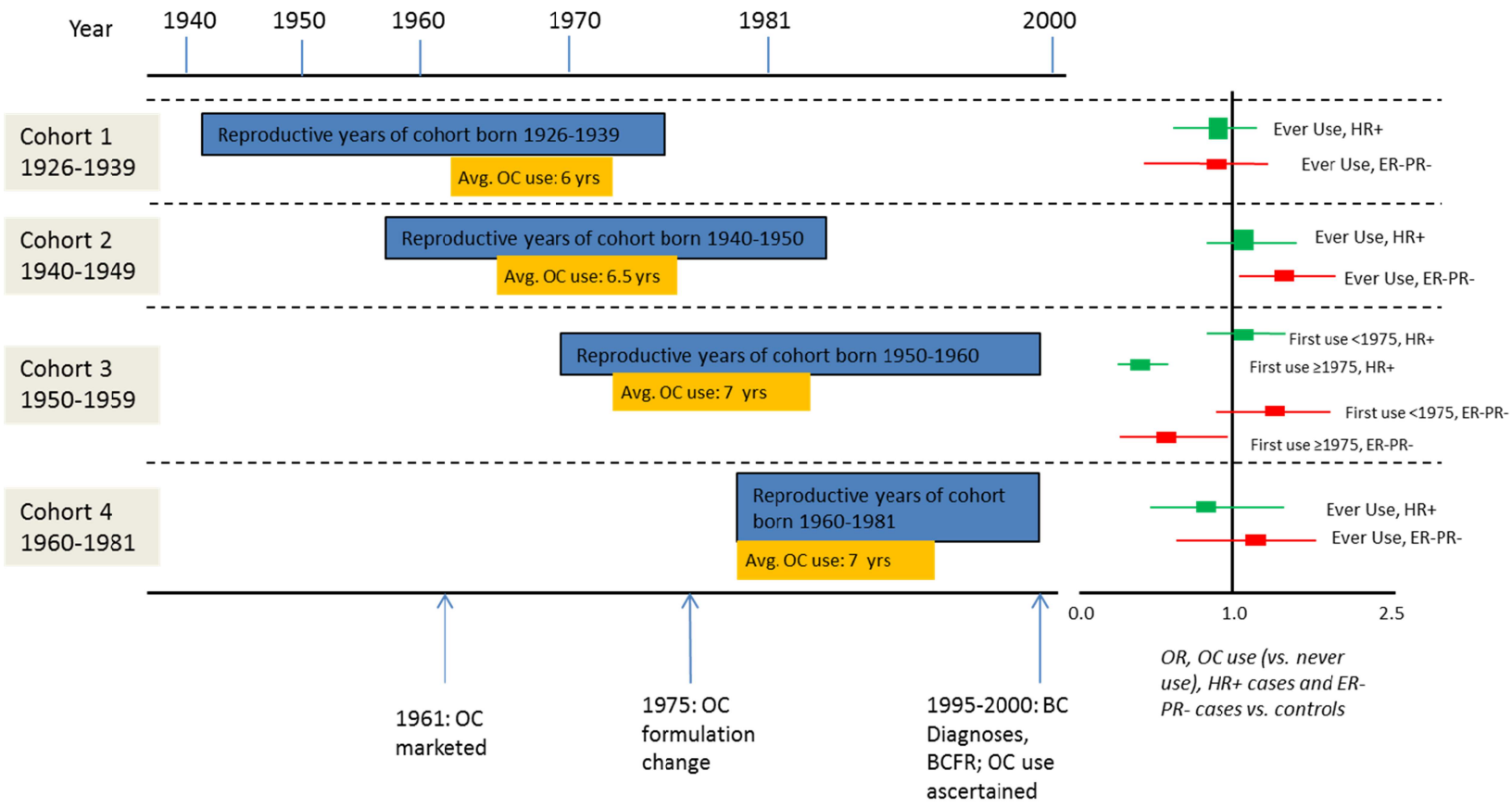


Table 2-4A: Associations between parity, breastfeeding and select tumor characteristics: case-case analysis and pseudo-conditional likelihood findings, Population-Based sites of the BCFR

	ER- (vs. ER+)		PR+ (vs. PR-)		Grade (3 vs. 1,2)	
	OR (95%CI) Logistic	OR (95%CI) PCL	OR (95%CI) Logistic	OR (95%CI) PCL	OR (95%CI) Logistic	OR (95%CI) PCL
Parity						
Nulliparous	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
1-2 live births	**1.45 (1.11-1.89)**	1.43 (0.98-2.09)	1.26 (0.98-1.61)	1.00 (0.72-1.40)	1.09 (0.85-1.40)	0.93 (0.70-1.22)
≥ 3 live births	**1.61 (1.19-2.17)**	**1.53 (1.00-2.33)**	1.32 (0.99-1.75)	0.97 (0.73-1.30)	1.22 (0.92-1.62)	1.01 (0.74-1.39)
Breastfeeding duration						
Never	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
< 12 mos.	**0.68 (0.54-0.85)**	**0.67 (0.48-0.92)**	**0.78 (0.63-0.97)**	0.97 (0.73-1.29)	0.95(0.77-1.17)	1.14 (0.90-1.45)
≥ 12 mos.	**0.65 (0.49-0.85)**	0.70 (0.48-1.03)	**0.72 (0.55-0.92)**	0.90 (0.64-1.26)	0.81 (0.63-1.04)	0.97 (0.73-1.28)
Parity and Breastfeeding						
Nulliparous	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
1, 2 live births, no BF	**1.42 (1.07-1.89)**	1.47 (0.98-2.20)	1.19 (0.91-1.55)	0.93 (0.65-1.33)	1.07 (0.82-1.40)	0.91 (0.68-1.23)
≥ 3 live births, no BF	**1.62 (1.15-2.27)**	1.54 (0.95-2.48)	**1.39 (1.01-1.92)**	1.08 (0.70-1.66)	1.20 (0.87-1.66)	0.98 0.69-1.41)
1, 2 live births, some BF	0.96 (0.74-1.25)	0.94 (0.65-1..36)	0.97 (0.76-1.24)	0.99 (0.72-1.36)	0.99 (0.78-1.25)	1.00 (0.77-1.30)
≥ 3 live births, some BF	1.03 (0.79-1.35)	1.08 (0.74, 1.56)	0.95 (0.74-1.22)	0.87(0.62-1.21)	1.06 (0.83-1.36)	1.07 (0.82-1.40)

*Note: All ORs adjusted for age, race, study site, education, oral contraceptive use, bmi, age at first birth, age at menarche , menopausal status, and other factors in the table. ORs in **bold** are statistically significant.*

Table 2-4B: Associations between oral contraceptive use and select tumor characteristics: case-case analysis and pseudo-conditional likelihood findings, Population-Based sites of the BCFR

	ER- (vs. ER+)		PR+ (vs. PR-)		Grade (3 vs. 1,2)	
	OR (95%CI) Logistic	OR (95%CI) PCL	OR (95%CI) Logistic	OR (95%CI) PCL	OR (95%CI) Logistic	OR (95%CI) PCL
OC Use						
Never	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
≤ 5 years	1.12 (0.90-1.41)	1.12 (0.81-1.54)	0.99 (0.81-1.23)	0.86 (0.65-1.13)	**1.21 (0.99-1.49)**	1.20 (0.96-1.51)
> 5 years	**1.26 (1.00-1.59)**	1.07 (0.77-1.50)	1.13 (0.91-1.40)	0.96 (0.72-1.28)	**1.41 (1.14-1.74)**	**1.37 (1.08-1.73)**
OC Use 1975						
Never Used OCs	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
Used OC before 1975	1.21 (0.96-1.52)	1.23 (0.89-1.70)	1.02 (0.82-1.25)	0.84 (0.64-1.11)	1.21 (0.98-1.49)	1.16 (0.92-1.46)
Used OC 1975 or later	1.12 (0.86-1.45)	0.89 (0.61-1.30)	1.13 (0.88-1.44)	1.05 (0.75-1.45)	**1.46 (1.15-1.86)**	**1.49 (1.15-1.94)**

*Note: All ORs adjusted for age, race, study site, education, parity, breastfeeding, bmi, age at first birth, and menopausal status; ORs in **bold** are statistically significant*

Chapter 3: Select Reproductive Risk Factors for Breast Cancer defined by Estrogen and Progesterone Status Within a Familial Breast Cancer Population

3.A. INTRODUCTION

The past two decades have transformed what we know about tumor biology and tumor markers for breast cancer, and the current paradigm for examining risk factor associations with breast cancer prevalence is to examine risk factors on disease outcome subdivided by relevant tumor characteristics or biomarkers. In breast cancer, most studies have focused on defining breast cancer by the tumor characteristics of estrogen-receptor (ER), and/or progesterone receptor (PR) expression, and more recently by human epidermal growth factor receptor 2 (HER2) expression. Tumor grade is a characteristic that is less commonly examined according to risk factor, however it is an important prognostic characteristic that has been demonstrated, in some studies, to differ according to risk factor [5, 14].

The bulk of published studies have analyzed average-risk populations, and associated most reproductive and hormonal risk factors, such as parity, age at first birth, and exogenous hormone use, with hormone-positive (ER+ and PR+) cancers [19, 27, 40, 55, 58, 63, 65, 70, 71, 90, 98]. By contrast, ER and PR negative breast cancer (ER-PR-) which is positively associated with younger age, high tumor grade, and poor prognosis compared with ER+PR+ cancer, does not demonstrate the same associations with reproductive and hormonal risk factors [6, 27, 40, 41, 65, 70, 71, 73, 81, 98], although breastfeeding is one of the few reproductive risk factors consistently associated with a reduction in both hormone receptor positive and negative breast cancer in a majority of studies [19, 27, 58, 70, 90, 98]. Evaluations of risk factor associations with ER- and PR-defined breast cancer are less common in high-risk populations, therefore findings have been less consistent across studies (*see Table 1-4*). Whether breastfeeding is protective against ER-PR- cancer in populations at high risk of breast cancer is critical for prevention, as there are few

prevention options available for these women apart from risk-reducing surgeries and chemoprevention.

Previous studies have also supported that another modifiable factor, oral contraceptive (OC) use is either positively associated, or not associated, with breast cancer, however, most studies have failed to examine year of commencing use of OCs in regards to effect on risk, even though previous studies have supported that year of OC use is an important variable in assessing breast cancer risk [152, 153], as estrogen and progestin concentrations were higher in older formulations of OCs (prior to 1975). Similar to breastfeeding, few studies have examined the role of oral contraceptive use in higher risk populations, by ER- and PR- status [67, 100, 101].

Additionally, few studies have examined risk factor associations with breast cancer tumor grade. Tumor grade classifies cancer cells according to their appearance under a microscope (how abnormal they look compared to normal breast tissue) and how quickly the tumor is likely to grow and spread. Poorly differentiated tumors are more aggressive, less amenable to treatment and have poorer prognosis compared with well differentiated tumors. While few studies have examined whether risk factors differ according to tumor grade, because prognosis differs for high and low grade, increased understanding of the role of different risk factors into the etiology of high or low grade tumors could be of value in determining cancer prevention measures.

In Chapter 2 of this dissertation, I focused on examination of breast cancer risk factors defined by joint ER and PR status, as well as molecular subtypes, using data from the population-based sites of the Breast Cancer Family Registry, and focusing on the risk factors of parity, breastfeeding, a combined parity-breastfeeding measure, and oral contraceptive use, with a control group serving as the primary reference group. In the analysis from Chapter 2, I found

that, within the population-based cohorts of the BCFR, high parity (3+ live births) without breastfeeding was positively associated with ER-PR- tumors (odds ratio [OR] =1.57, 95% confidence interval [CI], 1.10-2.24), but not with ER+PR+ tumors. Breastfeeding was associated with a reduced risk of all breast cancer subtypes, but most strongly with ER-PR- cancer (OR=0.52, 95% CI 0.40-0.68, ≥12 months of breastfeeding vs. never). High parity, when coupled with breastfeeding, was no longer associated with ER-PR- cancer (OR=0.93, 95% CI 0.71-1.22). Compared with controls, oral contraceptive (OC) use prior to 1975 was associated with an increased risk of ER-PR- cancer (OR=1.32, 95% CI 1.04-1.67), but not HR+ cancer. For women who began OC use in 1975 or later there was no increased risk conferred by OC use. Findings differed according to whether cases did or did not have a first-degree relative with breast cancer, as well as by pre- or postmenopausal status. Cases-only analyses of individual tumor characteristics indicated that high parity and breastfeeding were specifically associated with ER status, while OC use was associated with tumor grade, no risk factor correlated specifically with PR status after accounting for ER status and grade.

In this chapter, I will repeat the analyses from Chapter 2 using cases and controls from the clinic-based sites of the Breast Cancer Family Registry (sites in New York, NY, Philadelphia, PA, and Salt Lake City, UT). The clinic-ascertained cases differ from the population-based cases in that they were recruited through breast cancer clinics rather than identified through cancer registries. The clinic-based sites also recruited family members, rather than unrelated individuals, to serve as the control population. Because the controls are related to the cases, analysis will be performed using both traditional polytomous logistic regression, as well as using the method of generalized estimating equations (GEE) which accounts for the correlation occurring due to family relationships among the members of the dataset. I will then use the clinic-based datasets

to examine parity, breastfeeding and oral contraceptive use according to independent ER status, PR status, and tumor grade, and then adjust for the correlation between ER and PR status, and grade, to determine how correlation among these tumor characteristics may affect the interpretations of the differential role of etiologies on tumor characteristics. The two aims of this Chapter are therefore to 1) Determine whether the findings regarding breastfeeding, parity, and oral contraceptive use found in Chapter 2 are also observed in a clinic-based population and 2) to use a novel statistical approach, the pseudo-statistical likelihood method, to ascertain what effect correlation of various tumor characteristics with one another has on interpretation of findings, in a clinic-ascertained population. As in the previous chapter, the primary risk factors of interest will be parity, breastfeeding, the combination of parity and breastfeeding, and oral contraceptive use.

3.B. MATERIALS AND METHODS

Study Population

In 1995, the National Cancer Institute funded six international sites establishing the Breast Cancer Family Registry (BCFR), a resource for genetic studies of breast cancer. Six participating sites from the USA, Canada, and Australia ascertained families either from population-based cancer registries (San Francisco Bay, CA, Ontario, Canada, and Melbourne and Sydney, Australia) or from clinical and community settings (producing clinic-based families in New York, NY, Philadelphia, PA, and Salt Lake City, UT)[155]. The sample for this analysis is taken from clinic-based sites.

The three clinic-based sites enrolled families with multiple or early-onset cases of breast or ovarian cancer identified through community contacts and clinical settings including screening centers, family cancer clinics, surgical and medical oncology offices. Probands were defined as

the first family member enrolled in the Breast Cancer Family Registry and may or may not have had a personal history of breast or ovarian cancer. Eligibility for women was based on one or more of the following criteria: two or more relatives with a personal history of breast or ovarian cancer, a woman diagnosed with breast or ovarian cancer at a young age, a woman with a history of both breast and ovarian cancer, or known *BRCA1* or *BRCA2* mutation carriers.

Case and Control Ascertainment

At each of the three study sites, incident breast cancer cases were identified through clinical and community-based settings. There were 1647 clinic-based families with an affected proband, including 1379 females with a primary invasive breast cancer (the remaining families had females with a 2nd breast cancer or males with breast cancer) and there were 1187 clinic-based families without an affected proband, including 1163 females, for a total of 2834 probands. Of the total probands, 2666 completed the epidemiology questionnaire, and 2641 provided a blood or mouthwash sample. Of 8264 relatives, 4604 completed the epidemiology questionnaire, and 3973 provided a blood or mouthwash sample.

All sites used common questionnaires on family history and epidemiologic risk factors. The family history questionnaire was completed by the proband (initial person contacted in the family; this person was not required to have breast cancer in the clinic-based sites) and obtained information on vital status, dates of birth, dates of death, and dates of cancer diagnosis for all first-degree relatives, and more distant relatives with a personal history of cancer. The risk factor questionnaire was completed by participating probands and relatives and sought information on demographics, personal history of cancer, breast and ovarian surgeries, radiation exposure, smoking and alcohol consumption, menstrual and pregnancy history, breast feeding history,

hormone use, weight, height, and physical activity. Details of the recruitment criteria can be found in *Appendix Table A1-1*.

A total of 2627 women with a primary invasive breast cancer (1458 from New York, 725 from Philadelphia, and 444 from Salt Lake City) have been enrolled in the clinic-based sites and completed the epidemiology questionnaire. In addition, 3794 familial controls (2124 in New York, 882 in Philadelphia, and 788 in Utah), have been enrolled and completed the epidemiology questionnaire. The overall clinic-based study sample size is 2627 female cases, and 3794 related female controls.

Of the cases, 32% of women have both ER and PR information available and were therefore eligible for analysis, including 522 cases from New York, 150 from Philadelphia, and 178 from Salt Lake City. Because this is a familial study that includes previous generations, many of the cases were diagnosed with breast cancer prior to 1995, and ER and PR data for these cases was not commonly ascertained (N=1,719 66% of sample). In addition, several cases (N=30, 2% of sample) were described to have "mixed" ER+/- or PR+/- tumor structure, and were excluded. I used data from all 3794 related controls that completed the epidemiology questionnaire. *Appendix Figure A1-2* describes the ER and PR missingness data in further detail.

Risk Factor Data Collection

Cases and controls completed structured questionnaires assessing breast cancer risk factors and family history of cancer. In addition to detailed family history, information was collected on established and suspected breast cancer risk factors, including oral contraceptive use, hormone replacement therapy use, age at menarche, parity, age at first live birth, breastfeeding history, smoking history, alcohol use, education, body mass index, and menopausal status.

Tumor Marker Data Collection

BCFR study pathologists ascertained estrogen and progesterone status and tumor grade from patient tissue and/or pathology reports examined using a standardized protocol and pathology reporting forms, or through data available from the pertinent tumor registry.

Eligible cases had data available on ER status and PR status through either pathology samples (tumor samples) or pathology reports/medical records available for central review. The distribution of risk factors was no different between cases that did and did not have pathology data available for review. However, clinic-based cases who were diagnosed prior to 1995 were much less likely to have ER/PR information available than cases who were diagnosed in 1995 or later (86% of the 1521 cases diagnosed prior to 1995 did not have ER/PR information available, compared with 49% of the 982 cases diagnosed in 1995 or later). HER2 data were not available for the clinic-based sample. Details of missingness are available in *Appendix Figure A1-2*.

For cases for whom tissue samples were obtained, BCFR pathologists examined sections from histologic slides and/or paraffin tumor blocks and categorized tumors as ER or PR positive if ≥10% of tumor cells stained positive. Where tissue samples were not obtained, pathologists reviewed pathology reports and medical records and recorded the ER and PR status listed on the report, or, if information existed on the percent of cells staining positive, employed the same requirements that ≥10% of cells stained positive resulted in a definition of ER or PR positive. This cutoff for positivity was typical for samples collected and examined at the time of data collection, although current practice classifies tumors as ER or PR positive when greater than 1% of tumor cells stain positive. Of the 843 cases with ER/PR clearly coded as positive or negative (and not of "mixed" status), 436 are ER+PR+, 254 are ER-PR-, 100 are ER+PR-, 53 are ER-PR+.

For tumor grade, BCFR pathologists reviewed pathology reports and medical records to determine the tumor grade. Tumor grade was missing in 40% of the population with known ER/PR status, including all Philadelphia-based samples. Therefore for analyses including ER, PR, and Grade, only 524 subjects were available for analysis, all from the New York and Utah sites.

Statistical Analysis

I conducted both case-control and case-case analysis using several statistical techniques. To examine the association of risk factors with ER/PR status, I used unordered polytomous regression, resulting in 4 ER- and PR- defined subgroups, which were compared to a common control group. In the case-control analysis, ER+PR+ and ER-PR- patients were compared with familial controls. Because of the low incidence of ER+PR- and ER-PR+ subtypes, the findings for these subtypes are not reported in the Results section. In the case-only analysis, I additionally compared ER-PR- tumors to ER+PR+ tumors as the referent to assess case/case differences. Case-control analyses and were conducted using both polytomous logistic regression and GEE. Case-only analysis was conducted using binomial logistic regression and GEE.

To examine these tumor characteristics after accounting for their correlation with one another, I examined ER, PR, and the additional tumor characteristic of grade in case-only analyses using binary logistic regression, comparing ER- tumors to ER+ tumors, PR- tumors to PR+ tumors, and high-grade tumors to low-grade tumors (where high grade was considered grade 3, and low-grade was considered grade 1,2). I then accounted for the correlation between these factors by simultaneously adjusting for the correlation of ER status to PR status, for example, to see how findings changed, using the pseudo-conditional likelihood regression approach [125].

For the analysis, I concentrated on examining the following known or suspected breast cancer risk factors, adjusted for age, race, study site, first-degree family history (Yes/No), and menopausal status: oral contraceptive use (≥ 5 years, < 5 years, never), timing of oral contraceptive use (any use prior to 1975, all use after 1975, never); Parity (nulliparous, 1-2 live births, 3 or more live births), and a combined breastfeeding/parity measure. Findings were additionally stratified by first-degree family history status, and examined among cases that were positive for *BRCA1/BRCA2* mutation.

The multivariable models were adjusted for the variables of interest as well as for age, site, race, family history of breast cancer, and menopausal status. Other variables, including age at menarche (≤11, 12, ≥ 13), age at first birth (continuous), smoking (Never Smoker, Former Smoker, Current Smoker), BMI (continuous), education (< high school, ≥ high school), alcohol use (<7 drinks per week, ≥7 drinks per week, non-drinker) were evaluated to see if they were strongly associated with a risk factor of interest, such that they confounded the association between that factor and the outcome when added to a regression. I considered a risk factor to be a confounder if the suspected confounder changed the effect measure [β] of the risk factor of interest on the outcome by 10% or greater, or if a preponderance of previous literature supported an association. A risk factor was considered significantly related to the outcome if the 95% confidence interval did not include the value of "1".

Menopausal status was missing in for 138 cases and 264 controls. In order to estimate menopausal status for these subjects, cases and controls were coded as menopausal if they had experienced bilateral oophorectomy, or if they were age 51 or older, at the time of diagnosis for the cases, and at the time of interview for the controls. Subjects who were younger than 51 and had not had a bilateral oophorectomy were coded as premenopausal. Analyses including this

estimation of menopause did not significantly alter the study findings, compared to analyses excluding these subjects, but allowed for inclusion of these cases in analysis to improve study power.

All statistical analyses used SAS Version 9.4 Software (SAS Institute, Cary, NC), and MATLAB.

3.C. RESULTS

Table 3-1 summarizes demographic, risk factor and (for the cases) tumor characteristic frequencies by joint ER and PR status. The control population was young, with a mean age of 44, similar to the ages of ER-PR+ and ER-PR- cases, but younger than ER+PR+ and ER+PR- cases. Controls and cases that were ER- were more often premenopausal compared with ER+ cases, likely due to a lower mean age. ER+PR+ and ER+PR- cases were more likely to be never users of oral contraceptives. Nearly 30% of controls were nulliparous, compared to 17% of ER+PR+ and 12% of ER+PR- cases. The low relative age of the controls may indicate that some were nulliparous primarily due to young age. As parity among controls was low, a full 50% had never breastfed. Because controls were all familially related to a case, *BRCA1* positivity among controls was high, at 7%, higher than that for ER+PR+ and ER+PR- cases. ER-PR+ and ER-PR- had the highest rates of *BRCA1* positivity, at 11 and 22%, respectively. ER-PR+ and ER-PR- cases were also characterized by high tumor grade (72%, ER-PR+, and 82%, ER-PR-).

Appendix Table A1-6 summarizes demographic, risk factor and tumor characteristic frequencies, for cases categorized separately according to independent ER status, PR status, and grade. ER-, PR-, and high grade cases were more likely to be younger and more likely to be *BRCA1* positive, and were more likely to have ever used oral contraceptives and less likely to have ever used

HRT. Cases who were ER- were more often premenopausal. Cases who were ER- and had high grade tumors more often had a first-degree family history of breast cancer.

Parity and Breastfeeding

Table 3-2 presents adjusted ORs for parity, breastfeeding, and a combined parity/breastfeeding variable, comparing ER+PR+ cases to controls, ER-PR-cases to controls, and ER+PR+ cases to ER-PR- cases (Data are not shown for ER+PR- cases, or ER-PR+ cases, due to low sample size for these subtypes). Data are analyzed using both logistic regression, and the method of generalized estimating equations (GEE), which accounts for correlation of the data points resulting from using familial controls.

Both ER+PR+ and ER-PR- cases were significantly more likely to be parous than were controls, however for ER+PR+ cases, these findings were significant only for 1-2 live births, not for 3 or more live births, while ER-PR- cases were significantly more likely to be parous than controls, regardless of number of live births (OR=2.10, 95% CI 1.27-3.48, ER-PR- vs. control, 1-2 live births vs. none; OR=2.10, 95% CI 1.16-3.81, ER-PR- vs. controls, ≥3 live births vs. none). GEE findings were similar to those using logistic regression. Although point estimates were higher in ER-PR-, compared with ER+PR+ cases, the association between parity and increased likelihood of ER-PR-, rather than ER+PR+ breast cancer, was not significant (OR=1.37, 95% CI 0.72-2.59, ≥ 3 live births vs. nulliparous, ER-PR- cases vs. ER+PR+ cases).

A non-significant inverse association was found between ER-PR- cancer and breastfeeding, compared with controls, in logistic regression (OR=0.70, 95% CI 0.45-1.11 ≥12 months of breastfeeding vs. never), and GEE (OR=0.69, 95% CI 0.43-1.10, ≥12 months of breastfeeding vs. never). No association with breastfeeding was found for ER+PR+ cases. The association

estimate remained, when comparing ER-PR- patients to ER+PR+ patients (OR=0.60, 95% CI 0.35-1.02, ER-PR- compared to ER+PR+, ≥12 months of breastfeeding vs. never). When breastfeeding analysis was limited to parous women only, the association persisted and was statistically significant (OR=0.56, 95% CI 0.35-0.90, ER-PR- vs. controls, parous women only, ≥12 months of breastfeeding vs. never). Using a combined parity/breastfeeding variable, parity coupled with lack of breastfeeding, was significantly associated with ER-PR- breast cancer (OR=1.75, 95% CI 1.00-3.04 1-2 live births, no breastfeeding vs nulliparity, OR=2.07, 95% CI 1.09-3.33, ≥ 3 live births, no breastfeeding vs. nulliparity) and elevated, though not statistically significantly, among women with ER+PR+ cancer. Among parous women who breastfed, there was no longer a positive association between parity and ER-PR- cancer, however an association emerged for ER+PR+ cancer, only among women with 1-2 live births (OR=2.11, 95% CI 1.34-3.33, for 1-2 live births + any breastfeeding, vs. nulliparous).

Because the finding that parity was positively associated with ER+PR+ cancer was unexpected, given that most literature supports an inverse association, or no association, between ER+PR+ cancer and parity, I examined the association between ER and PR status and parity, in relation to the timing between diagnosis and interview, to understand whether the positive association could be associated with cancer survival, rather than incidence. Additionally, findings excluding *BRCA1* and *BRCA2* positive cases were examined, to determine whether the positive association between parity and cancer was related to the presence of *BRCA1* and 2+ cases in the case dataset, and time since last birth was examined, to determine if recent parity was elevating risk, as risk of breast cancer is typically elevated within the first several years after childbirth [172]. Details of these findings are available in *Appendix Table A1-7*. I found that when including only those cases interviewed within 2 years of diagnosis, that there was no association between parity and

ER+PR+ cancer (OR=1.04, 95% CI0.65-1.67, 1-2 live births vs. nulliparity). The association between parity and ER-PR- cancer was maintained regardless of time between diagnosis and interview. Omitting *BRCA1* and *2* cases from analysis, did not change point estimates or interpretation, and time since last birth analysis also did not materially affect point estimates (data not shown).

In further analysis, to determine whether risk-factor associations differed depending on whether cases had a first-degree family history of breast cancer, cases were stratified by whether they had at least one first-degree relative with breast cancer, vs. none. Among cases with a first degree family history, the positive association between ER-PR- and ER+PR+ breast cancer and parity, diminished and became non-significant. Among cases with *no* first-degree family history, parity was more strongly associated with both ER+PR+ and ER-PR- breast cancer (OR for 3+ live births: 3.06, 95% CI 1.46-6.42, for ER-PR- with no first-degree family history vs. control). Among cases with a first-degree family history, the inverse association between ER-PR- breast cancer, and at least 12 months of breastfeeding, became more negative (OR=0.51, 95% CI 0.24-1.07), while breastfeeding was not inversely associated with ER-PR- cancer among those with no first-degree family history of breast cancer (OR=0.90, 95% CI 0.50-1.63) *(Figure 3-1).*

Additional analysis of ER+PR+ and ER-PR- cases who were *BRCA1* positive or *BRCA2* positive indicated that among cases that were *BRCA1* or *2* positive (compared with *BRCA1* or *BRCA2* positive controls), parity was more positively associated with ER+PR+ tumors, than it was with ER-PR- tumors, although findings were non-significant. Most *BRCA1* positive women are ER-PR-, whereas most *BRCA2* women are ER+. Breastfeeding appeared to be more protective among *BRCA1/2+* cases, then among all ER-PR- cases (OR=0.35, 95% CI 0.14-0.93, *BRCA1/2+* cases vs. *BRCA 1/2+* controls, at least 12 months total breastfeeding vs. none) *(Table 3-4).*

Oral Contraceptive Use

Table 3-3 presents adjusted ORs for length and starting year of oral contraceptive use, comparing ER+PR+ cases to controls, ER-PR-cases to controls, and ER+PR+ cases to ER-PR- cases. Data are analyzed using both logistic regression and GEE. Oral contraceptive use was associated with ER-PR-, but not ER+PR+, breast cancer (Logistic regression, OR, 1.49, 95% CI 1.00-2.21; GEE, OR 1.50, 95% CI 1.01-2.23 OC Use > 5 years vs. Never). This association was also significant when comparing ER-PR- cases, to ER+PR+ cases (OR=1.90, 95% CI 1.17-3.06, OC use >5 years vs. never). In analysis of starting year of use, OC use was positively associated with ER-PR- cancer (but not ER+PR+ cancer), among cases who used oral contraceptives prior to 1975 (OR=1.60, 95% CI 1.08-2.37). Among cases who used OCs after 1975, there was no association with OC use and ER-PR- cancer, and there was a reduced odds of ER+PR+ cancer (OR=0.56, 95% CI 0.41-0.78). Findings did not differ significantly according to logistic regression or GEE.

As with the parity and breastfeeding variable, I examined whether the relation between OC use and ER-PR- or ER+PR+ breast cancer differed by family history of breast cancer (*Figure 3-1*). In cases with at least one first-degree relative with breast cancer, the association between OC use prior to 1975, and ER-PR- breast cancer, became non-significant, while it became more positive in those without a first-degree relative with breast cancer (OR=1.88, 95% CI 1.06-3.33, for ER-PR- cases vs. controls, OC use prior to 1975, vs. never use).

As with parity and breastfeeding, the association between OC use and ER- and PR- defined breast cancer was also examined among *BRCA1/BRCA2+* cases, versus *BRCA1/2+* controls. Among *BRCA1/2+* cases, >5 years of OC use, was positively associated with ER-PR- cancer , but not with ER+PR+ cancer (OR=3.53, 95% CI 1.59-7.86); similarly, use prior to 1975 was

associated with ER-PR- cancer among *BRCA1/2+* cases, compared with controls (OR=3.43, 95% CI 1.52-7.75) (*Table 3-4*).

Pseudo-Conditional Likelihood findings

Tables 3-5A and 3-5B present the multivariable ORs for ER+ vs. ER- tumors, PR+ vs. PR- tumors, and Grade 3 tumors vs. Grade 1,2 tumors, for the key risk factors of interest: parity and breastfeeding (*Table 3-5A*), and oral contraceptive use (*Table 3-5B*). For each tumor characteristic, the first (left-hand) column represents the multivariable OR unadjusted for correlation with the other tumor characteristics, and the 2nd (right-hand) column represents the multivariable OR, adjusted using the pseudo-conditional likelihood approach, to account for the correlation among tumor characteristics (i.e., ER status is adjusted for PR status and grade, PR status is adjusted for ER status and grade, and grade is adjusted for ER status and PR status.). Because grade was not available for the Philadelphia-based cases, the sample for this case-only analysis includes cases from New York and Utah.

Parity: Parity was not associated with an increased risk for ER- status, compared to ER+ status, in a multivariable model unadjusted for PR status and grade, or in a pseudo-conditional likelihood model adjusted for PR status and grade, nor was it associated with PR status in either the adjusted or unadjusted models. However, parity was associated with high grade (grade 3) vs. low grade (grade 1,2) tumors, in a model unadjusted for ER and PR status (OR=1.90, 95% CI 1.00-3.59, 1-2 live births vs. none and OR=1.96, 95% CI, 0.95-4.01, 3+ live births vs none), and in a model adjusted for both ER and PR status (OR=2.17, 95% CI 1.08-4.38, 1-2 live births vs. none and OR=2.23, 95% CI 1.02-4.86, $\geq$ 3 live births vs. none).

Breastfeeding: Breastfeeding for 12 months or longer was associated with reduced odds of ER- cancer, compared to ER+ cancer, and PR- cancer compared to PR+ cancer, in multivariable models unadjusted for PR status and grade (OR=0.49, 95% CI 0.28-0.84, ER- vs. ER+, OR=0.58, 95% CI 0.35-0.97, PR- vs. PR+). However, in multivariable models simultaneously adjusted for PR status and grade, breastfeeding was no longer associated with ER status. In models adjusted for ER status and grade, breastfeeding was no longer associated with PR status, although the point estimate remained similar to the logistic regression model. Breastfeeding of any duration was associated with reduced odds of high grade, vs. low grade cancer (OR=0.54, 95% CI 0.32-0.91, breastfeeding <12 months vs. never and OR=0.40, 95% CI 0.21-0.73, breastfeeding ≥12 months vs. never), and this association remained after adjustment for ER and PR status.

Oral contraceptive use: In the multivariable models in *Table 3-5B*, cases who used oral contraceptives were more likely to be ER-, rather than ER+ compared with never users (OR=1.56, 95% CI 1.02-2.40, ≤5 years use vs. never, OR=1.74, 95% CI 1.09-2.77 > 5 years use vs. never), however once the model was additionally adjusted for PR status and grade using the pseudo-conditional likelihood approach, ER status was no longer significantly associated with OC use, although point estimates changed only minimally. OC use prior to 1975 was associated with ER- vs. ER+ cancer, compared with never use (OR=1.75, 95% CI 1.15-2.67); upon adjustment for PR status and grade, this association became non-significant, although the point estimate remained similar (OR=1.90, 95% CI 0.95-3.80). By contrast, OC use in 1975 or later was positively associated with PR-, vs. PR+ status, compared with never use (OR=1.78, 95% CI 1.11-2.84), this association also became non-significant, but the point estimate remained similar,

after adjustment for ER status and grade (OR=1.88, 95% CI 0.92-3.83, for PR- vs. PR+ cancer). OC use was not associated with cancer grade in the clinic-based population.

3.D. DISCUSSION

Parity and Breastfeeding

In the clinic-based population of the BCFR, comprised of breast cancer cases and familial controls, I found that high parity was positively associated with both ER+PR+, and ER-PR- tumors, and breastfeeding was only associated with reduced odds of ER-PR- cancer. Having children and not breastfeeding them was associated with an increased risk of ER-PR- tumors. This risk was mitigated by breastfeeding in the ER-PR- tumors, but not in the ER+PR+ tumors. I found that the effect of these risk factors can differ depending on whether cases have a first-degree family history of breast cancer.

Analysis of the nature of the association between ER+PR+ cancer and parity indicated that among cases who were interviewed between 0-2 years after diagnosis, there was no association between parity and ER+PR+ cancer, with odds ratios close to 1. When time between diagnosis and interview was between 0-5 years, the association between ER+PR+ cancer and parity became positive, but was still non-significant. Only when all cases, even those interviewed more than 10 years after diagnosis, were included in the case group, was the significantly positive association between parity and ER+PR+ breast cancer maintained, indicating that among the ER+PR+ cases, parity is likely associated with breast cancer survival, rather than breast cancer incidence. By contrast, parity was positively associated with ER-PR- cancer, regardless of the timing between diagnosis and interview, indicating this association between parity and ER-PR- status is more likely to reflect risk of incidence (i.e., may have an etiological basis).

Most studies support that parity is inversely associated with ER+ breast cancer risk, whereas, after accounting for time between diagnosis and interview, I found no association between ER positivity and parity. Studies reporting an inverse association between parity and ER+PR+ cancer often contain a preponderance of postmenopausal women [19, 58], whereas the BCFR clinic-based sample has a high proportion of premenopausal women, particularly among controls. Studies of younger, primarily premenopausal women have found conflicting results regarding ER+ and parity, including several studies that have found no association [54, 69, 91].

Many studies have reported a positive association between parity and ER- breast cancer, particularly when parity is combined with a lack of breastfeeding [5, 56, 65, 67, 71, 75, 82, 177]. Most studies have found breastfeeding to be protective regardless of subtype, while this study does not show an inverse effect of breastfeeding on ER+PR+ cancers. However, some studies have indicated that breastfeeding confers greater protection against ER-PR-, or triple-negative cancer, and can mitigate the positive risk conferred by parity [81, 95, 108, 151]. The Collaborative Group on Hormonal Risk Factors in Breast Cancer has determined that breastfeeding is protective against breast cancer above and beyond the protection conferred by parity [162]. Hypothesized potential protective mechanisms include the removal of estrogens via breast fluid, excretion of carcinogenic agents through breast milk, delay in ovulation associated with breastfeeding, and induction of terminal differentiation of breast epithelial cells [163]. In addition, as mentioned in Chapter 2, involution of mammary tissue occurs after breastfeeding, but in the absence of breastfeeding, may occur sooner postpartum; this process has been associated with tumor development in animal breast cancer models [157].

Oral Contraceptive Use

Overall, oral contraceptive use was associated with an increased risk of ER-PR- breast cancer, however, by further defining OC use by starting year of use, I found that this increased risk was only present in women who began their oral contraceptive use prior to 1975. After 1975, OC use was not significantly associated with ER-PR- breast cancer, and was shown to be protective against ER+PR+ breast cancer. Earlier published studies reported positive associations between ER-PR- breast cancer and OC use (reviewed in[19]), whereas most recent studies have found no overall association between ER-PR- breast cancer and OC use [19, 27, 67, 73, 96, 98, 177]. A few studies have found, as this one did, an inverse association between OC use and ER+PR+ cancers [74, 81]. Analysis of year of initiation as an important variable in evaluating the association between OC use and breast cancer risk has become more common [12, 165, 166], but has not regularly been examined in previous research on OC use and breast cancer risk by hormone receptor status. OC use both before and after the year 1975 was associated with an increased risk of ER-PR- cancer, among cases who were interviewed soon after diagnosis. This could indicate that, among cases for whom use occurred after 1975, recency or total duration of use may be positively associated with ER-PR- cancer, as has been found in an additional study of young, triple-negative breast cancer cases [101]. Additionally, analysis in Chapter 2 indicated an age-cohort effect regarding OC use and ER-PR- cancer. The same age-cohort effect could be operating in this sample, although smaller sample size in this population did not allow for an age cohort analysis.

It is unclear why OCs used prior to 1975 would be more strongly associated with ER-PR- cancer. Studies of synthetic progestins used in OCs have generally found that the proliferative actions of progestins used in OCs are mediated through the ER [168, 178], which does not explain why ER-

breast cancer is more likely to be affected, unless the ER is effectively "turned off" by such proliferation. Typical estrogen doses used in the 1960s were more than double the doses used in the 1980s, and progestin doses were also higher and included different types of progestins than current OCs [166]. Biologic and clinical evidence support a role for exogenous estrogen effects on carcinogenesis mediated through estrogen-receptor α (ERα) receptor, yet evidence supports that receptor-independent pathways may also exist [170].

Role of family history

In this population with a high proportion of both cases and controls with a first-degree relative with breast cancer, it was feasible to examine findings stratified by family history. It should be noted that those cases and controls without a first degree relative with breast cancer, often have one or more 2nd degree relative with breast cancer, so cannot be considered to have non-familial breast cancer. Parity was more strongly associated with positive risk of breast cancer, among cases *without* a first-degree family history of breast cancer, particularly among ER-PR- cases. Breastfeeding was inversely associated with breast cancer, only among ER-PR- cases *with* a first-degree family history of breast cancer. And OC use (prior to 1975) was associated with increased risk of ER-PR- breast cancer, among both women *with and without* a first degree relative with breast cancer, but the association was more negative among those without first-degree family history. After 1975, OC use was protective against ER+PR+ cancer, only among women *without* a first degree relative with breast cancer.

Some studies have indicated that for women with a familial risk of breast cancer, the role of environmental risks (such as childbirth) in contributing to breast cancer etiology may be minimized [44, 47]. In cases without a predisposed genetic risk of breast cancer, environmental

factors may play a heightened role. My analysis comprised a relatively young sample of women, some of whom were of childbearing age, and experienced a cancer diagnosis within 5 years of childbirth. For cases with no genetic predisposition, recent childbirth might be considered causative (along with other factors) to breast cancer etiology [179]. However, for cases with an existing predisposition, breast cancer diagnosis at a young age may coincide with recent childbirth, but childbirth may have less of a causative role. This assumption is supported by the findings, that breast cancer molecular subtype distribution is not different in those with pregnancy-associated breast cancer, when family history is considered [180], and that those with family history have not been found at increased risk of breast cancer, within10 years of pregnancy [181]. This may be why, among cases with first degree family history, parity was less associated with increased odds of breast cancer, than among cases without.

Stratification by first-degree family history in this analysis demonstrated the value of breastfeeding for women with a first degree affected relative (and thus a likely genetic predisposition to breast cancer) in possibly modifying this risk. It has been shown in a recent meta-analysis that *BRCA1* mutation carriers, who are typically diagnosed with ER-PR- cancer, were significantly less likely to develop breast cancer if they breastfed for at least one year, compared with *BRCA1* mutation carriers who did not breastfeed; there was no association with breastfeeding among *BRCA2* mutation carriers, who usually have ER+ tumors [182]. The conclusion of the meta-analysis was that breastfeeding is inversely associated with *BRCA1* (but not *BRCA2*) carrier status, however, it could in fact be that breastfeeding is inversely associated with ER-PR- status, regardless of carrier status. Women who were *BRCA1* positive made up 13% of all clinic-based cases in this analysis, but 22% of ER-PR-cases, and breastfeeding was found to be protective against ER-PR- breast cancer cases that were *BRCA1/2* positive, indicating even

among *BRCA1/2* positive women, there is some differential pathway, potentially involving breastfeeding, by which cases become estrogen receptor or progesterone receptor negative, rather than positive.

In other analyses of parity and *BRCA1/2* carriers, including the meta-analysis, parity has been unassociated with *BRCA1* status [182, 183]; this study also found that parity was unassociated with ER-PR- status, which would be largely made up of *BRCA1* cases. Parity has been positively associated in a few studies with *BRCA2* status or *BRCA/2* status [184, 185]. These analyses did not classify cases by ER or PR subtype, potentially resulting in a finding confounded by heterogeneity of tumor characteristics. Age incidence curves for breast cancer tend to be younger for carriers of *BRCA1* or *2*, such that factors that occur close to the timing of diagnosis, may appear to be strongly related to risk. In this analysis, parity was positively (though non-significantly) associated with ER+PR+ *BRCA1/2* carriers, however, the average age of breast cancer diagnosis in *BRCA2* carriers (the bulk of whom are ER+), is less than 45, indicating that recent pregnancy and case status may be co-occurring, but not causally related.

Regarding oral contraceptive use, findings among cases with/without family history and among *BRCA1/2* carriers, were similar for ER-PR- cases. Thus findings may reflect that, prior to 1975, OCs conferred an increased risk of ER-PR- cancer, regardless of pre-existing genetic predisposition. Studies that have examined OC use among women with family history of breast cancer found increased risk of breast cancer only among women who began OC use prior to 1975 [166], as did my findings. Data on OC use and breast cancer risk in *BRCA1/2* mutation carriers, including some from the breast cancer family registry, have demonstrated no increased risk with OC use initiated after 1974, for use of ≥1 year [153, 154, 167, 186]. Additional studies have supported long duration of use, greater than 5 years, is positively associated with *BRCA1*

positivity in carriers, as was found in this study for ER-PR- cases with *BRCA1/2* positivity [187, 188]. These studies did not report findings by ER/PR subtype.

Case-Case Analysis of Tumor Characteristics

The challenges of understanding the possibly differential effects of estrogens and progestins on hormone positive and negative breast cancer risk can perhaps be better parsed out using the additional analyses in this chapter. There are few previous analyses of this type in breast cancer, and none that specifically consider ER status, PR status, and grade using the risk factors of parity, breastfeeding, and oral contraceptive use.

In this sample, OC use was positively associated with ER- (compared with ER+) cancer, but only in use prior to 1975. OC use was positively associated with PR- (compared with PR+) cancer, but only among users who initiated use after 1975. OC use was not associated with grade. OC use before 1975, when OCs contained high doses of estrogen and progestin, was positively associated with ER- tumors, while OC use after 1975 (when newer types of progestins were introduced into OCs) was positively associated with PR- tumors. Thus the specific formulation of the oral contraceptive over time, may have affected what subtype of cancer cases incurred, even if overall cancer risk was not affected.

Parity was positively associated with both ER+ and ER- cases, in case-control analysis, and thus not differentially associated with ER-, vs. ER+, in case-only analysis. However, parity was positively associated with high grade, even after accounting for ER and PR status (ER negativity and PR negativity are closely correlated to high grade). The association with high grade among parous women, regardless of ER/PR status, may be related to the high rate of *BRCA1* and *BRCA2* positive women in the sample, since presence of this mutation is associated with high grade

tumors regardless of ER/PR status. Publications have also noted that familial breast cancers are often of high grade, even when not associated with *BRCA1* or *BRCA2* positivity [189], indicating that the associations found in this analysis may be related to the high prevalence of familial breast cancer in the sample. Most interesting, breastfeeding was negatively associated with high grade vs. low grade tumors, and also with ER and PR negativity, even after these factors were adjusted for one another, giving further evidence that breastfeeding may truly reduce risk of acquiring poorer prognosis tumors, such as those that are ER-, PR- and high grade, even in cases where a familial predisposition to such cancers exists.

Methodologic Considerations

Selection Bias: Pathological data on hormonal status was available for review for only a subgroup of the clinic-based sample. If these women were not representative of all eligible cases, one or more findings could be biased, with the direction of the bias differing depending on the differences between those who participated and had pathology for review and those who did not. Distributions of parity and other risk factors for my sample and the entire case sample were similar (data not shown), improving the likelihood that cases with ER and PR data available are representative of the distribution of these hormonal subtypes for the entire case sample, however the requirement that ER and PR status be known resulted in a case sample with more recent diagnosis, and more recent history of relevant risk factors, such as parity, breastfeeding, and oral contraceptive use, resulting in small sample size for evaluating risk factors occurring further in the past (such as OC use prior to 1975). Additional follow-up and information gathering for the clinic-based sample, where case information is missing, would make findings more robust to selection bias. In addition, detection bias may have played a role in diagnosis of the cases. Although detection bias is more often considered an issue of prospective studies, in this instance,

cases may have screened more often and diagnosed early, because of a known family history of breast cancer; some cases may also have diagnosed prospectively, if controls with family history or *BRCA1/2* carriers, converted to cases status during the study. Detection bias, if present, would have tended to find cancers of smaller tumor size and earlier stage, and also resulted in younger age at diagnosis, resulting in poorer generalizability of the case sample to breast cancer cases found through more typical surveillance levels.

Comparison group: For this analysis, I used family-based controls as the common referent group. I did not observe some established associations between hormonal and reproductive factors and hormonal status. For example, I did not find high parity to be inversely associated with ER+PR+ cancer, while most studies have found parity to be protective against this subtype even among younger women (*Table 1-3, Table 1-4*). However, given the high familial component of cancer risk in this population, it is not surprising that risk factors associated with average-risk breast cancer populations, would not have the same association in a population with high familial risk and a sizable contingent of *BRCA* mutation carriers. Generalized estimating equations were performed, to minimize the correlation effect of related controls on case findings.

Case definitions: BCFR pathologists used common laboratory procedures and conducted a centralized pathology review to categorize the majority of cases. A recent study has demonstrated that cancer registry-provided data may undercount the rarer ER/PR combinations, such as ER-PR+ and ER+PR- tumors, and that centralized pathology review should be considered a gold-standard when classifying tumors by hormone receptor status [176]. The criteria for defining women as ER+ or PR+ were more stringent for this analysis than criteria typically used today. This may limit the ability to compare this study's findings to similar analyses in patients who have been classified as HR+ under less stringent criteria for positivity.

Information Bias: The possibility of recall bias exists, because I relied on participants' recalls of their exposures, and in some instances, interviews occurred several years after breast cancer diagnosis, which may not only affect accuracy of recall, but also introduced survivor bias. However, the purpose of this analysis was to determine whether risk factor associations differed by subtype, using controls as a common comparison group. Because it is unlikely that cases report exposures differently based on their ER status, PR status, or grade, it is unlikely that OR estimates would be affected by recall bias of exposures that are differential by subtype. Survivor bias can be minimized by limiting the analysis to cases that completed their interview within 2 years of initial breast cancer diagnosis.

Generalizability: My sample of cases is not representative of all women with breast cancer; they are younger, and are likely to have a strong family history of breast cancer and to be *BRCA1* or *BRCA2+*. Controls are also non-representative of a population-based control sample; the controls are young, and have higher than average risk of breast cancer due to family history of disease, or *BRCA1* or *2* positivity. As a result my distribution of the different ER and PR-defined subtypes is not representative of these subtypes in a sample of cases unselected for these characteristics. Regardless, the purpose of this analysis is to examine risk factor by tumor subtypes specifically in a high-risk, familial, population, therefore comparisons to "typical" populations are neither expected or of value versus comparisons to other high-risk populations. To this end, my findings do reflect previous reports that examine younger women with high familial risk, including *BRCA1* carriers.

A familial (related) sample is expected to have limited generalizability to a more typical population. Familial breast cancers have been found to differ in their prevalence of various clinical and immunohistochemical features [48]. For example, women with familial breast

cancers are more often younger, have microcalcifications on mammography [48], have tumors of smaller size on detection (likely a result of increased surveillance), and are less likely to be ER and PR+, than sporadic breast cancers [48]. Hispanics with a family history of breast cancer are more likely to have ER-, but not ER+ breast cancers, while whites with a family history of breast cancer are more likely to have ER+, but not ER- breast cancers [190]. In particular, generalizability to older patients may be inappropriate.

Summary

Overall, I found that multiparity is associated with an increased risk of ER-PR- and ER+PR+ cancer, in a high-risk population, compared to familial controls. Among cases interviewed within 2 years of breast cancer diagnosis, this finding was not maintained for ER+PR+ cases. I found that the risk for ER-PR- cancer can be mitigated by breastfeeding, such that multiparous women with a history of breastfeeding are no longer at increased risk of ER-PR- cancer. I found that oral contraceptive use is associated with increased risk of ER-PR- cancer, but only in populations who began use prior to 1975. In populations using newer formulations of oral contraceptive, a protective effect against ER+PR+ cancer was demonstrated. As 1975 is now more than 40 years in the past, more recent contraceptive use may not be a risk factor for breast cancer, according to this study, and may even be associated with a preventive effect against ER+PR+ cancer.

I found that in cases with a high genetic predisposition to breast cancer, the causal association of environmental factors may be minimized, resulting in different risk factor associations for high-risk populations, than may exist for average risk populations. Finally, I found that several risk factors were associated with high grade tumors (parity, oral contraceptive use) and that breast feeding was protective against ER-, PR-, and high grade tumors, in a population with a high genetic predisposition for cancer. The breastfeeding findings indicate that the acquisition of

certain poor-prognosis tumor characteristics in breast cancer, such as ER and PR negativity, and high grade, may be ameliorated through environmental actions, such as breastfeeding, even in women who have a high genetic predisposition to cancer, including *BRCA* carriers. These findings have significance for reducing the rate of poor-prognosis tumor characteristics, in women for whom eventual breast cancer diagnosis is likely.

Table 3-1: Demographic and Tumor Characteristics by ER/PR status, BCFR Clinic-Based Sample

	Controls N=3794 N (%)	**ER+PR+ N=436 N (%)**	**ER+PR- N=100 N (%)**	**ER-PR+ N=53 N (%)**	**ER-PR- N=254 N (%)**
Age (μ± s.d.)	44.4±15.0	50.2±12.3	51.6±15.0	43.2±10.3	44.2±11.1
Race					
White	3031 (80)	284 (74)	59 (71)	34 (72)	145 (66)
Black	66 (2)	17 (5)	6 (7)	1 (2)	20 (9)
Hispanic	474 (13)	61 (15)	14 (17)	7 (14)	39 (17)
Asian	85 (2)	9 (2)	2 (2)	7 (4)	149 (3)
Other	125 (3)	15 (4)	2 (2)	4 (8)	12 (5)
Site					
Philadelphia	882 (23)	69 (16)	26 (25)	3 (5)	51 (20)
New York	2124 (56)	287 (66)	58 (58)	39 (73)	137 (54)
Utah	788 (21)	80 (18)	16 (17)	11 (22)	66 (26)
Menopausal Status					
Pre	2530 (67)	159 (37)	36 (36)	22 (42)	127 (50)
Post	1264 (33)	277 (63)	64 (64)	31 (58)	127 (50)
Education					
< High school	756 (20)	103 (24)	20 (24)	9 (18)	64 (28)
≥ High school	3028 (80)	285 (76)	63 (76)	39 (82)	160 (72)
OC Use					
Never	1245 (36)	164 (46)	35 (46)	13 (32)	59 (30)
≤ 5 years	1319 (38)	112 (33)	25 (30)	14 (32)	80 (40)
> 5 years	867 (25)	76 (22)	19 (24)	16 (36)	59 (30)
Date of first OC use					
Never	1245 (34)	164 (42)	35 (44)	13 (28)	59 (26)
Before 1975	959 (26)	134 (35)	23 (28)	23 (48)	73 (34)
1975 or later	1479 (40)	85 (23)	25 (29)	12 (24)	90 (40)
HRT Use					
Never	2778 (77)	300 (74)	66 (76)	41 (82)	189 (80)
Former	335 (9)	7 (2)	1 (1)	3 (6)	37 (5)
Current	496 (14)	97 (24)	19 (22)	6 (12)	11 (16)
Age at menarche					
≤ 11	734 (20)	94 (23)	14 (16)	11 (22)	37 (15)
12	1020 (27)	118 (29)	25 (29)	11 (22)	89 (37)
≥ 13	1983 (53)	193 (48)	48 (55)	28 (56)	113 (48)
Parity					
Nulliparous	1114 (29)	66 (17)	10 (12)	14 (29)	49 (22)
1-2 live births	1517 (40)	179 (46)	44 (53)	30 (61)	99 (44)

≥3 live births	1157 (31)	143(37)	29 (35)	5 (10)	76 (34)
Age at first birth	25.1±5.1	25.3±5.3	25.1±4.9	26.2±6.5	24.3±5.2
Breastfeeding duration					
Never	1847 (50)	164 (42)	35 (43)	28 (55)	108 (47)
< 12 mos.	1072 (29)	137 (36)	33 (39)	18 (37)	74 (35)
≥ 12 mos.	757 (21)	82 (22)	15 (18)	3 (8)	39 (18)
BMI	25.2	26.1	25.6	24.5	26.5
Tumor Grade					
1, 2	NA	179 (63)	39 (66)	11 (28)	30 (18)
3	NA	106 (37)	20 (34)	28 (72)	135 (82)
BRCA1 status					
Status missing	1006 (25)	106 (22)	32 (32)	8 (15)	61 (24)
BRCA1 positive	262 (7)	14 (4)	5 (5)	6 (11)	56 (22)
BRCA1 negative	2526 (67)	324 (74)	63 (63)	39 (74)	137 (54)
BRCA2 status					
Status missing	1071 (28)	97 (23)	33 (33)	9 (17)	73 (29)
BRCA2 positive	173 (5)	32 (7)	6 (6)	6 (11)	18 (7)
BRCA2 negative	2550 (67)	307 (70)	61 (61)	38 (72)	163 (64)

Table 3-2: Association between parity and breastfeeding, and breast cancer classified by hormone status in the BCFR Clinic, adjusted models*

	ER+PR+ vs. Controls N=382	**ER-PR- vs. Controls N=223**	**ER-PR- vs. ER+PR+**
	OR (95%CI)	**OR (95%CI)**	**OR (95%CI)**
Parity, LOGISTIC			
Nulliparous	1.0 (ref)	1.0 (ref)	1.0 (ref)
1-2 live births	**1.63 (1.13-2.36)**	**1.90 (1.20-3.00)**	1.16 (0.66-2.04)
≥ 3 live births	1.35 (0.89-2.06)	**1.85 (1.09-3.13)**	1.37 (0.72-2.59)
Parity, GEE			
Nulliparous	1.0 (ref)	1.0 (ref)	1.0 (ref)
1-2 live births	**1.62 (1.10-2.38)**	**2.10 (1.27-3.48)**	1.01 (0.58-1.77)
≥ 3 live births	1.40 (0.90-2.19)	**2.10 (1.16-3.81)**	1.14 (0.59-2.18)
Breastfeeding duration, LOGISTIC			
Never	1.0 (ref)	1.0 (ref)	1.0 (ref)
< 12 mos.	1.23 (0.91-1.64)	0.88 (0.61-1.29)	0.72 (0.46-1.13)
≥ 12 mos.	1.17 (0.84-1.65)	0.70 (0.45-1.11)	0.60 (0.35-1.02)
Breastfeeding duration, GEE			
Never			
<12 mos.	1.19 (0.89-1.59)	0.83 (0.57-1.19)	0.73 (0.47-1.13)
≥ 12 mos.	1.18 (0.84-1.66)	0.69 (0.43-1.10)	0.70 (0.40-1.23)
Breastfeeding and parity, LOGISTIC			
Nulliparous	1.0 (ref)	1.0 (ref)	1.0 (ref)
1-2 live births, never BF	1.41 (0.94-2.13)	**1.65 (1.00-2.73)**	1.17 (0.63-2.16)
≥ 3 live births, never BF	1.47 (0.92-2.36)	1.72 (0.95-3.13)	1.17 (0.57-2.40)
1-2 live births, ever BF	**2.25 (1.45-3.49)**	1.44 (0.80-2.61)	0.64 (0.32-1.29)
≥ 3 live births, ever BF	1.39 (0.95-2.05)	1.22 (0.76-1.98)	0.88 (0.49-1.59)
Breastfeeding and parity, GEE			
Nulliparous			
1-2 live births, never BF	1.45 (0.95-2.21)	**1.75 (1.00-3.04)**	1.01 (0.56-1.80)
≥ 3 live births, never BF	1.63 (0.91-2.48)	**2.07 (1.09-3.91)**	1.03 (0.50-2.11)
1-2 live births, ever BF	**2.11 (1.34-3.33)**	1.56 (0.84-2.88)	0.60 (0.28-1.30)
≥ 3 live births, ever BF	1.47 (0.98-2.20)	1.31 (0.77-2.24)	0.76 (0.43-1.36)

**Adjusted for age, center, parity, oral contraceptive use, race, breastfeeding, menopausal status, family history*
*ORs in **bold** are statistically significant*

Table 3-3: Association between oral contraceptive use and breast cancer classified by hormone status in the BCFR Clinic, adjusted models*

	ER+PR+ N=382	**ER-PR- N=223**	**ER-PR- vs. ER+PR+**
	OR (95%CI)	**OR (95%CI)**	**OR (95%CI)**
OC Use , LOGISTIC			
Never	1.0 (ref)	1.0 (ref)	1.0 (ref)
≤ 5 years	0.77 (0.58-1.02)	1.40 (0.97-2.03)	**1.83 (1.17-2.84)**
> 5 years	0.79 (0.58-1.07)	**1.49 (1.00-2.21)**	**1.90 (1.17-3.06)**
OC Use, GEE			
Never	1.0 (ref)	1.0 (ref)	1.0 (ref)
≤ 5 years	0.76 (0.57-1.01)	1.45 (0.99-2.11)	**1.60(1.04-2.44)**
> 5 years	0.81 (059-1.11)	**1.50 (1.01-2.23)**	1.56 (0.97-2.53)
Date of first OC use, LOGISTIC			
Never	1.0 (ref)	1.0 (ref)	1.0 (ref)
Before 1975	0.94 (0.73-1.22)	**1.62 (1.11-2.36)**	**1.71 (1.11-2.64)**
1975 or later	**0.56 (0.41-0.78)**	1.18 (0.80-1.75)	**2.10 (1.29-3.42)**
Date of first OC use, GEE			
Never	1.0 (ref)	1.0 (ref)	1.0 (ref)
Before 1975	0.95 (0.72-1.23)	**1.60 (1.08-2.37)**	1.46 (0.95-2.25)
1975 or later	**0.56 (0.41-0.78)**	1.22 (0.83-1.80)	**1.80 (1.07-3.03)**

**Adjusted for age, center, parity, oral contraceptive use, race, breastfeeding, menopausal status, family history*
*ORs in **bold** are statistically significant*

Table 3-4: Association between parity, breastfeeding, and oral contraceptive use and breast cancer classified by ER/PR status in the BCFR Clinic, among BRCA1/2 cases, adjusted models*

	ER+PR+ N=48	**ER-PR- N=75**
	OR (95%CI)	**OR (95%CI)**
Parity, LOGISTIC		
Nulliparity	1.0 (ref)	1.0 (ref)
1-2 live births	3.21 (0.90-11.47)	1.42 (0.56-3.61)
3+ live births	2.78 (0.70-11.23)	1.99 (0.70-5.66)
Parity, GEE		
Nulliparity	1.0 (ref)	1.0 (ref)
1-2 live births	3.39 (0.80-14.36)	1.39 (0.53-3.68)
3+ live births	2.71 (0.55-13.31)	1.93 (0.66-5.63)
Breastfeeding duration, LOGISTIC		
Never	1.0 (ref)	1.0 (ref)
<12 mos.	1.65 (0.67-4.02)	1.28 (0.59-2.80)
≥ 12 mos.	0.62 (0.21-1.84)	**0.35 (0.14-0.93)**
Breastfeeding duration, GEE		
Never	1.0 (ref)	1.0 (ref)
<12 mos.	1.49 (0.60-3.67)	1.29 (0.61-2.71)
≥ 12 mos.	0.60 (0.19-1.92)	0.41 (0.16-1.06)
OC Use, LOGISTIC		
Never	1.0 (ref)	1.0 (ref)
≤ 5 years	1.61 (0.64-4.05)	1.65 (0.71-3.87)
> 5 years	1.17 (0.38-3.66)	**4.37 (1.85-10.36)**
OC Use, GEE		
Never	1.0 (ref)	1.0 (ref)
≤ 5 years	1.15 (0.52-2.55)	1.74 (0.78-3.87)
> 5 years	1.67 (0.73-3.80)	**3.53 (1.59-7.86)**
Date of first OC use, LOGISTIC		
Never	1.0 (ref)	1.0 (ref)
Before 1975	1.17 (0.52-2.63)	**4.10 (1.98-8.51)**
1975 or later	1.20 (0.52-2.80)	1.60 (0.77-3.35)
Date of first OC use, GEE		
Never	1.0 (ref)	1.0 (ref)
Before 1975	0.97 (0.41-2.32)	**3.43 (1.52-7.75)**
1975 or later	1.39 (0.60-3.21)	1.73 (0.81-3.71)

**Adjusted for age, center, menopausal status, family history and factors in the table*
*ORs in **bold** are statistically significant*

Figure 3_1: Comparison of Odds Ratios for those with and without a First-Degree Relative (FDR) with Breast Cancer

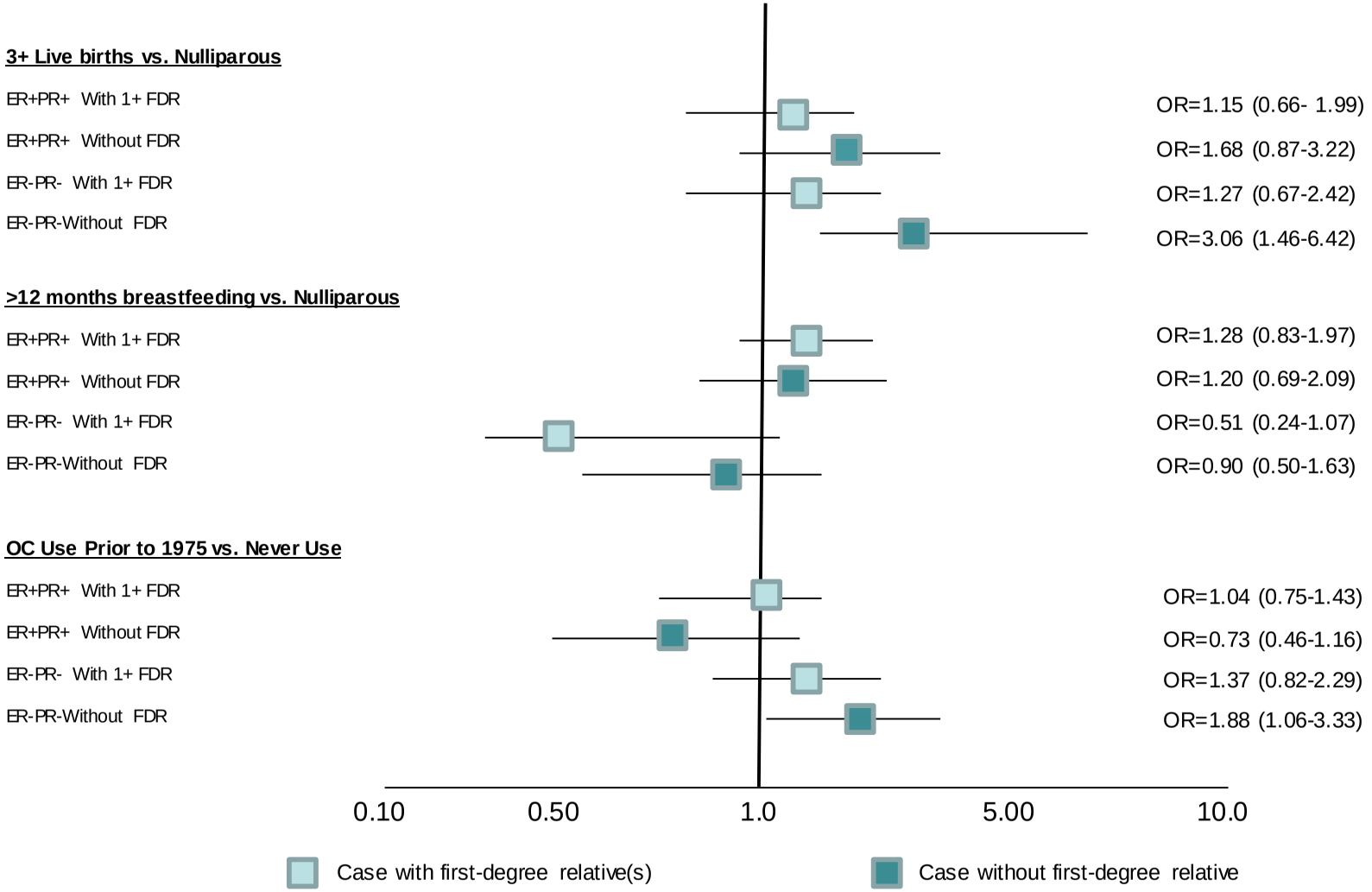


Table 3-5A: Associations between parity, breastfeeding and select tumor characteristics; case-case analysis and pseudo-conditional likelihood findings, Clinic-Based sites of the BCFR

	ER- (vs. ER+)		PR- (vs. PR+)		Grade (3 vs. 1,2)	
	OR (95%CI) Polytomous	**OR (95%CI) PCL**	**OR (95%CI) Polytomous**	**OR (95%CI) PCL**	**OR (95%CI) Polytomous**	**OR (95%CI) PCL**
Parity						
Nulliparous	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
1-2 live births	0.98 (0.58-1.66)	0.52 (0.22-1.27)	1.17 (0.70-1.95)	1.77 (0.80-3.90)	**1.90 (1.00-3.59)**	**2.17 (1.08-4.38)**
≥ 3 live births	1.00 (0.56-1.80)	0.46 (0.17-1.29)	1.24 (0.70-2.18)	1.50 (0.62-3.62)	1.96 (0.95-4.01**)**	**2.23 (1.02-4.86)**
Breastfeeding duration						
Never	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
<12 mos.	0.68 (0.44-1.05)	1.34 (0.65-2.74)	0.80 (0.53-1.21)	0.95 (0.51-1.90)	**0.54 (0.32-0.91)**	**0.48 (0.27-0.86)**
≥ 12 mos.	**0.49 (0.28-0.84)**	0.79 (0.33-1.89)	**0.58 (0.35-0.97)**	0.72 (0.34-1.56)	**0.40 (0.21-0.73)**	**0.47 (0.25-0.91)**
Parity and Breastfeeding						
Nulliparous	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
1, 2 live births, no BF	1.09 (0.62-1.93)	0.65 (0.24-1.80)	1.23 (0.70-2.15)	2.06 (0.84-5.08)	1.78 (0.89-3.57)	1.84 (0.85-3.96)
≥ 3 live births, no BF	0.82 (0.41-1.63)	0.29 (0.08-1.05)	1.23 (0.63-2.37**)**	1.96 (0.66-5.81)	1.53 (0.67-3.53)	1.96 (0.80-4.81)
1, 2 live births, some BF	**0.41 (0.20-0.85)**	0.42 (0.13-1.33)	0.58 (0.29-1.16)	1.24 (0.41-3.78)	0.62 (0.28-1.35)	0.84 (0.36-1.98)
≥ 3 live births, some BF	0.64 (0.36-1.13)	0.51 (0.18-1.41)	0.92 (0.53-1.61)	1.54 (0.63-3.76)	0.84 (0.44-1.62)	0.91 (0.44-1.88)

*Note: All ORs adjusted for age, race, study site, parity, breastfeeding, oral contraceptive use, family history, and menopausal status. ORs in **bold** are statistically significant*

Table 3-5B: Associations between oral contraceptive use and select tumor characteristics; case-case analysis and pseudo-conditional likelihood findings, Clinic-Based sites of the BCFR

	ER- (vs. ER+)		PR- (vs. PR+)		Grade (3 vs. 1,2)	
	OR (95%CI) Polytomous	**OR (95%CI) PCL**	**OR (95%CI) Polytomous**	**OR (95%CI) PCL**	**OR (95%CI) Polytomous**	**OR (95%CI) PCL**
OC Use						
Never	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
≤ 5 years	**1.56 (1.02-2.40)**	1.49 (0.75-2.97)	**1.57 (1.04-2.37)**	1.51 (0.81-2.83)	1.42 (0.86-2.33)	1.16 (0.67-2.01)
> 5 years	**1.74 (1.09-2.77)**	1.53 (0.70-3.34)	1.41 (0.90-2.20)	1.42 (0.71-2.82)	1.48 (0.84-2.63)	1.24 (0.66-2.33)
OC Use 1975						
Never Used OCs	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
Used OC before 1975	**1.75 (1.15-2.67)**	1.90 (0.95-3.80)	1.29 (0.87-1.92)	1.10 (0.60-2.02)	1.27 (0.79-2.06)	1.01 (0.60-1.71)
Used OC 1975 or later	1.33 (0.82-2.15)	1.16 (0.53-2.55)	**1.78 (1.11-2.84)**	1.88 (0.92-3.83)	1.09 (0.61-1.94)	0.96 (0.51-1.80)

*Note: All ORs adjusted for age, race, study site, parity, breastfeeding, oral contraceptive use, family history, and menopausal status ORs in **bold** are statistically significant*

Chapter 4: Comparison of Population-Based and Clinic-Based Findings, and Conclusion

In the previous two chapters, I examined select risk factors, primarily breastfeeding (and its relationship with parity) and oral contraceptive use, in high risk and familial populations. The changing nature of these risk factors was accounted for in the analysis of the population-based cases, as was the effect of these factors when paired with family history and the presence of genetic mutations that predispose to breast cancer (*BRCA1 and 2*).

Table 4-1 compares the demographic, risk factor and tumor characteristic frequencies for cases and controls at the population-based sites, with cases and controls at the clinic-based sites. Differences largely reflect that the clinic-based patients consist of a population with familial breast cancer, while population-based cases may or may not be familial. The clinic-based cases are more likely to be ER-PR- (30% of clinic-based cases vs. 23% of population-based cases), to have a high-tumor grade (53% of clinic-based cases grade 3, 41% of population-based cases grade 3), to be *BRCA1* positive (13% of clinic-based cases, vs 7% of population-based cases), and *BRCA2* positive (10% of clinic-based cases, vs. 5% of population-based cases). The population-based cases, for whom racial minorities were purposefully over-sampled, are more ethnically diverse (61% Non-Hispanic White, vs. 71% Non-Hispanic White among the clinic-based cases), however a higher percentage of clinic-based cases are Hispanic (16%, vs. 10% of population-based cases), likely due to the sites of recruitment for clinic-based populations. Clinic-based cases and controls were more likely to be never-users of oral contraceptives (40% and 36% of clinic-based cases and controls were never users, vs. 26% and 22% of population-based cases and controls). Twenty-nine percent of clinic-based controls were nulliparous, compared with 18% of population-based controls. Population-based cases were more likely to have been current users of HRT (16% compared to 3% of clinic-based) while clinic-based cases

were more likely to be former users (20% of clinic-based cases compared to 8% of population-based cases). Population-based controls were more likely to be age 13 or greater at menarche, than were clinic-based controls (68%, vs. 53%). Clinic-based controls were more likely to have never breast-fed (50%), compared with population-based controls (40%), which is unsurprising given the lower rate of parity among the clinic-based controls. Given differences between cases and controls in the two populations studied, it is perhaps unsurprising to find differences in risk factor associations with ER-PR- defined breast cancer subtypes.

Differences in Analytic Findings: Population-Based and Clinic-Based Data

Parity and Breastfeeding: In the analysis from Chapter 2, I found that, within the population-based cohorts of the BCFR, high parity (3+ live births) without breastfeeding was positively associated with ER-PR- tumors, but not with ER+PR+ tumors. Breastfeeding was associated with a reduced risk of all breast cancer subtypes, but most strongly with ER-PR- tumors. High parity, when coupled with breastfeeding, was no longer associated with ER-PR- cancer.

In Chapter 3, where the same analysis was completed with a clinic-based population of breast cancer cases and familial controls, high parity was associated with both ER+PR+, and ER-PR- tumors, and breastfeeding was only associated with reduced odds of ER-PR- cancer. However, after limiting the clinic-based analysis to cases who were interviewed within 2 years of diagnosis (to minimize potential survivorship bias), parity was no longer associated with ER+PR+ tumors. Childbirth associated with lack of breastfeeding was associated with an increased risk of ER-PR- tumors. This risk was mitigated by breastfeeding in the ER-PR- tumors, but not in the ER+PR+ tumors.

Thus, the polytomous logistic findings for the ER-PR- patients are generally consistent across the clinic-based and population-based samples in regards to parity and breastfeeding, despite the differences in the sample (*Table 4-2*).

Risk factors are less consistent in their associations with ER+PR+ cancers (*Table 4-2*). Parity and breastfeeding findings have different interpretations, across the population-based and clinic-based samples. The parity association in the clinic-based sample may have limited interpretability due to possible survivorship bias, but even among clinic-based cases interviewed close to diagnosis, when this bias is not present, there is no association (rather than an inverse association) between parity and ER+PR+ type, different from what was found in the population-based cases. Parity has been found to have a different association with ER/PR status among post-menopausal and premenopausal women, in other studies as well as in Chapter 2 of this dissertation, which found parity to be protective only among postmenopausal women.

Additionally, there is no protective association of breastfeeding on ER+PR+ cancer in the clinic-based sample, in contrast to the findings in the population-based sample, which found long-term breastfeeding to be protective against all subtypes. However, analysis stratified by menopause also indicated that there was no association between breastfeeding and postmenopausal ER+PR+ status. Additional analysis in premenopausal, vs. postmenopausal, cased in the clinic-based sample, should be undertaken to determine if findings by menopause are similar to those in the population-based sample.

Findings remain inconsistent when comparing findings from the pseudo-conditional likelihood approach, in the population-based and clinic-based samples. Parity is positively associated with ER negativity, but not PR negativity or grade, in the population-based cases. Parity is positively

associated with Grade, but not ER negativity or PR negativity, in the clinic-based cases. The association with breastfeeding is more consistently maintained: breastfeeding is inversely associated with ER negativity, in both population-based and clinic-based samples, and breastfeeding is unassociated with PR status, in both analyses.

In the population-based sample, associations between parity and ER- cancer, appear to be driven through estrogen receptor negativity, whereas in the clinic-based sample, apparent associations between parity and ER- cancer appear to in fact be an association between parity and high grade. (One or both associations could also be linked to another tumor characteristic not studied in-depth in this dissertation, that correlates to both ER status and grade, such as HER2 status).

Hypotheses about why parity would be associated with negative ER status, and high grade, are provided in the discussions in Chapters 2 and 3. The reasons behind the difference by sample type could be due to the "level" of familial risk in each sample. The differences related to parity, and its association with different tumor characteristics (ER-status in the population-based sample, and grade in the clinic-based sample) may result from structural differences of the sample constructs. Although both samples represent high-risk populations, the population-based cases had fewer first-degree (and total) relatives with breast cancer, than did clinic-based cases. They also were about half as likely to be *BRCA1* or *BRCA2* positive. *BRCA1* and *BRCA2* positive cases typically have high grade tumors, regardless of ER/PR status, and some studies have demonstrated that familial cancer (whether *BRCA*+ or not) tends to be of higher grade than non-familial cancer [38, 87]. It could be that in the familial sample, an apparent association between parity and grade, is simply the effect being a familial or *BRCA* positive case, and grade obfuscates (or minimizes) any individual risk factors.

In the population-based sample, because younger women (but not always women with family history) were purposefully recruited, the population-based cases may represent those who had a higher "environment-driven" predilection for breast cancer, while the clinic-based cases had a higher genetic component to their breast cancer, thus limiting the role of environmental factors in contributing to breast cancer etiology. In such a case, a factor (such as parity) may have an apparent link to tumor characteristic such as grade, but may in fact not be causative. Among cases with a first-degree family history, across both population-based and clinic-based samples, findings were more similar: parity remained positively associated with ER-PR- cancer, only in those cases without a first degree family history, in both samples. The similarity of findings when stratifying by family history lends further support to the idea that considering family history is of value when comparing risk factor associations with tumor characteristics, across different samples of cases, and also demonstrates that in the presence of family history, environmental risk factor effects on disease incidence may be diminished.

The findings regarding breastfeeding are more consistent across samples, and in line with findings of previous studies, demonstrating that breastfeeding appears to provide the strongest protection against ER- cancer, with pseudo-conditional findings indicating that the estrogen receptor status is the tumor characteristic (among ER, PR, or grade) most influenced by breastfeeding. This protective effect may be due to breastfeeding's effect on alterations in hormones such as androgens, which may suppress cell proliferation in ER+ tumors, but can promote tumorigenesis in ER-tumors [191].

Oral contraceptive use: In the population-based sample in cases compared with controls, oral contraceptive (OC) use prior to 1975 was associated with an increased risk of ER-PR- cancer but not ER+PR+ cancer. For women who began use in 1975 or later there was no increased risk

conferred by OC use, in the ER-PR- cases, and an inverse association between OC use and ER+PR+ cancer. In the clinic-based sample, compared with controls, oral contraceptive use was associated only with a risk of ER-PR- tumors, and in case-only analysis, was associated with ER-PR- tumors compared with ER+PR+ tumors. Similar to women in the population-based findings, for women who used oral contraceptives prior to 1975, there was an increased risk of ER-PR- cancer, compared with controls, and no such association with ER+PR+ cancer. For women who began using contraceptives in 1975 or later, there was no significant association with ER-PR- cancer, when compared with controls, and there was an inverse association between OC use and ER+PR+ cancer, among women who began using OCs after 1975. Thus, the findings regarding OC use are generally consistent across the population and clinic-based samples, when comparing ER+PR+ cases from each sample set, and ER-PR- cases from each sample set. The consistency of findings indicates that the finding is robust to study design and sample composition, and that OCs can have null, protective, and negative effects, depending on start date and duration of use, in populations regardless of their existing levels of genetic predisposition to breast cancer.

As was the case with parity and breastfeeding, the findings between cases in the population-based sample, and in the clinic-based sample, are less consistent when examining the association for ER- vs. ER+, PR- vs. PR+, and low grade vs. high grade tumors, after accounting for correlation of these factors. Oral contraceptive use of greater than 5 years was associated with ER negativity, PR negativity, and high grade, in the clinic-based sample, but with only high grade, in the population-based sample. Oral contraceptive use prior to 1975 was associated with ER- in the clinic-based sample, but only slightly elevated in the population-based sample. Oral contraceptive use *after* 1975 was associated with high grade in the population-based, but not the

clinic-based sample, but OC use after 1975 was associated with PR negativity in the clinic-based sample, but not the population-based sample.

Because some of these estimates are non-significant, particularly in the clinic-based sample where there are fewer cases for analysis, interpretability of these differences is challenging. However it is feasible that oral contraceptives operate differently, in women with high genetic predisposition to cancer [167, 187, 192]. Thus although both populations are affected by oral contraceptive use, the high rate of BRCA1 and BRCA2 carriers and individuals with multiple family members with breast cancer, in the clinic-based cases, may affect both the mechanism by which oral contraceptives affect breast cancer risk, as well as familial control (and case) decisions to use oral contraceptives. It is notable that in cases with at least one first-degree relative with breast cancer, the association between oral contraceptive use and breast cancer was minimized or non-significant, and this was true for both clinic-based and population-based cases. Therefore oral contraceptive use may be a bigger driver of breast cancer risk (or protection) among women who do not already have an elevated genetic predisposition to breast cancer.

Conclusion

In this dissertation, I summarized existing literature on reproductive and hormonal risk factors for breast cancer defined by estrogen receptor and progesterone receptor status and molecular subtype, among average-risk and high-risk populations. I also independently examined the role of parity, breastfeeding, and oral contraceptive use, in development of different cancer subtypes and tumor characteristics, in two populations at high risk for breast cancer. Findings regarding breastfeeding and oral contraceptive use were generally consistent across studies, as were findings related to parity for the ER-PR- subtype. Parity findings regarding the ER+PR+ subtype

were inconsistent, for reasons that may be related to case ascertainment in the clinic-based sample. Additional analyses using a two-stage regression approach, the pseudo-conditional likelihood method, revealed that apparent associations with parity and ER negativity or oral contraceptive use, may actually represent associations with the correlated tumor characteristic of grade, a finding that allows for hypothesis generation around mechanism of action in terms of how the risk factor leads to specific tumor subtype. Specific analyses in the population-based sample further demonstrated the changing nature of oral contraceptive use and breast cancer risk, according to age cohort, demonstrated that the protective nature of breastfeeding against ER-PR- cancer exists regardless of race, and also confirmed findings in previous literature, regarding the associations between breastfeeding, parity, and triple-negative subtypes. Analyses in both the population-based and clinic-based sample described the way in which risk factor associations may differ in regard to outcome, for those with and without first-degree relatives with cancer, and analysis of *BRCA1/2* cases demonstrated the importance of modifiable risk factors in reducing risk of poor prognosis disease. They also contribute to a relatively small body of literature evaluating risk factor associations with tumor characteristics in higher risk populations. Inclusion of these populations in such analyses is of value for understanding the relative importance of risk factors in all populations, as well as understanding biological mechanisms by which such factors may operate.

Due to the high missingness of ER and PR status in the clinic-based sample and unavailability of HER2 status, I was unable to confirm all of the findings of the population-based sample, nor was I able to conduct age-cohort analyses, stratify by race, or conduct molecular subtype analyses. Grade was also missing for a large portion of the sample, limiting the power of the case-only analyses to determine significant findings. An update of both tumor and epidemiological data

from the clinic-based sites of the Breast Cancer Family Registry, should be undertaken to improve the power of the sample to detect associations or confirm null findings.

Despite these limitations, these studies confirm a previous body of literature indicating that breastfeeding confers protection against ER-PR- and cancers, even in populations with existing genetic predisposition for breast cancer, and across all races and ethnicities. They also indicate that breastfeeding may protect against development of higher grade tumors. The studies also demonstrate that oral contraceptive use may be associated with risk primarily in cases with low to no genetic predisposition to breast cancer, but also that in general, oral contraceptive use is not associated with breast cancer risk in women whose OC use primarily occurred after 1975, thus recent formulations of contraceptives may confer little or no increased risk for breast cancer. Additional studies should continue to examine risk factor associations with tumor characteristics associated with prognosis, such as grade, to add to this limited body of literature, and provide hypothesis generation to understand possible mechanisms by which OC use may contribute to high grade.

Many studies have examined the relation between breast cancer risk factors and breast cancer tumor markers. Despite the wealth of information on this topic, few studies have examined risk factors in high-risk or familial populations. If we can understand how environmental breast cancer risk factors differentially affect risk in high risk populations, women at high risk of breast cancer may be better able to make decisions that allow for reduction in overall risk. If, in addition, we can understand the role that these risk factors play in the etiology of subtypes with prognostic ramifications, we could tailor behavioral modifications to reduce the risk of those types of breast cancer associated with fewer treatment options and poorer survival, such as ER-PR- or triple-negative breast cancers. For example, the World Cancer Research Fund/American

Institute for Cancer Research has estimated that over 40% of post-menopausal breast cancer could be prevented by reductions in alcohol, excess body weight, and inactivity [193]. However, most women at higher than average risk are more likely to be diagnosed prior to menopause, and alcohol and BMI have not been as strongly linked to premenopausal cancers [109, 111, 122, 194, 195], thus the presence of these factors in higher risk women may not adequately aid in risk assessment, and risk may not be ameliorated by reducing such factors. Popular risk assessment models, such as the Gail model, include as part of risk assessment factors such as age at first live birth and age at menarche. Older age at first birth and younger age at menarche are known risk factors in average-risk women, but have not been consistently associated with increased risk in higher risk women, or consistently associated with development of poorer prognosis, ER-PR- tumors [19, 40, 54, 70, 98] . It is therefore unclear that incorporating such factors in risk assessment models is helpful in assessing risk for women at higher than average risk for not only breast cancer overall, but for poorer prognosis breast cancers. Thus, examining risk factor heterogeneity in the presence or absence of known risks such as family history, is not only important for improving understanding of disease etiology, but may also allow for more tailored evaluation of risk, as well as improve the ability to prevent cancers associated with poorer prognosis, among those for whom breast cancer may be less avoidable.

The findings of this dissertation therefore have important implications, not only for generating hypotheses in regard to the etiology of different breast cancer subtypes, but also when considering which factors to evaluate in risk assessments of higher-risk women, and when counseling women at high risk for breast cancer on environmental factors that may reduce their risk for poorer prognosis tumors. The findings also add to the increasing body of literature that

demonstrates the value of breastfeeding on reduction of poorer prognosis tumors, across the risk spectrum, and regardless of race/ethnicity.

Table 4-1: Comparison Risk Factor Frequencies in Population-Based and Clinic-Based BCFR Populations

	Cases				Controls			
	Population-Based Sites		Clinic-Based Sites		Population-Based Sites		Clinic-Based Sites	
	#	%	#	%	#	%	#	%
Sample Size	**4011**	**100**	**843**	**100**	**2997**	**100**	**3794**	**100**
ERPR Status								
ER+PR+	2486	62	436	52	NA		NA	
ER+PR-	397	10	100	12	NA		NA	
ER-PR+	208	5	53	6	NA		NA	
ER-PR-	920	23	254	30	NA		NA	
Age at dx* (μ± s.d.)	47.5±9.5		48.1±12.6		47.6±10.3		44.4±15.0	
Tumor Grade								
1, 2	1980	59	245	47	NA		NA	
3	1384	41	273	53	NA		NA	
First Degree FH								
No	2886	72	344	46	2732	91	1252	33
Yes	1109	28	403	54	263	9	2357	67
BRCA1 positivity								
Negative	1264	93	571	87	NA		2526	91
Positive	95	7	83	13	NA		262	9
BRCA2 positivity								
Negative	1606	95	575	90	NA		2550	94
Positive	78	5	65	10	NA		173	6
Race								
White	2428	61	522	71	2487	86	3031	80
Black	413	10	44	6	96	3	66	2
Hispanic	395	10	121	16	72	2	474	13
Asian	696	17	21	3	165	6	85	2
Other	58	1	32	4	82	3	125	3
Menopausal Status								
Pre	2326	61	344	41	1566	55	2530	67
Post	1513	39	499	59	1262	45	1264	33
Education								
≤ High school	1169	30	196	26	908	30	756	20
> High school	2767	70	547	74	2082	70	3028	80
OC Use								
Never	1019	26	287	40	646	22	1245	36
≤ 5 years	1476	38	241	34	1117	37	1319	38
> 5 years	1417	36	180	26	1216	41	867	25
Date of first OC use								
Never	1019	26	287	37	646	22	1245	34
Before 1975	1799	46	254	34	1435	48	959	26
1975 or later	1093	28	218	29	898	30	1479	40
HRT Use								
Never	2894	76	571	77	2081	70	2778	77

Former	304	8	148	20	246	8	335	9
Current	625	16	21	3	663	22	496	14
Age at menarche								
≤ 11	818	21	146	20	406	14	734	20
12	949	24	232	31	711	28	1020	27
≥ 13	2149	55	365	49	1760	68	1983	53
Parity								
Nulliparous	902	22	141	19	531	18	1114	29
1-2 Live Births	1643	41	355	47	1334	45	1517	40
≥ 3 Live Births	1466	37	259	34	1348	46	1157	31
Age at first birth	25.0		25.0		24.8		25.1	
Breastfeeding duration								
Never	1842	46	340	46	1203	40	1847	50
<12 mos.	1195	30	267	36	991	33	1072	29
≥ 12 mos.	928	23	140	19	803	27	757	21
Parity+Breastfeeding								
Nulliparous	902	23	141	20	531	19	1114	32
1-2 live births, never BF	589	15	116	16	448	16	460	13
3+ live births, never BF	351	9	83	11	224	8	272	8
1-2 live births, ever BF	1029	26	208	29	768	27	891	25
3+ live births, ever BF	1094	28	167	23	836	30	795	23
BMI	26.1		25.9		25.9		25.2	

Table 4-2: Consistency of Findings in the BCFR population, across population-based and clinic-based sites

Risk Factor	*ER+PR+ (compared to control)*		*ER-PR- (compared to control)*		*ER- (compared to ER +)*		*PR- (compared to PR+)*		*High Grade (compared to low)*	
Population or Clinic?	***Pop***	***Clin***	***Pop***	***Clin***	***Pop***	***Clin***	***Pop***	***Clin***	***Pop***	***Clin***
Parity (1-2) (vs. Nulliparity)	↓*0.80*	↑*1.62*	↑*1.33*	↑*2.10*	↑*1.43*	↓*0.52*	—*1.00*	↑*1.77*	—*0.93*	↑*2.17*
High parity (≥3) (vs. Nulliparity)	—*0.93*	↑*1.40*	↑*1.59*	↑*2.10*	↑*1.43*	↓*0.46*	—*0.97*	↑*1.50*	—*1.01*	↑*2.23*
Breastfeeding >12 mos vs. nulliparity	↓*0.80*	—*1.18*	↓*0.52*	↓*0.69*	↓*0.70*	↓*0.79*	—*0.90*	—*0.72*	—*0.97*	↓*0.48*
High parity (≥3), no breastfeeding, vs. nulliparity	—*1.05*	↑*1.63*	↑*1.49*	↑*2.07*	↑*1.54*	↓*0.29*	—*1.08*	↑*1.96*	—*0.98*	↑*1.96*
Oral contraceptive use > 5 years vs. None	↓*0.83*	↓*0.81*	—*1.13*	↑*1.50*	—*1.07*	↑*1.53*	—*0.96*	↑*1.42*	↑*1.37*	↑*1.24*
Oral contraceptive use before 1975	—*1.06*	—*0.95*	↑*1.32*	↑*1.60*	↑*1.23*	↑*1.90*	—*0.84*	—*1.10*	—*1.16*	—*1.01*
Oral contraceptive use 1975 or later	↓*0.59*	↓*0.56*	—*0.82*	—*1.22*	—*0.89*	—*1.16*	—*1.05*	↑*1.88*	↑*1.49*	—*0.96*

Point estimates for population-based and clinic-based samples shown, with arrows representing positive or negative associations. Where estimates are similar and both positive or inverse, (or not associated) squares are marked in green. Where there is an association for one population, and no association for another, squares are marked yellow. Where there is a positive association for one population, and an inverse association for the other, squares are marked pink.

REFERENCES

1. Jemal, A., E. Ward, and M.J. Thun, *Recent trends in breast cancer incidence rates by age and tumor characteristics among U.S. women.* Breast Cancer Res, 2007. **9**(3): p. R28.
2. Anderson, W.F., H.A. Katki, and P.S. Rosenberg, *Incidence of breast cancer in the United States: current and future trends.* J Natl Cancer Inst, 2011. **103**(18): p. 1397-402.
3. Brinton, L.A., et al., *Recent trends in breast cancer among younger women in the United States.* J Natl Cancer Inst, 2008. **100**(22): p. 1643-8.
4. Albain, K.S., et al., *Racial disparities in cancer survival among randomized clinical trials patients of the Southwest Oncology Group.* J Natl Cancer Inst, 2009. **101**(14): p. 984-92.
5. Carey, L.A., et al., *Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study.* Jama, 2006. **295**(21): p. 2492-502.
6. Millikan, R.C., et al., *Epidemiology of basal-like breast cancer.* Breast Cancer Res Treat, 2007.
7. DeSantis, C.E., et al., *Breast cancer statistics, 2015: Convergence of incidence rates between black and white women.* CA Cancer J Clin, 2016. **66**(1): p. 31-42.
8. Kohler, B.A., et al., *Annual Report to the Nation on the Status of Cancer, 1975-2011, Featuring Incidence of Breast Cancer Subtypes by Race/Ethnicity, Poverty, and State.* J Natl Cancer Inst, 2015. **107**(6): p. djv048.
9. Blows, F.M., et al., *Subtyping of breast cancer by immunohistochemistry to investigate a relationship between subtype and short and long term survival: a collaborative analysis of data for 10,159 cases from 12 studies.* PLoS Med. **7**(5): p. e1000279.
10. Cancello, G., et al., *Prognosis and adjuvant treatment effects in selected breast cancer subtypes of very young women (<35 years) with operable breast cancer.* Ann Oncol.
11. Garcia-Closas, M., et al., *Heterogeneity of breast cancer associations with five susceptibility loci by clinical and pathological characteristics.* PLoS Genet, 2008. **4**(4): p. e1000054.
12. *Breast cancer and hormonal contraceptives: collaborative reanalysis of individual data on 53 297 women with breast cancer and 100 239 women without breast cancer from 54 epidemiological studies. Collaborative Group on Hormonal Factors in Breast Cancer.* Lancet, 1996. **347**(9017): p. 1713-27.
13. Anderson, D.E. and M.D. Badzioch, *Combined effect of family history and reproductive factors on breast cancer risk.* Cancer, 1989. **63**(2): p. 349-53.
14. Garcia-Closas, M., et al., *Established breast cancer risk factors by clinically important tumour characteristics.* Br J Cancer, 2006. **95**(1): p. 123-9.
15. Ricks, L.J., et al., *Family history of cancer associated with breast tumor clinicopathological features.* J Community Genet, 2014. **5**(3): p. 233-40.
16. Foulkes, W.D., et al., *Estrogen receptor status in BRCA1- and BRCA2-related breast cancer: the influence of age, grade, and histological type.* Clin Cancer Res, 2004. **10**(6): p. 2029-34.
17. Piccart-Gebhart, M.J., et al., *Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer.* N Engl J Med, 2005. **353**(16): p. 1659-72.
18. Romond, E.H., et al., *Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer.* N Engl J Med, 2005. **353**(16): p. 1673-84.

19. Althuis, M.D., et al., *Etiology of hormone receptor-defined breast cancer: a systematic review of the literature.* Cancer Epidemiol Biomarkers Prev, 2004. **13**(10): p. 1558-68.
20. Bardou, V.J., et al., *Progesterone receptor status significantly improves outcome prediction over estrogen receptor status alone for adjuvant endocrine therapy in two large breast cancer databases.* J Clin Oncol, 2003. **21**(10): p. 1973-9.
21. Yu, K.D., et al., *Breast cancer patients with estrogen receptor-negative/progesterone receptor-positive tumors: being younger and getting less benefit from adjuvant tamoxifen treatment.* J Cancer Res Clin Oncol, 2008. **134**(12): p. 1347-54.
22. Yang, X.R., et al., *Differences in risk factors for breast cancer molecular subtypes in a population-based study.* Cancer Epidemiol Biomarkers Prev, 2007. **16**(3): p. 439-43.
23. Foulkes, W.D., I.E. Smith, and J.S. Reis-Filho, *Triple-negative breast cancer.* N Engl J Med, 2010. **363**(20): p. 1938-48.
24. Prat, A., et al., *Clinical implications of the intrinsic molecular subtypes of breast cancer.* Breast, 2015. **24 Suppl 2**: p. S26-35.
25. Ishitha, G., et al., *Clinicopathological Study of Triple Negative Breast Cancers.* J Clin Diagn Res, 2016. **10**(9): p. EC05-EC09.
26. Gammon, M.D., et al., *Oral contraceptive use and other risk factors in relation to HER-2/neu overexpression in breast cancer among young women.* Cancer Epidemiol Biomarkers Prev, 1999. **8**(5): p. 413-9.
27. Ma, H., et al., *Hormone-related risk factors for breast cancer in women under age 50 years by estrogen and progesterone receptor status: results from a case-control and a case-case comparison.* Breast Cancer Res, 2006. **8**(4): p. R39.
28. Ellingjord-Dale, M., et al., *Parity, hormones and breast cancer subtypes - results from a large nested case-control study in a national screening program.* Breast Cancer Res, 2017. **19**(1): p. 10.
29. Islam, T., et al., *Reproductive and hormonal risk factors for luminal, HER2-overexpressing, and triple-negative breast cancer in Japanese women.* Ann Oncol, 2012.
30. Lambertini, M., et al., *Reproductive behaviors and risk of developing breast cancer according to tumor subtype: A systematic review and meta-analysis of epidemiological studies.* Cancer Treat Rev, 2016. **49**: p. 65-76.
31. Reeves, G.K., et al., *Hormonal therapy for menopause and breast-cancer risk by histological type: a cohort study and meta-analysis.* Lancet Oncol, 2006. **7**(11): p. 910-8.
32. Reeves, G.K., et al., *Reproductive factors and specific histological types of breast cancer: prospective study and meta-analysis.* Br J Cancer, 2009. **100**(3): p. 538-44.
33. Li, C.I., et al., *Relationship between established breast cancer risk factors and risk of seven different histologic types of invasive breast cancer.* Cancer Epidemiol Biomarkers Prev, 2006. **15**(5): p. 946-54.
34. Work, M.E., et al., *Risk factors for uncommon histologic subtypes of breast cancer using centralized pathology review in the Breast Cancer Family Registry.* Breast Cancer Res Treat, 2012.
35. Yiangou, C., S. Shousha, and H.D. Sinnett, *Primary tumour characteristics and axillary lymph node status in breast cancer.* Br J Cancer, 1999. **80**(12): p. 1974-8.
36. Terry, M.B., et al., *Oral contraceptive use and cyclin D1 overexpression in breast cancer among young women.* Cancer Epidemiol Biomarkers Prev, 2002. **11**(10 Pt 1): p. 1100-3.
37. Schwartz, A.M., et al., *Histologic grade remains a prognostic factor for breast cancer regardless of the number of positive lymph nodes and tumor size: a study of 161 708*

cases of breast cancer from the SEER Program. Arch Pathol Lab Med, 2014. **138**(8): p. 1048-52.
38. *Familial breast cancer: collaborative reanalysis of individual data from 52 epidemiological studies including 58,209 women with breast cancer and 101,986 women without the disease.* Lancet, 2001. **358**(9291): p. 1389-99.
39. Pharoah, P.D., et al., *Family history and the risk of breast cancer: a systematic review and meta-analysis.* Int J Cancer, 1997. **71**(5): p. 800-9.
40. Setiawan, V.W., et al., *Breast cancer risk factors defined by estrogen and progesterone receptor status: the multiethnic cohort study.* Am J Epidemiol, 2009. **169**(10): p. 1251-9.
41. Rosenberg, L.U., et al., *Risk factors for hormone receptor-defined breast cancer in postmenopausal women.* Cancer Epidemiol Biomarkers Prev, 2006. **15**(12): p. 2482-8.
42. Colditz, G.A., et al., *Risk factors for breast cancer according to estrogen and progesterone receptor status.* J Natl Cancer Inst, 2004. **96**(3): p. 218-28.
43. McCredie, M.R., et al., *Risk factors for breast cancer in young women by oestrogen receptor and progesterone receptor status.* Br J Cancer, 2003. **89**(9): p. 1661-3.
44. Jiang, X., et al., *Family history and breast cancer hormone receptor status in a Spanish cohort.* PLoS One, 2012. **7**(1): p. e29459.
45. Melchor, L., et al., *Distinct genomic aberration patterns are found in familial breast cancer associated with different immunohistochemical subtypes.* Oncogene, 2008. **27**(22): p. 3165-75.
46. Phipps, A.I., et al., *Family history of breast cancer in first-degree relatives and triple-negative breast cancer risk.* Breast Cancer Res Treat, 2011. **126**(3): p. 671-8.
47. Welsh, M.L., et al., *Population-based estimates of the relation between breast cancer risk, tumor subtype, and family history.* Breast Cancer Res Treat, 2009. **114**(3): p. 549-58.
48. D'Eredita, G., et al., *Familial and Sporadic Breast Cancers: Differences in Clinical, Histopathological and Immunohistochemical Features.* Int J Surg Pathol.
49. Colditz, G.A., B.A. Rosner, and F.E. Speizer, *Risk factors for breast cancer according to family history of breast cancer. For the Nurses' Health Study Research Group.* J Natl Cancer Inst, 1996. **88**(6): p. 365-71.
50. Anderson, K.N., R.B. Schwab, and M.E. Martinez, *Reproductive risk factors and breast cancer subtypes: a review of the literature.* Breast Cancer Res Treat, 2014. **144**(1): p. 1-10.
51. Carey, L., et al., *Triple-negative breast cancer: disease entity or title of convenience?* Nat Rev Clin Oncol, 2010. **7**(12): p. 683-92.
52. Gierach, G.L., A. Burke, and W.F. Anderson, *Epidemiology of triple negative breast cancers.* Breast Dis, 2010. **32**(1-2): p. 5-24.
53. Li, L., et al., *Association between oral contraceptive use as a risk factor and triple-negative breast cancer: A systematic review and meta-analysis.* Mol Clin Oncol, 2017. **7**(1): p. 76-80.
54. Largent, J.A., A. Ziogas, and H. Anton-Culver, *Effect of reproductive factors on stage, grade and hormone receptor status in early-onset breast cancer.* Breast Cancer Res, 2005. **7**(4): p. R541-54.
55. Nichols, H.B., et al., *Differences in breast cancer risk factors by tumor marker subtypes among premenopausal Vietnamese and Chinese women.* Cancer Epidemiol Biomarkers Prev, 2005. **14**(1): p. 41-7.

56. Ambrosone, C.B., et al., *Parity and breastfeeding among African-American women: differential effects on breast cancer risk by estrogen receptor status in the Women's Circle of Health Study.* Cancer Causes Control, 2014. **25**(2): p. 259-65.
57. Cui, Y., et al., *Associations of hormone-related factors with breast cancer risk according to hormone receptor status among white and African American women.* Clin Breast Cancer, 2014. **14**(6): p. 417-25.
58. Ma, H., et al., *Pregnancy-related factors and the risk of breast carcinoma in situ and invasive breast cancer among postmenopausal women in the California Teachers Study cohort.* Breast Cancer Res, 2010. **12**(3): p. R35.
59. Ma, H., et al., *Use of four biomarkers to evaluate the risk of breast cancer subtypes in the women's contraceptive and reproductive experiences study.* Cancer Res, 2010. **70**(2): p. 575-87.
60. Palmer, J.R., et al., *Parity, lactation, and breast cancer subtypes in African American women: results from the AMBER Consortium.* J Natl Cancer Inst, 2014. **106**(10).
61. Ritte, R., et al., *Reproductive factors and risk of hormone receptor positive and negative breast cancer: a cohort study.* BMC Cancer, 2013. **13**: p. 584.
62. Sweeney, C., et al., *Reproductive history in relation to breast cancer risk among Hispanic and non-Hispanic white women.* Cancer Causes Control, 2008. **19**(4): p. 391-401.
63. Ursin, G., et al., *Reproductive factors and subtypes of breast cancer defined by hormone receptor and histology.* Br J Cancer, 2005. **93**(3): p. 364-71.
64. Warner, E.T., et al., *Estrogen receptor positive tumors: do reproductive factors explain differences in incidence between black and white women?* Cancer Causes Control, 2013. **24**(4): p. 731-9.
65. Yang, X.R., et al., *Associations of breast cancer risk factors with tumor subtypes: a pooled analysis from the Breast Cancer Association Consortium studies.* J Natl Cancer Inst, 2011. **103**(3): p. 250-63.
66. Iwasaki, M., et al., *Body size and risk for breast cancer in relation to estrogen and progesterone receptor status in Japan.* Ann Epidemiol, 2007. **17**(4): p. 304-12.
67. Bertrand, K.A., et al., *Differential Patterns of Risk Factors for Early-Onset Breast Cancer by ER Status in African American Women.* Cancer Epidemiol Biomarkers Prev, 2017. **26**(2): p. 270-277.
68. Dolle, J.M., et al., *Risk factors for triple-negative breast cancer in women under the age of 45 years.* Cancer Epidemiol Biomarkers Prev, 2009. **18**(4): p. 1157-66.
69. Jia, X., et al., *Reproductive factors and hormone receptor status among very young (<35 years) breast cancer patients.* Oncotarget, 2015. **6**(27): p. 24571-80.
70. Ma, H., et al., *Reproductive factors and breast cancer risk according to joint estrogen and progesterone receptor status: a meta-analysis of epidemiological studies.* Breast Cancer Res, 2006. **8**(4): p. R43.
71. Palmer, J.R., et al., *Parity and lactation in relation to estrogen receptor negative breast cancer in African American women.* Cancer Epidemiol Biomarkers Prev, 2011. **20**(9): p. 1883-91.
72. Song, Q., et al., *The diverse distribution of risk factors between breast cancer subtypes of ER, PR and HER2: a 10-year retrospective multi-center study in China.* PLoS One, 2013. **8**(8): p. e72175.

73. Rusiecki, J.A., et al., *Breast cancer risk factors according to joint estrogen receptor and progesterone receptor status.* Cancer Detect Prev, 2005. **29**(5): p. 419-26.
74. Phipps, A.I., et al., *Reproductive history and oral contraceptive use in relation to risk of triple-negative breast cancer.* J Natl Cancer Inst, 2011. **103**(6): p. 470-7.
75. Phipps, A.I., et al., *Reproductive history and risk of three breast cancer subtypes defined by three biomarkers.* Cancer Causes Control, 2011. **22**(3): p. 399-405.
76. Lara-Medina F, P.-S.V., Saavedra-Perez D, Blake-Cerda M, Arce C, Motola-Kuba D, Villarreal-Garza C, Gonzalez-Angulo AM, Bargallo E, Aquilar JL, Mohar A, Arrieta O, *Triple-negative breast cancer in Hispanic patients: high prevalence, poor prognosis, and association with menopausal status, body mass index, and parity.* Cancer, 2011. **117**(16): p. 3658-3669.
77. Bartow, S.A., et al., *Breast mammographic pattern: a concatenation of confounding and breast cancer risk factors.* Am J Epidemiol, 1995. **142**(8): p. 813-9.
78. von Au, A., et al., *Impact of reproductive factors on breast cancer subtypes in postmenopausal women: a retrospective single-center study.* Arch Gynecol Obstet, 2017. **295**(4): p. 971-978.
79. Ma, H., et al., *Reproductive factors and the risk of triple-negative breast cancer in white women and African-American women: a pooled analysis.* Breast Cancer Res, 2017. **19**(1): p. 6.
80. Li, H., et al., *BMI, reproductive factors, and breast cancer molecular subtypes: A case-control study and meta-analysis.* J Epidemiol, 2017. **27**(4): p. 143-151.
81. Kwan, M.L., et al., *Epidemiology of breast cancer subtypes in two prospective cohort studies of breast cancer survivors.* Breast Cancer Res, 2009. **11**(3): p. R31.
82. Phipps, A.I., et al., *Reproductive and hormonal risk factors for postmenopausal luminal, HER-2-overexpressing, and triple-negative breast cancer.* Cancer, 2008. **113**(7): p. 1521-6.
83. Xing, P., J. Li, and F. Jin, *A case-control study of reproductive factors associated with subtypes of breast cancer in Northeast China.* Med Oncol, 2010. **27**(3): p. 926-31.
84. Lee, J.S., et al., *Reproductive factors and subtypes of breast cancer defined by estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2: a register-based study from Korea.* Clin Breast Cancer, 2014. **14**(6): p. 426-34.
85. Chen, L., et al., *Reproductive Factors and Risk of Luminal, HER2-Overexpressing, and Triple-Negative Breast Cancer Among Multiethnic Women.* Cancer Epidemiol Biomarkers Prev, 2016. **25**(9): p. 1297-304.
86. Butt, S., et al., *Parity and age at first childbirth in relation to the risk of different breast cancer subgroups.* Int J Cancer, 2009. **125**(8): p. 1926-34.
87. Albrektsen, G., I. Heuch, and S.O. Thoresen, *Histological type and grade of breast cancer tumors by parity, age at birth, and time since birth: a register-based study in Norway.* BMC Cancer, 2010. **10**: p. 226.
88. Somasegar, S., L. Li, and C.L. Thompson, *No association of reproductive risk factors with breast cancer tumor grade.* Eur J Cancer Prev, 2016.
89. Iwasaki, M., et al., *Role and impact of menstrual and reproductive factors on breast cancer risk in Japan.* Eur J Cancer Prev, 2007. **16**(2): p. 116-23.
90. Lord, S.J., et al., *Breast cancer risk and hormone receptor status in older women by parity, age of first birth, and breastfeeding: a case-control study.* Cancer Epidemiol Biomarkers Prev, 2008. **17**(7): p. 1723-30.

91. Li, C.I., et al., *Reproductive factors and risk of estrogen receptor positive, triple-negative, and HER2-neu overexpressing breast cancer among women 20-44 years of age.* Breast Cancer Res Treat, 2013. **137**(2): p. 579-87.
92. Ambrosone, C.B., et al., *Important Role of Menarche in Development of Estrogen Receptor-Negative Breast Cancer in African American Women.* J Natl Cancer Inst, 2015. **107**(9).
93. Ritte, R., et al., *Height, age at menarche and risk of hormone receptor-positive and -negative breast cancer: a cohort study.* Int J Cancer, 2013. **132**(11): p. 2619-29.
94. Orgeas, C.C., et al., *The influence of menstrual risk factors on tumor characteristics and survival in postmenopausal breast cancer.* Breast Cancer Res, 2008. **10**(6): p. R107.
95. Islami, F., et al., *Breastfeeding and breast cancer risk by receptor status--a systematic review and meta-analysis.* Ann Oncol, 2015. **26**(12): p. 2398-407.
96. Gaudet, M.M., et al., *Risk factors by molecular subtypes of breast cancer across a population-based study of women 56 years or younger.* Breast Cancer Res Treat, 2011. **130**(2): p. 587-97.
97. Butt, S., et al., *Breastfeeding in relation to risk of different breast cancer characteristics.* BMC Res Notes, 2014. **7**: p. 216.
98. Bao, P.P., et al., *Association of hormone-related characteristics and breast cancer risk by estrogen receptor/progesterone receptor status in the shanghai breast cancer study.* Am J Epidemiol, 2011. **174**(6): p. 661-71.
99. Rosenberg, L., et al., *Oral contraceptive use and estrogen/progesterone receptor-negative breast cancer among African American women.* Cancer Epidemiol Biomarkers Prev, 2010. **19**(8): p. 2073-9.
100. Beaber, E.F., et al., *Recent oral contraceptive use by formulation and breast cancer risk among women 20 to 49 years of age.* Cancer Res, 2014. **74**(15): p. 4078-89.
101. Beaber, E.F., et al., *Oral contraceptives and breast cancer risk overall and by molecular subtype among young women.* Cancer Epidemiol Biomarkers Prev, 2014. **23**(5): p. 755-64.
102. Veneroso, C., R. Siegel, and P.H. Levine, *Early age at first childbirth associated with advanced tumor grade in breast cancer.* Cancer Detect Prev, 2008. **32**(3): p. 215-23.
103. Li, C.I., et al., *Trends in incidence rates of invasive lobular and ductal breast carcinoma.* Jama, 2003. **289**(11): p. 1421-4.
104. Slanger, T.E., et al., *Menopausal hormone therapy and risk of clinical breast cancer subtypes.* Cancer Epidemiol Biomarkers Prev, 2009. **18**(4): p. 1188-96.
105. Rosenberg, L.U., et al., *Menopausal hormone therapy in relation to breast cancer characteristics and prognosis: a cohort study.* Breast Cancer Res, 2008. **10**(5): p. R78.
106. Cui, Y., et al., *Interactions of hormone replacement therapy, body weight, and bilateral oophorectomy in breast cancer risk.* Clin Cancer Res, 2014. **20**(5): p. 1169-78.
107. Collins, L.C., et al., *Pathologic features and molecular phenotype by patient age in a large cohort of young women with breast cancer.* Breast Cancer Res Treat, 2012. **131**(3): p. 1061-6.
108. Bauer, K.R., et al., *Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype: a population-based study from the California cancer Registry.* Cancer, 2007. **109**(9): p. 1721-8.

109. Stead, L.A., et al., *Triple-negative breast cancers are increased in black women regardless of age or body mass index.* Breast Cancer Res, 2009. **11**(2): p. R18.
110. Ambrosone, C.B., et al., *Associations between estrogen receptor-negative breast cancer and timing of reproductive events differ between African American and European American women.* Cancer Epidemiol Biomarkers Prev, 2014. **23**(6): p. 1115-20.
111. Chen, L., et al., *Body mass index and risk of luminal, HER2-overexpressing, and triple negative breast cancer.* Breast Cancer Res Treat, 2016. **157**(3): p. 545-54.
112. Borgquist, S., et al., *Anthropometric factors in relation to different tumor biological subgroups of postmenopausal breast cancer.* Int J Cancer, 2009. **124**(2): p. 402-11.
113. Canchola, A.J., et al., *Body size and the risk of postmenopausal breast cancer subtypes in the California Teachers Study cohort.* Cancer Causes Control, 2012.
114. Vona-Davis, L., et al., *Triple-negative breast cancer and obesity in a rural Appalachian population.* Cancer Epidemiol Biomarkers Prev, 2008. **17**(12): p. 3319-24.
115. Maiti, B., et al., *The association of metabolic syndrome with triple-negative breast cancer.* Breast Cancer Res Treat, 2009.
116. Rosenberg, L.U., et al., *Menopausal hormone therapy and other breast cancer risk factors in relation to the risk of different histological subtypes of breast cancer: a case-control study.* Breast Cancer Res, 2006. **8**(1): p. R11.
117. Daling, J.R., et al., *Relation of body mass index to tumor markers and survival among young women with invasive ductal breast carcinoma.* Cancer, 2001. **92**(4): p. 720-9.
118. Brinton, L.A., et al., *Anthropometric and hormonal risk factors for male breast cancer: male breast cancer pooling project results.* J Natl Cancer Inst, 2014. **106**(3): p. djt465.
119. Kabat, G.C., et al., *Smoking and alcohol consumption in relation to risk of triple-negative breast cancer in a cohort of postmenopausal women.* Cancer Causes Control, 2011. **22**(5): p. 775-83.
120. Manjer, J., et al., *Smoking associated with hormone receptor negative breast cancer.* Int J Cancer, 2001. **91**(4): p. 580-4.
121. Suzuki, R., et al., *Alcohol and postmenopausal breast cancer risk defined by estrogen and progesterone receptor status: a prospective cohort study.* J Natl Cancer Inst, 2005. **97**(21): p. 1601-8.
122. Suzuki, R., et al., *Alcohol intake and risk of breast cancer defined by estrogen and progesterone receptor status--a meta-analysis of epidemiological studies.* Int J Cancer, 2008. **122**(8): p. 1832-41.
123. Lew, J.Q., et al., *Alcohol and risk of breast cancer by histologic type and hormone receptor status in postmenopausal women: the NIH-AARP Diet and Health Study.* Am J Epidemiol, 2009. **170**(3): p. 308-17.
124. Li, C.I., et al., *The relationship between alcohol use and risk of breast cancer by histology and hormone receptor status among women 65-79 years of age.* Cancer Epidemiol Biomarkers Prev, 2003. **12**(10): p. 1061-6.
125. Chatterjee, N., *A two-stage regression model for epidemiologic studies with multivariate disease classification data.* J American Statistical Association, 2004. **99**(465): p. 127-138.
126. Miao, J., et al., *Analysis of Multivariate Disease Classification Data in the Presence of Partially Missing Disease Traits.* J Biom Biostat, 2014. **5**.
127. Sherman, M.E., et al., *Variation in breast cancer hormone receptor and HER2 levels by etiologic factors: a population-based analysis.* Int J Cancer, 2007. **121**(5): p. 1079-85.

128. Berndt, S.I., et al., *Transforming growth factor beta 1 (TGFB1) gene polymorphisms and risk of advanced colorectal adenoma.* Carcinogenesis, 2007. **28**(9): p. 1965-70.
129. Moore, L.E., et al., *GSTM1, GSTT1, and GSTP1 polymorphisms and risk of advanced colorectal adenoma.* Cancer Epidemiol Biomarkers Prev, 2005. **14**(7): p. 1823-7.
130. Hou, L., et al., *CYP1A1 Val462 and NQO1 Ser187 polymorphisms, cigarette use, and risk for colorectal adenoma.* Carcinogenesis, 2005. **26**(6): p. 1122-8.
131. Show classification by ER/PR, and molecular, and grade?
132. Allen-Brady, K., et al., *Lobular breast cancer: excess familiality observed in the Utah Population Database.* Int J Cancer, 2005. **117**(4): p. 655-61.
133. Mavaddat, N., et al., *Familial relative risks for breast cancer by pathological subtype: a population-based cohort study.* Breast Cancer Res, 2010. **12**(1): p. R10.
134. Li, C.I., A.J. Littman, and E. White, *Relationship between age maximum height is attained, age at menarche, and age at first full-term birth and breast cancer risk.* Cancer Epidemiol Biomarkers Prev, 2007. **16**(10): p. 2144-9.
135. Nagatsuma, A.K., et al., *Impact of recent parity on histopathological tumor features and breast cancer outcome in premenopausal Japanese women.* Breast Cancer Res Treat, 2013. **138**(3): p. 941-50.
136. McTiernan, A., et al., *Risk factors for estrogen receptor-rich and estrogen receptor-poor breast cancers.* J Natl Cancer Inst, 1986. **77**(4): p. 849-54.
137. Stanford, J.L., et al., *A case-control study of breast cancer stratified by estrogen receptor status.* Am J Epidemiol, 1987. **125**(2): p. 184-94.
138. Cooper, J.A., et al., *Risk factors for breast cancer by oestrogen receptor status: a population-based case-control study.* Br J Cancer, 1989. **59**(1): p. 119-25.
139. Nasca, P.C., et al., *Alcohol consumption and breast cancer: estrogen receptor status and histology.* Am J Epidemiol, 1994. **140**(11): p. 980-8.
140. Potter, J.D., et al., *Progesterone and estrogen receptors and mammary neoplasia in the Iowa Women's Health Study: how many kinds of breast cancer are there?* Cancer Epidemiol Biomarkers Prev, 1995. **4**(4): p. 319-26.
141. Yoo, K.Y., et al., *Breast cancer risk factors according to combined estrogen and progesterone receptor status: a case-control analysis.* Am J Epidemiol, 1997. **146**(4): p. 307-14.
142. Morabia, A., et al., *Relation of smoking to breast cancer by estrogen receptor status.* Int J Cancer, 1998. **75**(3): p. 339-42.
143. Wohlfahrt, J., et al., *Reproductive risk factors for breast cancer by receptor status, histology, laterality and location.* Int J Cancer, 1999. **81**(1): p. 49-55.
144. Enger, S.M., et al., *Body size, physical activity, and breast cancer hormone receptor status: results from two case-control studies.* Cancer Epidemiol Biomarkers Prev, 2000. **9**(7): p. 681-7.
145. Huang, W.Y., et al., *Hormone-related factors and risk of breast cancer in relation to estrogen receptor and progesterone receptor status.* Am J Epidemiol, 2000. **151**(7): p. 703-14.
146. Britton, J.A., et al., *Risk of breast cancer classified by joint estrogen receptor and progesterone receptor status among women 20-44 years of age.* Am J Epidemiol, 2002. **156**(6): p. 507-16.

147. Li, C.I., K.E. Malone, and J.R. Daling, *Differences in breast cancer hormone receptor status and histology by race and ethnicity among women 50 years of age and older.* Cancer Epidemiol Biomarkers Prev, 2002. **11**(7): p. 601-7.
148. Chen, W.Y., et al., *Association of hormone replacement therapy to estrogen and progesterone receptor status in invasive breast carcinoma.* Cancer, 2004. **101**(7): p. 1490-500.
149. Chu, K.C., et al., *Frequency distributions of breast cancer characteristics classified by estrogen receptor and progesterone receptor status for eight racial/ethnic groups.* Cancer, 2001. **92**(1): p. 37-45.
150. Clarke, C.A., et al., *Age-specific incidence of breast cancer subtypes: understanding the black-white crossover.* J Natl Cancer Inst, 2012. **104**(14): p. 1094-101.
151. Redondo, C.M., et al., *Breast feeding, parity and breast cancer subtypes in a spanish cohort.* PLoS One, 2012. **7**(7): p. e40543.
152. Iodice, S., et al., *ABO blood group and cancer.* Eur J Cancer, 2010. **46**(18): p. 3345-50.
153. Milne, R.L., et al., *Oral contraceptive use and risk of early-onset breast cancer in carriers and noncarriers of BRCA1 and BRCA2 mutations.* Cancer Epidemiol Biomarkers Prev, 2005. **14**(2): p. 350-6.
154. Iodice, S., et al., *Oral contraceptive use and breast or ovarian cancer risk in BRCA1/2 carriers: a meta-analysis.* Eur J Cancer, 2010. **46**(12): p. 2275-84.
155. John, E.M., et al., *The Breast Cancer Family Registry: an infrastructure for cooperative multinational, interdisciplinary and translational studies of the genetic epidemiology of breast cancer.* Breast Cancer Res, 2004. **6**(4): p. R375-89.
156. John, E.M., et al., *Medical radiation exposure and breast cancer risk: findings from the Breast Cancer Family Registry.* Int J Cancer, 2007. **121**(2): p. 386-94.
157. Fornetti, J., et al., *Mammary gland involution as an immunotherapeutic target for postpartum breast cancer.* J Mammary Gland Biol Neoplasia, 2014. **19**(2): p. 213-28.
158. Callihan, E.B., et al., *Postpartum diagnosis demonstrates a high risk for metastasis and merits an expanded definition of pregnancy-associated breast cancer.* Breast Cancer Res Treat, 2013. **138**(2): p. 549-59.
159. Lyons, T.R., et al., *Postpartum mammary gland involution drives progression of ductal carcinoma in situ through collagen and COX-2.* Nat Med, 2011. **17**(9): p. 1109-15.
160. Anderson, W.F., I. Jatoi, and S.S. Devesa, *Distinct breast cancer incidence and prognostic patterns in the NCI's SEER program: suggesting a possible link between etiology and outcome.* Breast Cancer Res Treat, 2005. **90**(2): p. 127-37.
161. Bernards, R. and R.A. Weinberg, *A progression puzzle.* Nature, 2002. **418**(6900): p. 823.
162. *Breast cancer and breastfeeding: collaborative reanalysis of individual data from 47 epidemiological studies in 30 countries, including 50302 women with breast cancer and 96973 women without the disease.* Lancet, 2002. **360**(9328): p. 187-95.
163. Lipworth, L., L.R. Bailey, and D. Trichopoulos, *History of breast-feeding in relation to breast cancer risk: a review of the epidemiologic literature.* J Natl Cancer Inst, 2000. **92**(4): p. 302-12.
164. Jernstrom, H., et al., *Breast-feeding and the risk of breast cancer in BRCA1 and BRCA2 mutation carriers.* J Natl Cancer Inst, 2004. **96**(14): p. 1094-8.
165. Kahlenborn, C., et al., *Oral contraceptive use as a risk factor for premenopausal breast cancer: a meta-analysis.* Mayo Clin Proc, 2006. **81**(10): p. 1290-302.

166. Grabrick, D.M., et al., *Risk of breast cancer with oral contraceptive use in women with a family history of breast cancer.* Jama, 2000. **284**(14): p. 1791-8.
167. Haile, R.W., et al., *BRCA1 and BRCA2 mutation carriers, oral contraceptive use, and breast cancer before age 50.* Cancer Epidemiol Biomarkers Prev, 2006. **15**(10): p. 1863-70.
168. Jeng, M.H., C.J. Parker, and V.C. Jordan, *Estrogenic potential of progestins in oral contraceptives to stimulate human breast cancer cell proliferation.* Cancer Res, 1992. **52**(23): p. 6539-46.
169. Jordan, V.C., et al., *The estrogenic activity of synthetic progestins used in oral contraceptives.* Cancer, 1993. **71**(4 Suppl): p. 1501-5.
170. Yue, W., et al., *Effects of estrogen on breast cancer development: Role of estrogen receptor independent mechanisms.* Int J Cancer, 2010. **127**(8): p. 1748-57.
171. Tryggvadottir, L., H. Tulinius, and G.B. Gudmundsdottir, *Oral contraceptive use at a young age and the risk of breast cancer: an Icelandic, population-based cohort study of the effect of birth year.* Br J Cancer, 1997. **75**(1): p. 139-43.
172. Dall, G., G. Risbridger, and K. Britt, *Mammary stem cells and parity-induced breast cancer protection- new insights.* J Steroid Biochem Mol Biol, 2017. **170**: p. 54-60.
173. Brinton, L.A., et al., *Oral contraceptives and breast cancer risk among younger women.* J Natl Cancer Inst, 1995. **87**(11): p. 827-35.
174. Olsson, H., et al., *Early oral contraceptive use and premenopausal breast cancer--a review of studies performed in southern Sweden.* Cancer Detect Prev, 1991. **15**(4): p. 265-71.
175. Milne, R.L., et al., *The potential value of sibling controls compared with population controls for association studies of lifestyle-related risk factors: an example from the Breast Cancer Family Registry.* Int J Epidemiol, 2011.
176. Ma, H., et al., *Breast cancer receptor status: do results from a centralized pathology laboratory agree with SEER registry reports?* Cancer Epidemiol Biomarkers Prev, 2009. **18**(8): p. 2214-20.
177. Work, M.E., et al., *Reproductive risk factors and oestrogen/progesterone receptor-negative breast cancer in the Breast Cancer Family Registry.* Br J Cancer, 2014. **110**(5): p. 1367-77.
178. Jordan, V.C., *Growth factor regulation by tamoxifen is demonstrated in patients with breast cancer.* Cancer, 1993. **72**(1): p. 1-2.
179. Ruiz, R., et al., *Epidemiology and pathophysiology of pregnancy-associated breast cancer: A review.* Breast, 2017. **35**: p. 136-141.
180. Collins, L.C., et al., *Molecular Phenotype of Breast Cancer According to Time Since Last Pregnancy in a Large Cohort of Young Women.* Oncologist, 2015. **20**(7): p. 713-8.
181. Johansson, A.L., et al., *Family history and risk of pregnancy-associated breast cancer (PABC).* Breast Cancer Res Treat, 2015. **151**(1): p. 209-17.
182. Pan, H., et al., *Reproductive factors and breast cancer risk among BRCA1 or BRCA2 mutation carriers: results from ten studies.* Cancer Epidemiol, 2014. **38**(1): p. 1-8.
183. Andrieu, N., et al., *Pregnancies, breast-feeding, and breast cancer risk in the International BRCA1/2 Carrier Cohort Study (IBCCS).* J Natl Cancer Inst, 2006. **98**(8): p. 535-44.
184. Cullinane, C.A., et al., *Effect of pregnancy as a risk factor for breast cancer in BRCA1/BRCA2 mutation carriers.* Int J Cancer, 2005. **117**(6): p. 988-91.

185. Jernstrom, H., et al., *Pregnancy and risk of early breast cancer in carriers of BRCA1 and BRCA2.* Lancet, 1999. **354**(9193): p. 1846-50.
186. Lee, E., et al., *Effect of reproductive factors and oral contraceptives on breast cancer risk in BRCA1/2 mutation carriers and noncarriers: results from a population-based study.* Cancer Epidemiol Biomarkers Prev, 2008. **17**(11): p. 3170-8.
187. Brohet, R.M., et al., *Oral contraceptives and breast cancer risk in the international BRCA1/2 carrier cohort study: a report from EMBRACE, GENEPSO, GEO-HEBON, and the IBCCS Collaborating Group.* J Clin Oncol, 2007. **25**(25): p. 3831-6.
188. Narod, S.A., et al., *Oral contraceptives and the risk of breast cancer in BRCA1 and BRCA2 mutation carriers.* J Natl Cancer Inst, 2002. **94**(23): p. 1773-9.
189. Arpino, G., et al., *Tumor characteristics and prognosis in familial breast cancer.* BMC Cancer, 2016. **16**(1): p. 924.
190. Hines, L.M., et al., *Differences in estrogen receptor subtype according to family history of breast cancer among Hispanic, but not non-Hispanic White women.* Cancer Epidemiol Biomarkers Prev, 2008. **17**(10): p. 2700-6.
191. McNamara, K.M., et al., *Androgen receptor in triple negative breast cancer.* J Steroid Biochem Mol Biol, 2013. **133**: p. 66-76.
192. Silvera, S.A., A.B. Miller, and T.E. Rohan, *Oral contraceptive use and risk of breast cancer among women with a family history of breast cancer: a prospective cohort study.* Cancer Causes Control, 2005. **16**(9): p. 1059-63.
193. Howell, A., et al., *Risk determination and prevention of breast cancer.* Breast Cancer Res, 2014. **16**(5): p. 446.
194. Ellingjord-Dale, M., et al., *Alcohol, Physical Activity, Smoking, and Breast Cancer Subtypes in a Large, Nested Case-Control Study from the Norwegian Breast Cancer Screening Program.* Cancer Epidemiol Biomarkers Prev, 2017. **26**(12): p. 1736-1744.
195. McGuire, V., et al., *No increased risk of breast cancer associated with alcohol consumption among carriers of BRCA1 and BRCA2 mutations ages <50 years.* Cancer Epidemiol Biomarkers Prev, 2006. **15**(8): p. 1565-7.

APPENDIX 1: Supplementary Tables and Additional Details of Methodology

A. Description of Pseudo-Conditional Likelihood Approach:

Most previous research regarding risk factor and breast cancer tumor characteristics has been via case-control design, and the examination of the association between the risk factor(s) of interest and the outcome of breast cancer performed using polytomous logistic regression, where the outcome of breast cancer is divided into several sub-outcomes defined, for example, by joint ER and PR status. Etiologic heterogeneity is measured as the difference in the regression parameters across subtype. The number of regression parameters will be large due to several disease characteristics, each with multiple levels [126]. However, with each additional tumor characteristic of interest (such as HER2 status and grade), conducting analysis for each subtype defined by each characteristic results in loss of statistical power and may be clinically irrelevant. For example, an analysis of ER status, PR status, grade, and nodal score using polytomous logistic regression yields 14 categorical subtypes (See figure *A1-1*). As a result, one might choose to focus on one or two tumor characteristics; however this method ignores the fact that these characteristics are correlated, and that a risk factor's association with a tumor characteristic may in fact be due to that characteristic's correlation with a different tumor characteristic.

An alternative analytic technique exists that allows for examination of the association of multiple tumor characteristics by specific risk factors. This technique is known as the pseudo-conditional likelihood method, and it is an offshoot of polytomous logistic regression [125]. This type of regression allows for the adjustment of correlated tumor characteristics, when examining a risk factor's association with a cancer outcome more specifically defined by the presence or absence of a tumor marker. Using this method, allows for adjustment of the "dependent" variable side of

the equation for correlated tumor characteristics, such that multiple tumor characteristics represent the outcome variables, and multiple exposures represent the explanatory variables.

$$\left.\begin{array}{r}\text{Set of tumor characteristics (T):} \\ \text{ER status, PR status, Grade, Nodes, etc}\end{array}\right\} = \beta_1 x_1 + \beta_2 x_2 + \dot{\varepsilon}$$

The method allows for the determination of which risk factor associated with several correlated tumor characteristics is “most important”. A limitation of the method is that it can only be conducted as a case/case analysis, there is no intercept in the model, and therefore no estimate of baseline risk. Additionally, the tumor characteristics must be defined in a binary fashion (e.g. high grade vs. low grade, rather than as grade 1, 2, 3).

Recently, an additional publication has describe a means for performing the pseudo-conditional likelihood method, in datasets with missing traits, further improving the applicability of the method [126].

***Figure A1-1*: Example of subtype sample in a categorization of ER Status, PR status, Grade, and Nodal status (Using population-based cases from the Breast Cancer Family Registry)**

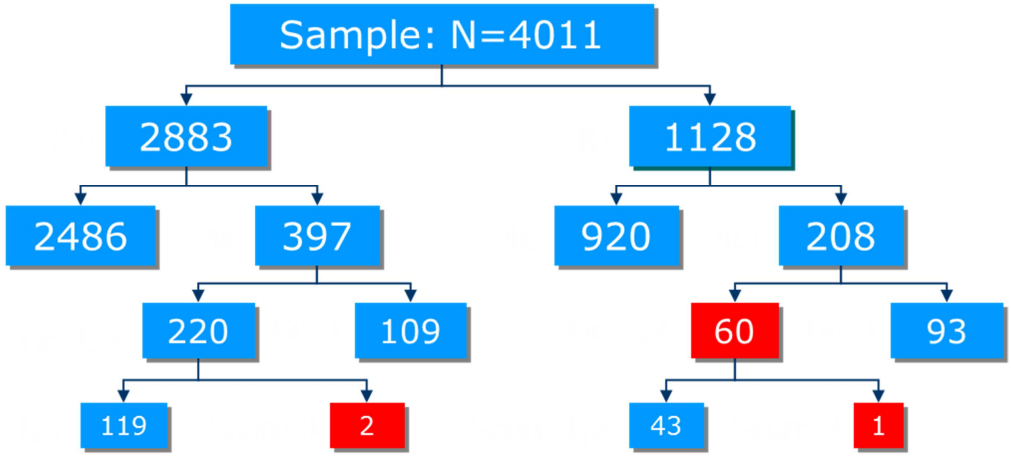


Table A1-1a: Ascertainment criteria for women in the 3 population-based BCFR sites identified from 1996-2005

Geographic Area	Greater San Francisco, CA, USA	Toronto, ON, Canada	Melbourne and Sydney, Australia
Recruitment Criteria: Cases	• Age 18-34: all • Age 35-64: at least one marker of high or familial cancer risk, including: o Bilateral breast cancer dx'd before age 50 o Previous dx of ovarian or childhood cancer o 1+ first degree relative with breast, ovarian or childhood cancer • Age 35-64: 2.5% of Whites, 15% of minorities not meeting high risk criteria	• Age 18-35: all • Age 36-54: at least one marker of high or familial cancer risk, including: o Previous diagnosis of breast or ovarian cancer o 1+ first or 2+ 2nd – degree relatives with breast or ovarian cancer o Multiple primary breast cancers o Breast and ovarian cancer • Age 55-69: 35% of those meeting high risk criteria; 8.75% of those not meeting high risk criteria	• Age 18-39: all • Age 40-49: 50% random sample • Age 50-59: 25% random sample
Recruitment Criteria: Controls	Random digit dialing, no personal history of breast or ovarian cancer; in same catchment area as cases	Random digit dialing, no personal history of breast or ovarian cancer, in same catchment area as cases	Electoral rolls, no personal history of breast or ovarian cancer, in same catchment area as cases

Table A1-1b: Ascertainment criteria for women in clinic-based BCFR families identified at 3 sites from 1996-2000

Geographic Area	New York, NY, USA	Philadelphia, PA, USA	Utah, USA
Recruitment Criteria for Families	• Breast or ovarian cancer diagnosed at age <45 • Breast and ovarian cancer (diagnosed at any age) • Multiple first or 2nd degree relatives with breast or ovarian cancer • BRCA1 or BRCA2 mutation carrier	• Breast or ovarian cancer diagnosed at age <35 • Breast and ovarian cancer (diagnosed at any age) • Bilateral breast cancer and 1st diagnosis at age <50 • 2 individuals in 2 generations with breast or ovarian cancer • BRCA1 or BRCA2 mutation carrier	• Breast or ovarian cancer diagnosed at age <45 • Breast and ovarian cancer (diagnosed at any age) • Multiple 1st or 2nd degree relatives with breast or ovarian cancer • BRCA1 or BRCA2 mutation carrier
Relationship to Proband	First- second- and third-degree relative	First- and –second degree relative	Any relative

Both Figures Adapted from John, E. et al [155]. "The Breast Cancer Family Registry: an infrastructure for cooperative multinational, interdisciplinary and translational studies of the genetic epidemiology of breast cancer" Breast Cancer Research and Treatment, 2004

Figure A1-2a. Cases in the population-based sites of the BCFR, included in analysis

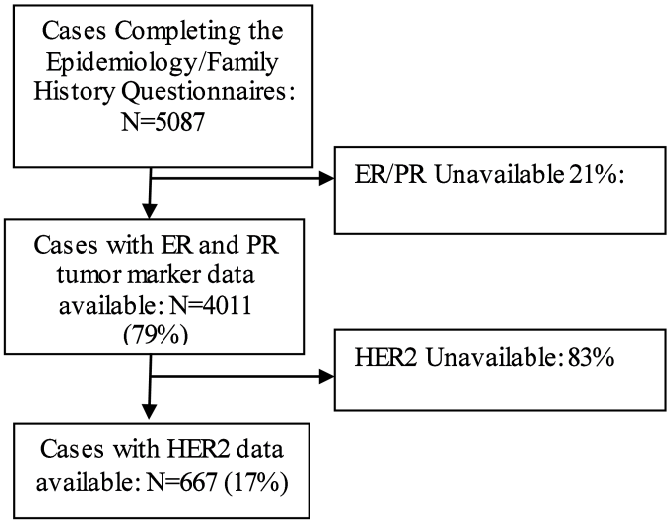


Figure A1-2b. Cases in the clinic-based sites of the BCFR, with ER/PR data available

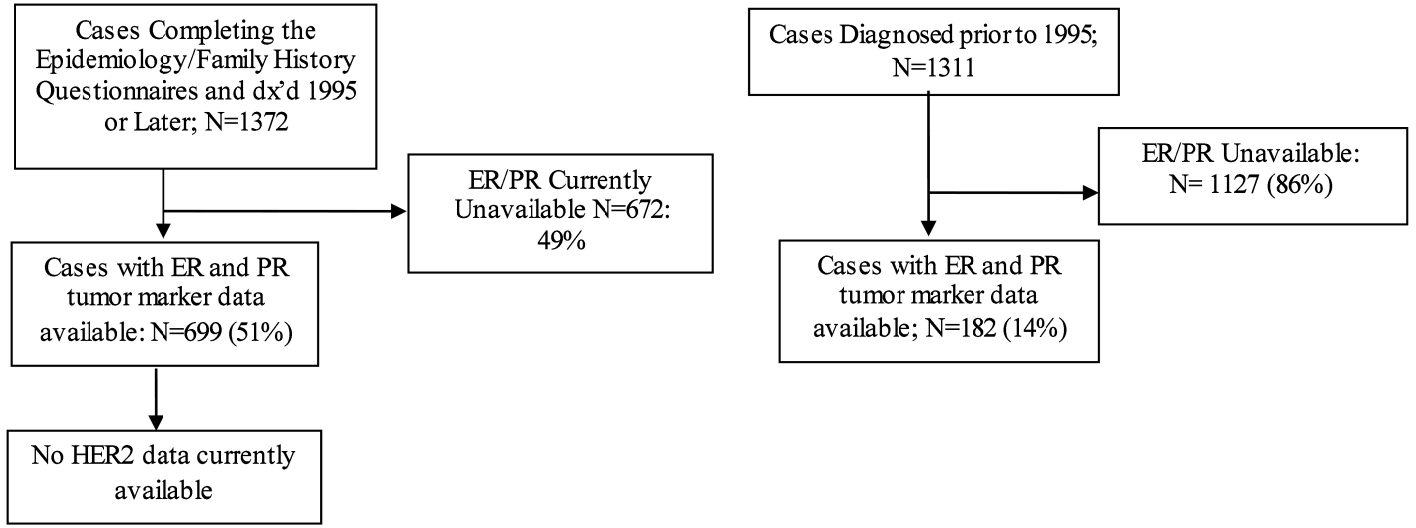


Table A1-2: Demographic and Tumor Characteristics by ER status, PR status, and grade, Population-Based Sites of the BCFR (N=3364)

	ER+ `N=2429 N (%)	ER- N=935 N (%)	p-value	PR+ N=2253 N (%)	PR- N=1111 N (%)	p-value	Grade 1,2 N=1980 N (%)	Grade 3 N=1384 N (%)	p-value
Age (μ± s.d.)	47.9±9.5	44.9±9.6	**<0.0001**	47.5±9.4	46.3±10.1	**=0.0006**	48.8±9.2	44.5±9.7	**<0.0001**
Race									
White	1415 (58)	525 (56)	**=0.0003**	1352 (60)	588 (53)	**<0.0001**	1160 (59)	780 (57)	**=0.0004**
Black	241 (10)	139 (15)		220 (10)	160 (14)		184 (9)	196 (14)	
Hispanic	254 (10)	104 (11)		215 (10)	143 (13)		220 (11)	138 (10)	
Asian	478 (20)	148 (16)		426 (19)	200 (18)		383 (19)	243 (18)	
Other	33 (1)	14 (2)		32 (1)	15 (1)		27 (1)	20 (1)	
Site									
Ontario	733 (30)	251 (27)	**=0.002**	688 (31)	296 (27)	**=0.022**	604 (31)	380 (27)	**<0.0001**
Australia	362 (15)	184 (20)		373 (17)	173 (16)		271 (14)	275 (20)	
California	1334 (55)	500 (53)		1192 (53)	642 (58)		1105 (56)	729 (53)	
Menopausal Status									
Pre	1347 (58)	593 (66)	**<0.0001**	1313 (62)	627 (60)	=0.23	1105 (55)	917 (69)	**<0.0001**
Post	979 (42)	305 (34)		843 (38)	441 (40)		868 (45)	416 (31)	
Education									
< High school	685 (29)	273 (29)	=0.41	628 (29)	330 (30)	=0.17	557 (29)	401 (29)	=0.40
⩾ High school	1706 (71)	634 (71)		1592 (71)	748 (70)		1398 (71)	942 (71)	
First degree family history									
No	1693 (70)	679 (73)	=0.09	1563 (70)	809 (73)	=0.052	1355 (69)	1017 (74)	**=0.0011**
Yes	725 (30)	251 (27)		677 (30)	299 (27)		617 (31)	359 (26)	
OC Use									
Never	673 (28)	205 (23)	**=0.0011**	604 (27)	274 (26)	=0.069	577 (28)	301 (21)	**<0.0001**
⩽ 5 years	903 (38)	335 (37)		855 (39)	383 (36)		744 (39)	494 (38)	
> 5 years	804 (34)	356 (40)		754 (34)	406 (38)		632 (33)	528 (41)	
OC Use 1975									
Never Used OCs	673 (28)	205 (23)	**<0.0001**	604 (27)	274 (26)	**=0.002**	577 (30)	301 (23)	**<0.0001**
Used OC before 1975	1098 (46)	381 (43)		1031 (47)	448 (42)		955 (49)	524 (40)	
Used OC 1975 or later	609 (26)	310 (35)		578 (26)	341 (32)		421 (22)	498 (38)	
HRT Use									
Never	1708 (74)	721 (81)	**<0.0001**	1617 (75)	812 (78)	=0.24	1351 (72)	1078 (82)	**<0.0001**
Former	199 (9)	56 (6)		181 (8)	74 (6)		172 (9)	83 (6)	
Current	414 (18)	108 (12)		360 (16)	162 (16)		366 (19)	156 (12)	
Age at menarche									
⩽ 11	505 (21)	184 (21)	=0.55	483 (22)	206 (19)	=0.10	400 (21)	289 (22)	=0.91

12	582 (24)	215 (24)		533 (24)	264 (25)		483 (25)	314 (24)	
⩾ 13	1291 (55)	494 (55)		1188 (54)	597 (56)		1063 (55)	722 (54)	
Parity									
3+ Live Births	805 (33)	326 (35)	=0.49	757 (34)	374 (34)	=0.66	668 (34)	463 (33)	=0.24
1,2 Live Births	1064 (44)	399 (43)		979 (43)	484 (44)		876 (44)	587 (42)	
Nulliparous	560 (23)	210 (22)		517 (23)	253 (23)		436 (22)	334 (24)	
Age at first birth	25.1	24.6	**=0.046**	25.0	24.7	=0.16	25.1	24.7	=0.096
Breastfeeding duration									
Never	1123 (47)	470 (51)	=0.058	1035 (46)	558 (51)	**=0.022**	928 (47)	665 (49)	=0.53
<12 mos.	758 (32)	262 (29)		696 (31)	324 (30)		607 (31)	413 (30)	
⩾ 12 mos.	527 (22)	185 (20)		502 (22)	210 (19)		432 (22)	280 (21)	
Parity and Breastfeeding									
Nulliparous	560 (25)	210 (24)	**=0.033**	517(24)	253(24)	**=0.025**	436(23)	334(26)	0.604
1,2 live births no BF	364 (16)	162 (19)		338(16)	188(18)		317(17)	209(16)	
3+ live births no BF	199 (9)	98 (11)		180(9)	117(11)		175(9)	122(9)	
1,2 live births, some BF	605(27)	199 (23)		554(26)	250(24)		485(26)	319(25)	
3+ lives births, some BF	555 (24)	204 (23)		529(25)	230(22)		443(23)	311(24)	
Time since last pregnancy									
Nulliparous	428 (18)	154 (16)	**<0.0001**	396 (18)	186 (17)	=0.056	324 (16)	258 (19)	**<0.0001**
⩽10 years	557 (23)	286 (31)		540 (24)	303 (27)		412 (21)	431 (31)	
>10 yrs, ⩽ 20 yrs	571(25)	201 (21)		538 (24)	234 (21)		459 (23)	313 (23)	
> 20 years	873 (39)	294 (31)		779 (35)	388 (35)		785 (40)	382 (28)	
Smoking									
Never Smoker	1450 (57)	566 (58)	=0.32	1348 (60)	668 (61)	=0.78	1181 (60)	835 (61)	=0.39
Former Smoker	588 (26)	204 (24)		539 (24)	253 (23)		483 (25)	309 (23)	
Current Smoker	375 (17)	155 (18)		352 (16)	178 (16)		305 (15)	225 (16)	
Alcohol Use									
Never	1407 (59)	545 (60)	=0.28	1281 (58)	671 (62)	=0.07	1156 (59)	796 (59)	=0.93
<7 drinks/week	598 (25)	242 (25)		573 (26)	267 (25)		492 (25)	348 (26)	

⩾7 drinks/week	386 (16)	128 (14)		363 (16)	151 (14)		301 (15)	213 (16)	
BMI	26.2	26.3	=0.45	26.1	26.5	=0.056	26.1	26.4	=0.19
BRCA1 status									
missing	723 (30)	264 (28)	**<0.0001**	677 (30)	310 (28)	**<0.0001**	576 (29)	411 (30)	**<0.0001**
positive	18 (1)	67 (7)		18 (1)	67 (6)		11 (1)	74 (5)	
negative	1688 (69)	604 (65)		1558 (69)	734 (66)		1393 (70)	899 (65)	
BRCA2 status									
missing	913 (38)	323 (35)	=0.23	844 (37)	392 (35)	=0.44	737 (37)	499 (36)	**=0.006**
positive	54 (2)	19 (2)		47 (2)	26 (2)		25 (1)	48 (3)	
negative	1462 (60)	593 (63)		1362 (60)	693 (62)		1218 (62)	837 (60)	

Table A1-3: Frequency Table of Risk factors by birth cohort, BCFR Population-based Sites

	Year of birth							
	1926-1939		1940-1949		1950-1959		1960-1981	
	Cases (N=628)	Controls (N=450)	Cases (N=1446)	Controls (N=951)	Cases (N=1205)	Controls (N=958)	Cases (N=732)	Controls (N=638)
	N (%)		N (%)		N (%)		N (%)	
Age (μ± s.d.)	60.5±2.9	63.6±3.2	51.8±3.6	53.8±3.0	42.8±3.7	44.9±3.1	32.9±3.7	33.6±4.3
Study Site								
Australia	85 (14)	96 (21)	302 (20)	222 (23)	336 (28)	191 (20)	206 (28)	159 (25)
Ontario	193 (31)	274 (61)	443 (31)	518 (55)	315 (26)	586 (61)	137 (19)	328 (51)
California	350 (56)	80 (18)	701 (48)	211 (22)	554 (46)	181 (19)	389 (53)	151 (24)
First degree family history								
No	364 (58)	400 (89)	980 (68)	851 (89)	905 (75)	879 (92)	637 (87)	602 (95)
Yes	263 (42)	50 (11)	462 (32)	100 (11)	294 (25)	78 (8)	92 (13)	35 (5)
OC Use								
Never	270 (45)	210 (47)	335 (24)	214 (23)	273 (23)	139 (15)	141 (20)	83(13)
≤ 5 years	201 (33)	139 (31)	594 (42)	382 (40)	440 (37)	384 (40)	241 (34)	212 (26)
> 5 years	133 (22)	99 (22)	487 (34)	352 (37)	467 (40)	430 (45)	329 (46)	335 (53)
mean length OC use (yrs) among users	5.78	6.07	6.39	6.30	7.29	7.02	7.24	7.29
Year of first OC use								
Never	270 (45)	210 (47)	335 (24)	214 (23)	273 (23)	139 (15)	141 (20)	83 (13)
Before 1975	312 (52)	231(52)	1008 (71)	697 (73)	478 (41)	505 (53)	1 (0)	2 (0)
1975 or later	22 (4)	7 (2)	73 (5)	37 (4)	429 (36)	309 (32)	569 (80)	545 (87)
Age (yrs) at first OC use (mean)	28.9±5.3	29.4±5.4	22.8±4.2	22.5±3.7	20.8±4.6	20.2±4.3	19.8±4.3	19.2±3.8
% of OC users starting after first live birth	266 (74%)	198 (83%)	500 (45%)	432 (41%)	315 (34%)	(230) 28%	254 (43%)	195 (35%)
Age at menarche								
≤ 11	111 (18)	71 (16)	303 (21)	205 (22)	271 (23)	192 (20)	133 (19)	130 (21)
12	145 (24)	91 (20)	314 (22)	216 (23)	290 (25)	245 (26)	200 (28)	159 (25)
≥ 13	352 (58)	284 (64)	795 (56)	519 (55)	615 (52)	509 (54)	376 (53)	341(54)
Parity								
Parous	535 (85)	408 (91)	1185 (82)	837 (88)	935 (78)	796 (83)	454 (62)	425 (67)
Nulliparous	93 (15)	42 (9)	261 (18)	114 (12)	270 (22)	162 (17)	278 (38)	213 (33)
Age at first live birth	23.6	24.0	24.5	24.2	26.1	25.4	25.6	25.8
Breastfeeding (among parous)								
Never	193 (36)	116 (28)	425 (36)	285 (34)	227 (24)	198 (25)	80 (18)	68 (16)
Ever	337 (64)	292 (72)	754 (64)	552 (66)	704 (76)	598 (75)	370 (82)	357 (84)
% with 3+ Live births, no BF	33%	25%	29%	27%	19%	19%	12%	10%
Median dur. BF (mos)	8.5	11.5	9.0	9.0	11.5	11.5	8.5	9.5
Avg. Parity (among parous)	3.2	3.4	2.8	2.9	2.7	2.6	2.4	2.4

Smoking								
Never Smoker	347 (56)	220 (49)	833 (58)	511 (54)	723 (60)	461 (48)	476 (65)	350 (55)
Former Smoker	194 (31)	165 (37)	369 (26)	290 (30)	277 (23)	318 (33)	117 (16)	146 (23)
Current Smoker	80 (13)	63 (14)	232 (16)	150 (16)	196 (16)	178 (19)	137 (19)	142 (22)
Alcohol Use								
Nondrinkers	357 (58)	223 (51)	796 (56)	505 (53)	697 (59)	474 (50)	445 (61)	336 (53)
<7 drinks/week	143 (23)	118 (27)	374 (26)	262 (28)	304 (26)	302 (32)	181 (25)	208 (33)
≥ drinks/week	113 (18)	98 (22)	257 (18)	179 (19)	182 (15)	171 (18)	99 (14)	93 (15)
Menopausal status								
Premenopausal	47 (7)	3(1)	562(41)	216 (14)	1010 (90)	738 (89)	707 (98)	609 (98)
Postmenopausal	581 (93)	447 (99)	803 (59)	710 (56)	112 (10)	95 (11)	17 (2)	10 (2)
ER/PR status								
ER+ and/or PR+	518 (82)		1161 (80)		833 (69)		499 (68)	
ER-PR-	110 (18)		285 (20)		292 (31)		233 (32)	
Tumor Grade								
1, 2	382 (71)		796 (66)		534 (54)		268 (42)	
3	156 (29)		408 (34)		452 (46)		368 (58)	
BRCA1 status								
Status missing	195		520		414		135	
BRCA1 positive	4 (1)		19 (2)		31 (4)		41 (7)	
BRCA1 negative	429 (99)		907 (98)		760 (96)		556 (93)	

Table A1-4: Association of selected risk factors by birth cohort, population-based sites of the BCFR

A1-4a: Oral Contraceptive Use

	1926-1939*					1940-1949**				
Hormone Status	Controls N=448	HR+ N=498		ER-PR- N=106		Controls N=948	HR+ N=1140		ER-PR- N=276	
Never Use	210	223	1.00	47	1.00	214	276	1.00	59	1.00
Ever Use	238	275	0.75 (0.53-1.06)	59	0.78 (0.47-1.32)	734	864	1.15 (0.89-1.49)	217	**1.53 (1.04-2.24)**
Never Use	210	223	1.00	47	1.00	214	276	1.00	59	1.00
First Use pre 1975	231	254	0.80 (0.54-1.15)	58	0.78 (0.46-1.35)	697	805	1.15 (0.89-1.50)	203	**1.55 (1.04-2.29)**
First Use 1975/later	7	21	NA	1	NA	37	59	1.09 (0.63-1.90)	14	1.31 (0.59-2.90)

	1950-1959**					1960-1981*				
Hormone Status	Controls N=953	HR+ N=904		ER-PR- N=276		Controls N=630	HR+ N=491		ER-PR- N=220	
Never Use	139	220	1.00	53	1.00	83	102	1.00	39	1.00
Ever Use	814	684	**0.66 (0.48-0.91)**	223	0.90 (0.58-1.38)	547	389	0.90 (0.62-1.31)	181	1.21(0.75-1.96)
Never Use	139	220	1.00	53	1.00	83	102	1.00	39	1.00
First Use pre 1975	505	369	1.01 (0.71-1.43)	109	1.27 (0.78-2.07)	2	1	NA	0	NA
First Use 1975/later	309	315	**0.40 (0.28-0.58)**	114	**0.58 (0.36-0.94)**	545	388	All use post 1975	181	All use post 1975

**adjusted for age, site, race, parity, age at first birth, breastfeeding, BMI, age at menarche, family history*

***adjusted for age, site, race, parity, age at first birth, breastfeeding, BMI, age at menarche, family history, menopausal status*

A1-4b: Parity

	1926-1939*					1940-1949**				
Hormone Status	Controls N=450	HR+ N=518		ER-PR- N=110		Controls N=951	HR+ N=1161		ER-PR- N=285	
Nulliparous	42	81	1.00	12	1.00	114	226	1.00	35	1.00
1-2 Live Births	148	180	**0.54 (0.29-0.99)**	33	1.10 (0.44-2.76)	433	494	**0.65 (0.45-0.92**	132	1.22 (0.72-2.06)
3+ Live Births	260	257	0.66 (0.35-1.23)	65	1.82 (0.73-4.49)	404	441	0.77 (0.52-1.13)	118	1.58 (0.89-2.81)

	1950-1959**					1960-1981*				
Hormone Status	Controls N=958	HR+ N=913		ER-PR- N=292		Controls N=638	HR+ N=499		ER-PR- N=233	
Nulliparous	162	214	1.00	56	1.00	213	190	1.00	88	1.00
1-2 Live Births	471	387	1.00 (0.68-1.48)	126	1.59 (0.94-2.70)	282	191	1.13 (0.68-1.86)	100	1.68 (0.93-3.03)
3+ Live Births	325	312	1.03 (0.66-1.61)	110	1.66 (0.92-3.00)	143	118	1.54 (0.86-2.78)	45	1.96 (0.96-4.00)

**adjusted for age, site, race, OC use, age at first birth, breastfeeding, BMI, age at menarche, family history*

***adjusted for age, site, r ace, OC use, age at first birth, breastfeeding, BMI, age at menarche, family history menopausal status*

***A1-4c*: Breastfeeding**

	1926-1939*					1940-1949**				
Hormone Status	Controls	HR+		ER-PR-		Controls	HR+		ER-PR-	
Never Brfed	159	229	1.00	63	1.00	401	546	1.00	145	1.00
Brfed <12 mos.	156	172	1.13 (0.76-1.69)	31	**0.53 (0.30-0.94)**	315	339	0.96 (0.63-1.27)	82	0.72 (0.49-1.06)
Brfed ≥12 mos.	135	104	0.82 (0.52-1.28)	14	**0.24 (0.12-0.50)**	235	268	0.77 (0.56-1.07)	54	**0.50 (0.31-0.80)**

	1950-1959**					1960-1981*				
Hormone Status	Controls N=958	HR+ N=910		ER-PR- N=285		Controls N=638	HR+ N=495		ER-PR- N=228	
Never Brfed	362	379	1.00	119	1.00	281	240	1.00	121	1.00
Brfed <12 mos.	314	264	0.84 (0.59-1.20)	86	0.72 (0.46-1.13)	206	153	0.95 (0.57-1.52)	68	0.63 (0.36-1.11)
Brfed ≥12 mos.	282	267	0.79 (0.54-1.16)	80	0.65 (0.39-1.06)	151	102	0.64 (0.38-1.10)	39	**0.41 (0.21-0.78)**

**adjusted for age, site, race, parity, age at first birth, OC use, BMI, age at menarche, family history*
***adjusted for age, site, race, parity, age at first birth, OC use, BMI, age at menarche, family history, menopausal status*

Table A1-5: Association among oral contraceptive use, parity and breastfeeding and breast cancer classified by molecular status, Breast Cancer Family Registry (compared with Luminal A cases, N=470)

	Luminal B N=119	**HER2+ N=67**	**Triple-negative N=142**
	OR (95%CI)	**OR (95%CI)**	**OR (95%CI)**
OC use			
Never	1.0 (ref)	1.0 (ref)	1.0 (ref)
≤ 5 years	0.85 (0.50-1.46)	1.34 (0.66-2.71)	1.24 (0.72-2.12)
> 5 years	0.84 (0.49-1.44)	1.61 (0.81-3.20)	**1.73(1.03-2.90)**
Timing of first OC			
Never	1.0 (ref)	1.0 (ref)	1.0 (ref)
Before 1975	0.94 (0.54-1.63)	1.11 (0.54-2.28)	1.15 (0.67-1.95)
1975 or later	0.77 (0.43-1.39)	1.69 (0.78-3.68)	**2.17 (1.10-3.94)**
Parity (number of live births)			
Nulliparous	1.0 (ref)	1.0 (ref)	1.0 (ref)
1-2	1.42 (0.72-2.83)	**3.48 (1.30-9.48)**	**2.08 (1.08-4.01)**
≥3	1.29 (0.59-2.80)	2.85 (0.97-8.42)	**2.72 (1.33-5.55)**
Breastfeeding duration (months)			
Never	1.0 (ref)	1.0 (ref)	1.0 (ref)
<12	0.78 (0.44-1.41)	0.70 (0.35-1.39)	**0.48 (0.28-0.81)**
≥12	1.10 (0.57-2.09)	0.76 (0.35-1.68)	0.56 (0.31-1.01)
Breastfeeding and parity			
Nulliparous	1.0 (ref)	1.0 (ref)	1.0 (ref)
1-2 live births, never BF	1.38 (0.66-2.89)	**3.37 (1.21-9.40)**	**2.44 (1.24-4.82)**
≥3 live births, never BF	1.28 (0.42-3.16)	3.01 (0.92-9.86)	2.10 (0.93-4.76)
1-2 live births, some BF	1.25 (0.67-2.33)	**2.61 (1.03-6.61)**	0.93 (0.48-1.79)
≥3 lives births, some BF	1.23 (0.58-2.58)	2.10 (0.93-4.76)	1.80 (0.89-3.62)

Note: Odds ratios (OR) and 95% confidence interval (CI), adjusted for age, race, study site, and menopausal status. OC findings adjusted for breastfeeding and parity; parity findings adjusted for OC use and breastfeeding; breastfeeding findings adjusted for OC use and parity, combined parity-breastfeeding findings adjusted for OC use.

***Bold** indicates* $p < 0.05$

Table A1-6: Demographic and Tumor Characteristics by ER status, PR status, and grade, Clinic-Based Sites of the BCFR, for (N=515)

	ER+ N=326 N (%)	**ER- N=189 N (%)**	**p-value**	**PR+ N=307 N (%)**	**PR- N=208 N (%)**	**p-value**	**Grade 1,2 N=243 N (%)**	**Grade 3 N=272 N (%)**	**p-value**
Age (μ± s.d)	50.5±12.4	44.0±10.3	**<0.0001**	49.5±12.1	46.1±11.9	**=0.002**	51.0±12.9	45.6±10.7	**<0.0001**
Race									
White	216 (75)	116 (67)	=0.30	205 (75)	127 (68)	=0.39	170 (77)	162 (67)	**=0.005**
Black	5 (2)	6 (3)		6 (2)	5 (3)		2 (1)	9 (4)	
Hispanic	51 (18)	36 (21)		45 (16)	42 (22)		30 (14)	57 (24)	
Asian	7 (2)	7 (3)		7 (3)	6 (3)		6 (3)	7 (3)	
Other	10 (3)	8 (5)		11 (4)	7 (4)		12 (5)	6 (3)	
Site									
Philadelphia	NA	NA	=0.082	NA	NA	=0.13	NA	NA	=0.82
New York	256 (79)	135 (72)		242 (79)	152 (73)		187 (77)	207 (756	
Utah	70 (21)	54 (28)		65 (21)	56 (27)		56 (23)	65 (24)	
Menopausal Status									
Pre	116 (36)	90 (48)	**=0.007**	114 (37)	92 (44)	=0.11	88 (36)	118 (44)	=0.10
Post	210 (64)	99 (52)		193 (63)	116 (56)		155 (64)	154 (56)	
Education									
High school	79 (26)	42 (22)	**=0.005**	72 (25)	49 (24)	**=0.012**	49 (21)	72 (28)	=0.216
> High school	224 (74)	146 (78)		217 (75)	154 (76)		184 (79)	186 (72)	
First degree family history									
None	120 (41)	97 (56)	**=0.025**	116 (42)	101 (54)	=0.081	89 (40)	128 (53)	**=0.025**
1 FDR	116 (40)	56 (32)		109 (40)	63 (34)		92 (41)	80 (33)	
2+ FDR	54 (19)	20 (12)		50 (18)	24 (12)		39 (19)	35 (14)	
OC Use									
Never	126 (49)	42 (28)	**=0.002**	117 (48)	51 (31)	**=0.002**	96 (50)	72 (33)	**=0.002**
≤ 5 years	81 (31)	62 (42)		78 (32)	65 (40)		59 (31)	84 (39)	
> 5 years	51 (20)	45 (30)		48 (20)	48 (29)		36 (19)	60 (28)	
OC Use 1975									
Never Used OCs	126 (44)	42 (24)	**<0.0001**	117 (43)	51 (27)	**<0.001**	96 (44)	72 (30)	**=0.0016**
Used OC before 1975	96 (33)	64 (37)		95 (35)	65 (35)		75 (34)	85 (35)	
Used OC 1975 or later	66 (23)	67 (39)		61 (22)	72 (38)		48 (22)	85 (35)	
HRT Use									
Never	210 (74)	134 (79)	**=0.0009**	201 (74)	143 (78)	**=0.0052**	144 (67)	200 (83)	**=0.0001**
Former	72 (25)	26 (15)		67 (25)	31 (17)		65 (30)	33 (14)	
Current	3 (1)	10 (6)		3 (1)	10 (5)		6 (3)	7 (3)	
Age at menarche									
≤11	55 (19)	27 (14)	=0.400	56 (20)	26 (14)	=0.215	47 (21)	35 (13)	=0.280
12	90 (31)	59 (31)		84 (30)	65 (34)		63 (29)	86 (32)	
≥13	146 (50)	107 (55)		136 (50)	99 (52)		111 (50)	151 (55)	
Parity									
Nulliparous	49 (16)	38 (20)	=0.332	53 (18)	34 (17)	=0.556	42 (18)	45 (17)	=0.626

1,2 Live Births	140 (46)	90 (48)		129 (45)	101 (50)		104 (45)	126 (49)	
3+ Live Births	114 (38)	60 (32)		106 (37)	68 (34)		87 (37)	87 (34)	
Age at first birth	25.4±5.5	25.1±5.0	**=0.63**	25.1±5.2	24.9±5.1	=0.22	25.1±4.7	25.5±5.6	=0.43
Breastfeeding duration									
Never	125 (44)	84 (49)	=0.069	122 (45)	87 (46)	=0.166	90 (41)	119 (49)	=0.171
<12 mos.	95 (33)	64 (37)		88 (32)	71 (38)		78 (36)	81 (33)	
≥12 mos.	67 (23)	25 (14)		62 (23)	30 (16)		50 (23)	42 (17)	
Parity and Breastfeeding									
Nulliparous	49 (21)	38 (27)	=0.224	53 (23)	34 (22)	=0.588	42 (23)	45 (23)	=0.200
1,2 live births no BF	45 (19)	33 (23)		41 (18)	37 (24)		30 (17)	48 (24)	
3+ live births no BF	35 (15)	16 (11)		31 (14)	20 (13)		21 (12)	30 (15)	
1,2 live births, some BF	32 (13)	11 (8)		29 (13)	14 (9)		24 (13)	19 (10)	
3+ live births, some BF	77 (32)	44 (31)		73 (32)	48 (31)		64 (35)	57 (29)	
Time since last live birth									
Nulliparous	49 (17)	37 (23)	=0.054	53 (19)	33 (19)	=0.923	42 (19)	44 (19)	**=0.003**
≤5 years	29 (10)	22 (14)		28 (10)	23 (13)		17 (8)	34 (15)	
>5 yrs, ≤10 yrs	30 (11)	24 (15)		34 (12)	20 (11)		20 (9)	34 (14)	
>10 yrs, ≤20 yrs	74 (26)	41 (25)		70 (26)	45 (26)		51 (24)	64 (27)	
>20 yrs	104 (36)	39 (24)		88 (32)	55 (31)		86 (40)	57 (24)	
Smoking									
Never Smoker	167 (57)	110 (62)	=0.512	159(57)	118 (61)	=0.678	129 (58)	148 (60)	=0.922
Former Smoker	109 (37)	60 (34)		104 (38)	65 (34)		82 (37)	87 (35)	
Current Smoker	16 (5)	7 (4)		14 (5)	9 (5)		11 (5)	12 (5)	
Alcohol Use									
Non-drinker	162(56)	105 (60)	=0.495	155 (56)	112 (59)	=0.586	126 (57)	141(58)	=0.428
Former drinker	53(18)	34 (19)		50 (18)	37 (19)		37 (17)	50 (20)	
Current drinker	75 (26)	37 (21)		71 (26)	41 (22)		58 (26)	54 (22)	
BMI	25.6±5.2	25.9±5.2	=0.496	25.5±5.3	25.9±5.1	=0.482	25.0±4.5	26.3 ±5.8	**=0.007**
BRCA1 status									
missing	69 (21)	32 (17)	**<0.0001**	62 (20)	39 (19)	**<0.0001**	52 (21)	49 (18)	**<0.0001**
positive	11 (3)	44 (23)		12 (4)	43 (21)		7 (3)	48 (18)	
negative	246 (75)	111 (60)		233 (76)	126 (61)		184 (76)	175 (64)	
BRCA2 status									
missing	66 (20)	40 (21)	=0.682	59 (19)	47 (23)	=0.593	46 (19)	60 (22)	=0.076
positive	23 (7)	17 (9)		23 (7)	17 (8)		13 (5)	27 (10)	
negative	237 (73)	132 (70)		225 (73)	144 (69)		184 (76)	185 (68)	

Table A1-7: Sensitivity Analysis: Parity and ER/PR status

	ER+PR+ǂ	**ER-PR-ǂ**
	OR (95%CI)	**OR (95%CI)**
Cases with Age at Interview ≤ 2 years after Diagnosis, vs. Controls		
Parity, LOGISTIC,	**N=210**	**N=117**
Nulliparity	1.0 (ref)	1.0 (ref)
1-2 live births	1.04 (0.65-1.67)	**2.02 (1.11-3.66)**
≥3 live births	0.99 (0.58-1.67)	1.78 (0.89-3.56)
Cases with Age at Interview ≤ 5 years after Diagnosis, vs. Controls		
Parity, LOGISTIC	**N=304**	**N=167**
Nulliparity	1.0 (ref)	1.0 (ref)
1-2 live births	1.31 (0.87-1.97)	**2.20 (1.32-3.69)**
≥3 live births	1.15 (0.73-1.82)	**2.22 (1.23-4.00)**
Cases with Age at Interview at any Date after Diagnosis (full case sample)		
Parity, LOGISTIC	**N=377**	**N=219**
Nulliparous	1.0 (ref)	1.0 (ref)
1-2 live births	**1.63 (1.13-2.36)**	**1.90 (1.20-3.00)**
≥3 live births	1.35 (0.90-2.19)	**1.85 (1.09-3.13)**

Cases vs. Controls, BRCA1+ and BRCA2+ omitted		
Parity, LOGISTIC	**N=334**	**N=146**
Nulliparous	1.0 (ref)	1.0 (ref)
1-2 live births	**1.54 (1.05-2.27)**	**2.18 (1.27-3.75)**
≥3 live births	1.32 (0.85-2.04)	**1.93 (1.03-3.61)**

**Adjusted for age, center, parity, oral contraceptive use, race, breastfeeding, menopausal status, family history*

FULL PAPER

BJC

British Journal of Cancer (2014) 110, 1367–1377 | doi: 10.1038/bjc.2013.80

Keywords: breast cancer; epidemiology; breastfeeding; oral contraceptives

Reproductive risk factors and oestrogen/progesterone receptor-negative breast cancer in the Breast Cancer Family Registry

M E Work[1], E M John[2,3], I L Andrulis[4], J A Knight[4,5], Y Liao[1], A M Mulligan[6,7], M C Southey[8], G G Giles[9,10], G S Dite[10], C Apicella[10], H Hibshoosh[11], J L Hopper[10,11] and M B Terry*[,1,12]

[1]Department of Epidemiology, Mailman School of Public Health, Columbia University, 722 West 168th Street, New York, NY 10032, USA; [2]Cancer Prevention Institute of California, Fremont, CA 94538, USA; [3]Division of Epidemiology, Department of Health Research and Policy, and Stanford Cancer Institute, Stanford University School of Medicine, Stanford, CA 94305-5101, USA; [4]Department of Molecular Genetics, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, University of Toronto, Toronto, Ontario, Canada M5G 1X5; [5]Division of Epidemiology, Dalla Lana School of Public Health, University of Toronto, Toronto, Ontario, Canada M5T 3M7; [6]Laboratory Medicine Program, University Health Network, Toronto, Ontario, Canada M5G 1X5; [7]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada M5G 1X5; [8]Department of Pathology, The University of Melbourne, Melbourne, VIC 3010, Australia; [9]Cancer Epidemiology Centre, Cancer Council Victoria, Carlton, VIC 3053, Australia; [10]Centre for Molecular, Environmental, Genetic and Analytic Epidemiology, University of Melbourne, Melbourne, VIC 3010, Australia; [11]Department of Epidemiology and Institute of Health and Environment, School of Public Health, Seoul National University, 110-799 Seoul, Korea and [12]Herbert Irving Comprehensive Cancer Center, Columbia University, New York, NY 10032, USA

Background: Oestrogen receptor (ER)- and progesterone receptor (PR)-negative (ER − PR −) breast cancer is associated with poorer prognosis compared with other breast cancer subtypes. High parity has been associated with an increased risk of ER − PR − cancer, but emerging evidence suggests that breastfeeding may reduce this risk. Whether this potential breastfeeding benefit extends to women at high risk of breast cancer remains critical to understand for prevention.

Methods: Using population-based ascertained cases ($n = 4011$) and controls (2997) from the Breast Cancer Family Registry, we examined reproductive risk factors in relation to ER and PR status.

Results: High parity (⩾3 live births) without breastfeeding was positively associated only with ER − PR − tumours (odds ratio (OR) = 1.57, 95% confidence interval (CI), 1.10–2.24); there was no association with parity in women who breastfed (OR = 0.93, 95% CI 0.71–1.22). Across all race/ethnicities, associations for ER − PR − cancer were higher among women who did not breastfeed than among women who did. Oral contraceptive (OC) use before 1975 was associated with an increased risk of ER − PR − cancer only (OR = 1.32, 95% CI 1.04–1.67). For women who began OC use in 1975 or later there was no increased risk.

Conclusions: Our findings support that there are modifiable factors for ER − PR − breast cancer and that breastfeeding in particular may mitigate the increased risk of ER − PR − cancers seen from multiparity.

The extensive epidemiologic literature supports that risk factors vary by subtypes of breast cancer defined by oestrogen receptor (ER) and progesterone receptor (PR) expression (Mctiernan *et al*, 1986; Stanford *et al*, 1987; Potter *et al*, 1995; Yoo *et al*, 1997; Britton *et al*, 2002; Mccredie *et al*, 2003; Althuis *et al*, 2004; Colditz *et al*, 2004; Largent *et al*, 2005; Rusiecki *et al*, 2005;

*Correspondence: Dr MB Terry; E-mail: mt146@columbia.edu

Received 1 August 2013; revised 3 December 2013; accepted 4 December 2013; published online 18 February 2014

Ursin *et al*, 2005; Ma *et al*, 2006a; Rosenberg *et al*, 2006; Lord *et al*, 2008; Kwan *et al*, 2009; Setiawan *et al*, 2009; Yang *et al*, 2011) and that many established breast cancer risk factors are more strongly associated with hormone receptor-positive (ER+ and/or PR+) cancers; for example, high parity, earlier age at first birth, and later age at menarche have been associated with reduced risk of ER+ and/or PR+ cancers (Althuis *et al*, 2004; Nichols *et al*, 2005; Ursin *et al*, 2005; Ma *et al*, 2006a, b, 2010a; Lord *et al*, 2008; Setiawan *et al*, 2009; Bao *et al*, 2011; Palmer *et al*, 2011; Yang *et al*, 2011), and postmenopausal hormone therapy use has been associated with an increased risk of ER+ and/or PR+ cancer (Althuis *et al*, 2004; Rosenberg *et al*, 2008; Setiawan *et al*, 2009; Slanger *et al*, 2009; Bao *et al*, 2011). In contrast, ER- and PR-negative breast cancer (ER−PR−), which is associated with a higher tumour grade and poorer prognosis, and is more prevalent in women of African-American race, and in younger age groups (Britton *et al*, 2002; Carey *et al*, 2006; Bauer *et al*, 2007; Brinton *et al*, 2008; Stead *et al*, 2009; Clarke *et al*, 2012), is not associated with reproductive and hormonal risk factors in the same way as hormone receptor-positive cancers. For example, age at first birth appears to be unrelated to ER−PR− cancer, and high parity has been associated with increased, rather than decreased risk (Rusiecki *et al*, 2005; Ma *et al*, 2006a, b; Rosenberg *et al*, 2006; Millikan *et al*, 2007; Kwan *et al*, 2009; Setiawan *et al*, 2009; Bao *et al*, 2011; Palmer *et al*, 2011; Yang *et al*, 2011).

Breastfeeding is one of the few factors found by a majority of studies to be consistently associated with a reduction in both hormone receptor-positive and -negative breast cancer (Althuis *et al*, 2004; Ma *et al*, 2006a, b, 2010a; Lord *et al*, 2008; Bao *et al*, 2011). For ER−PR− or triple-negative (ER−PR− human epidermal growth factor receptor 2 (HER2−)) cancer, in particular, breastfeeding may mitigate the increased risk of ER−PR− cancer associated with multiparity (Millikan *et al*, 2007; Kwan *et al*, 2009; Palmer *et al*, 2011; Redondo *et al*, 2012). Whether this risk reduction in ER−PR− cancer extends to women at high risk of breast cancer remains critical for prevention, as there are few prevention options available to these women apart from risk-reducing surgeries and chemoprevention; options that are particularly difficult to implement during childbearing age.

Given the consistent protective association between breastfeeding and ER−PR− and triple-negative cancers in populations unselected for family history of breast cancer, we evaluated associations between reproductive and hormonal risk factors and risk of breast cancer categorised by joint ER/PR status, using population-based data from the Breast Cancer Family Registry (BCFR). In particular, we focused on the associations with parity and breastfeeding and, more importantly, evaluated whether the reduction in risk from breastfeeding in the presence of multiparity extended to higher risk women. We also focused on evaluating oral contraceptive (OC) use, which has previously been associated with an increased risk of ER−PR− cancer (Althuis *et al*, 2004; Rosenberg *et al*, 2010).

MATERIALS AND METHODS

Study sample. We included population-based ascertained breast cancer cases and controls from three sites of the BCFR: Northern California, USA; Ontario, Canada; and Melbourne and Sydney, Australia. The details of the BCFR have been published elsewhere (John *et al*, 2004; Knight *et al*, 2006; Milne *et al*, 2011; Work *et al*, 2012). Briefly, cases included women aged 18–69 years diagnosed with a first primary invasive breast cancer from 1995 to 2004, with the sample enriched for women at increased genetic and/or familial risk of breast cancer, based on age at breast cancer diagnosis and family history of breast and other cancers (John *et al*, 2004). Questionnaire data were obtained for 76%, 72%, and 75% of eligible cases from Northern California, Ontario, and Australia, respectively. Controls were randomly sampled from the population living in the same catchment area as the cases and frequency matched according to 5-year age groupings. Of the eligible controls, 67%, 64%, and 74% participated from Northern California, Ontario, and Australia, respectively, for a total of 5107 cases and 2997 unrelated controls. The ER/PR information was available for 4011 (79%) cases, including 1994 from Northern California, 1088 from Ontario, and 929 from Australia. We also had data available on HER2 status for a subgroup of these women from Northern California and Ontario ($N=792$).

Risk factor data collection. We collected epidemiologic data through structured questionnaire interviews (conducted either in-person or by telephone) assessing breast cancer risk factors before diagnosis, including OC use, menopausal hormone therapy use, age at menarche, parity, age at first childbirth, breastfeeding history, smoking history, alcohol use, education, body mass index (BMI), and menopausal status.

Tumour marker data collection. For 2351 cases, BCFR study pathologists ascertained ER and PR status from patient tumour tissue using immunohistochemistry (IHC) and/or pathology reports using a standardised protocol and pathology reporting forms. For the remaining cases ($N=1660$), ER and PR status was provided by the relevant Cancer Registry for that population, or through patient medical records. For all cases with HER2 status available ($N=792$), the information on HER2 status was provided by the California Cancer Registry ($N=639$), or patient medical records ($N=153$). The distribution of risk factors did not differ between cases that did or did not have ER/PR data available for review (data not shown).

Where tumour tissue was available, BCFR study pathologists used IHC testing for ER and PR, and categorised tumours as ER or PR positive if $\geqslant 10\%$ of tumour cells stained positive. Where tissue samples were not obtained, pathologists reviewed pathology reports and recorded the ER and PR status listed on the report, or, if information existed on the percent of cells staining positive, employed the same requirements that $\geqslant 10\%$ of cells stained positive resulted in a definition of ER or PR positive.

Of the cases, 2486 were ER+PR+, 920 were ER−PR−, 397 were ER+PR−, and 208 were ER−PR+. Of the sub-population for whom HER2 data were available, 468 were classified as Luminal A (ER+ and/or PR+, HER2−), 118 as Luminal B (ER+ and/or PR+, HER2+), 67 as HER2+ (ER− and PR−, HER2+), and 139 as triple negative (ER−, PR−, and HER2−).

Statistical analysis. Using multivariable unordered polytomous regression, adjusted for age, race/ethnicity, and study site, we compared known or suspected breast cancer risk factors, including OC use (never, $\leqslant 5$ years, >5 years), starting date of OC use (never, any use before 1975, all use in 1975 or later; the year 1975 was chosen as a cutpoint because oestrogen and progesterone doses in OC brands had a marked change in formulation in 1975); time since last OC use (never, $\leqslant 10$ years, >10–$\leqslant 20$ years, >20 years); age at menarche ($\leqslant 11$, 12, $\geqslant 13$ years); parity (nulliparous, 1–2 live births, $\geqslant 3$ live births); age at first birth (continuous); lifetime breastfeeding duration (never, 0–<12 months, $\geqslant 12$ months); combined parity and breastfeeding (nulliparous, 1–2 children never breastfed, 1–2 children ever breastfed, $\geqslant 3$ children never breastfed, $\geqslant 3$ children ever breastfed); smoking history (never smoker, former smoker, current smoker), BMI (continuous), education (< high school, completed high school), alcohol consumption (<7 drinks per week, $\geqslant 7$ drinks per week, current non-drinker), history of $\geqslant 1$ first-degree relative with breast cancer (yes, no), and menopausal status (premenopausal or postmenopausal). Cutpoints for categorical variables were selected

based on meaningful cutpoints (e.g., education defined by high school graduation, as well as selected cutpoints used in the prior literature for replication purposes).

We compared each of the four subgroups defined by ER and PR status with the reference group of controls, for the total population as well as by site (Northern California, Ontario, Australia). Findings did not differ by site (results not shown). We also examined associations separately for premenopausal and postmenopausal women. Because some associations with ER − PR − cancer differed from associations with ER + PR + cancer when using controls as the referent group, we also conducted a case-only analysis directly comparing ER − PR − cases with ER + PR + cases. For the molecular subtypes, we conducted a case-only analysis comparing Luminal B, HER2 +, and triple-negative cases with Luminal A cases.

Because we examined multiple risk factors, we focused on patterns in risk factor associations as well as formal tests for trends. We did not formally adjust for multiple comparisons by altering the significance level but regarded associations that did not follow patterns (by increasing levels of the covariate) as more likely to be spurious.

We analysed the level of missingness for each of the variables used in the multivariable regression. Rates of missingness were very low, $<2\%$ of the sample, for most variables modelled: there was 0% missingness for parity, 1.7% missingness for OC use, and 0.7% missingness for breastfeeding. Menopausal status was missing for 12% of the participants, however, when we considered the ages and/or surgical history (i.e., bi-lateral oophorectomy) of the participants, we were able to classify menopausal status for 61% of the women missing data by assigning postmenopausal status to women over the age of 50 or those who had undergone surgical menopause, and included them in the analysis as postmenopausal. Findings did not differ when these women were excluded from the analysis (results not shown).

We considered results statistically significant if the 95% confidence interval (CI) did not include the value of '1'. All statistical analyses used SAS Version 9.2 Software (SAS Institute, Cary, NC, USA).

RESULTS

Table 1 summarises frequencies of demographic characteristics, risk factors, and tumour characteristics for breast cancer cases categorised by joint ER/PR status. The ER − cases were more likely to be younger and premenopausal compared with ER + cases, and were more likely than ER + cases to have grade 3 cancer. ER and PR status was very similar across sites (ER + PR +: 64%, 60%, and 61%, ER + PR −: 9%, 10%, and 11%; ER − PR +: 5%, 8%, and 4%; and ER − PR − 22%, 21%, and 24% for Ontario, Australia, and California, respectively). Compared with controls, cases were more likely to be non-white and to have a family history of breast cancer, partly reflecting enrollment criteria for cases that favoured racial minorities and those with family history. Cases regardless of hormone status had a higher rate of nulliparity and were less likely to breastfeed than controls, reflecting differences in known breast cancer risk factors.

Table 2 presents the multivariate-adjusted ORs for each breast cancer subtype, categorised as ER + PR +, ER + PR −, ER − PR +, or ER − PR −, compared with the control group, and also includes the findings for parity and breastfeeding from case-only analyses comparing ER − PR − cases with ER + PR + cases.

High parity ($\geqslant 3$ live births) was associated with an increased risk of ER − PR − cancer (odds ratio (OR) = 1.59, 95% CI 1.15–2.18, *vs* nulliparity). When stratified by menopausal status, high parity was associated with an increased risk in premenopausal women only (OR = 1.68, 95% CI 1.10–2.56, $\geqslant 3$ live births, *vs* nulliparity). Breastfeeding was associated with a reduced risk of all breast cancer subtypes, but most strongly with ER − PR − cancer (OR = 0.52, 95% CI 0.40–0.68, $\geqslant 12$ months of breastfeeding *vs* never), with even greater risk reduction found in postmenopausal women (OR = 0.34, 95% CI 0.21–0.54, $\geqslant 12$ months of breastfeeding *vs* never). When combined with breastfeeding behaviour, the increased risk of ER − PR − breast cancer associated with high parity was only found in women who had children but did not breastfeed (OR = 1.57, 95% CI 1.10–2.24, $\geqslant 3$ live births, no breastfeeding, *vs* nulliparity). Case-only comparisons (with ER + PR + tumours as the referent) showed an increased risk of ER − PR − tumours for parity combined with a lack of breastfeeding (OR = 1.59, 95% CI 1.19–2.13, 1 − 2 live births, no breastfeeding and OR = 1.69, 95% CI 1.20–2.38, $\geqslant 3$ live births, no breastfeeding, *vs* nulliparity). These associations were not materially different by study site and the tests for statistical interaction by site were not significant (data not shown).

Table 3 presents the multivariate-adjusted ORs for each breast cancer subtype, compared with the control group, for OC use and OC start date, and also includes the findings on OC use for the case-only comparisons comparing ER − PR − cases with ER + PR + cases.

Oral contraceptive use was not associated with ER − PR − breast cancer (OR = 1.13, 95% CI 0.89–1.44 for use >5 years *vs* never). However, first OC use before 1975 compared with never use was positively associated with ER − PR − breast cancer (OR = 1.32, 95% CI 1.04–1.67), but not with hormone receptor-positive cancers. Use in 1975 or later was not associated with ER − PR − cancer.

Oral contraceptive use was inversely associated with ER + PR +, ER + PR −, and ER − PR + breast cancer, with OR estimates statistically significant for ER + PR + cancer (OC use >5 years *vs* none: OR = 0.83, 95% CI = 0.69–0.98). Inverse associations with hormone receptor-positive subtypes were stronger when OC use began in 1975 or later (OR = 0.59, 95% CI 0.48–0.73, ER + PR +; OR = 0.52, 95% CI, 0.36–0.76, ER + PR −, OR = 0.34, 95% CI, 0.21–0.56, ER − PR +). Findings did not differ for cancer diagnosed premenopausally or postmenopausally. There was a stronger association between OC use and ER − PR − cancer compared with ER + PR + cancer (OR = 1.35, 95% CI = 1.07–1.70, OC use >5 years *vs* none). Case–case differences also existed for OC use pre- or post-1975, with statistically significant associations for ER − PR − cancer compared with ER + PR + cancer.

Differences by race/ethnicity. African-American women (OR = 1.71, 95% CI 1.22–2.40) and Hispanic women (OR = 1.43, 95% CI 1.02–2.00) were more likely to be ER − PR − than ER + PR +, compared with non-Hispanic White women. We found that the trend for the combined parity–breastfeeding measure held across race/ethnicities, with our findings supporting higher associations for ER − PR − cancer among women who did not breastfeed than among women who did, for all races/ethnicities examined (non-Hispanic Whites, African Americans, Hispanics, and Asians) (Figure 1).

Differences by molecular subtype. Table 4 presents findings by molecular subtype. Three or more live births were associated with an increased risk of HER2 + and triple-negative breast cancer (OR = 2.88, 95% CI 0.98–8.51, for HER2 *vs* Luminal A cancer; OR = 2.82, 95% CI 1.37–5.83, for triple-negative *vs* Luminal A cancer), whereas breastfeeding was inversely associated with triple-negative cancer (OR = 0.49, 95% CI 0.29–0.82, <12 months of breastfeeding *vs* none; OR = 0.57, 95% CI 0.31–1.04, $\geqslant 12$ months of breastfeeding *vs* none). Parous women who did not breastfeed were more likely to have HER2 + (OR = 3.32, 95% CI 1.26–8.73, HER2 + *vs* Luminal A, for parous, no breastfeeding) or

Table 1. Demographic and tumour characteristics by ER/PR status, Breast Cancer Family Registry

	Controls $N=2997$ N (%)	ER+PR+ $N=2486$ N (%)	ER+PR− $N=397$ N (%)	ER−PR+ $N=208$ N (%)	ER−PR− $N=920$ N (%)
Age					
Age (mean ± s.d.)	47.6 ± 10.3	47.1 ± 9.3	48.6 ± 9.8	43.8 ± 8.0	44.5 ± 9.8
Race/Ethnicity					
Non-Hispanic White	2487 (86)	1542 (62)	222 (56)	158 (76)	506 (55)
African American	96 (3)	221 (9)	45 (11)	16 (8)	131 (14)
Hispanic	72 (2)	229 (9)	46 (11)	7 (3)	113 (12)
Asian	165 (6)	445 (18)	79 (20)	23 (11)	149 (16)
Other	82 (3)	35 (1)	5 (1)	4 (2)	14 (2)
First-degree family history of breast cancer					
No	2732 (91)	1761 (71)	291 (73)	161 (78)	673 (73)
Yes	263 (9)	714 (29)	106 (27)	45 (22)	244 (27)
Menopausal status					
Premenopausal	1566 (55)	1431 (60)	172 (46)	149 (76)	574 (65)
Postmenopausal	1262 (45)	951 (40)	205 (54)	47 (24)	310 (35)
Education					
<High school	908 (30)	710 (29)	114 (29)	56 (27)	289 (32)
High school or more	2082 (70)	1740 (71)	275 (71)	150 (73)	602 (68)
Oral contraceptive (OC) use					
Never	646 (22)	648 (27)	124 (32)	49 (24)	198 (23)
⩽5 years	1117 (37)	948 (39)	129 (34)	71 (34)	328 (37)
>5 years	1216 (41)	847 (35)	131 (34)	86 (42)	353 (40)
Year of first OC use					
Never	646 (22)	648 (27)	124 (32)	49 (24)	198 (23)
Before 1975	1435 (48)	1165 (48)	167 (43)	97 (47)	370 (42)
1975 or later	898 (30)	630 (26)	93 (24)	60 (29)	310 (35)
Time of last OC use					
Never user	646 (24)	648 (30)	124 (36)	49 (27)	198 (26)
⩾10 years ago	489 (18)	340 (15)	42 (12)	42 (23)	152 (20)
>10, ⩽20 years ago	704 (26)	613 (28)	80 (23)	52 (29)	199 (27)
>20 years ago	913 (33)	604 (27)	98 (28)	39 (21)	202 (27)
Menopausal hormone therapy use					
Never	2081 (70)	1756 (74)	264 (70)	175 (88)	699 (80)
Former	246 (8)	199 (8)	37 (10)	9 (5)	59 (7)
Current	663 (22)	424 (18)	74 (20)	16 (8)	111 (13)
Age at menarche (years)					
⩽11	598 (20)	528 (22)	64 (16)	43 (20)	183 (21)
12	711 (24)	590 (24)	100 (26)	44 (21)	215 (24)
⩾13	1670 (56)	1317 (54)	225 (58)	125 (59)	482 (55)
Parity (number of live births)					
Nulliparous	531 (18)	565 (23)	95 (24)	51 (25)	191 (21)
1–2	1334 (45)	1015 (41)	166 (42)	71 (34)	391 (42)
⩾3	1132 (38)	906 (36)	136 (34)	86 (41)	338 (37)
Mean age at first birth					
Mean age at first birth	24.8 ± 5.1	25.1 ± 5.3	25.0 ± 5.3	24.7 ± 5.0	24.6 ± 5.5
Breastfeeding duration (months)					
Never	1203 (40)	1105 (45)	194 (49)	95 (46)	448 (50)
<12	991 (33)	764 (31)	113 (29)	51 (25)	267 (30)
⩾12	803 (27)	595 (24)	86 (22)	60 (29)	187 (21)

Table 1. (Continued)

	Controls *N* = 2997 *N* (%)	ER + PR + *N* = 2486 *N* (%)	ER + PR − *N* = 397 *N* (%)	ER − PR + *N* = 208 *N* (%)	ER − PR − *N* = 920 *N* (%)
Parity and breastfeeding (BF)					
Nulliparous	531 (15)	565 (23)	95 (24)	51 (25)	191 (21)
1–2 live births, never BF	448 (15)	340 (14)	61 (16)	31 (15)	157 (17)
⩾3 live births, never BF	224 (7)	200 (8)	38 (10)	13 (6)	100 (11)
1–2 live births, ever BF	886 (30)	663 (27)	103(26)	39 (19)	201 (25)
⩾3 live births, ever BF	908 (30)	696 (28)	96 (24)	72 (35)	221 (25)
Mean BMI (kg m^{-2})					
Mean BMI (kg m^{-2})	25.9 ± 5.5	26.0 ± 5.5	26.0 ± 5.5	24.7 ± 5.1	26.6 ± 5.7
Tumour grade					
1, 2	NA	1546 (74)	220 (67)	60 (39)	154 (20)
3	NA	554 (26)	109 (33)	93 (61)	628 (80)

Abbreviations: BMI = body mass index; ER = oestrogen receptor; NA = Not Applicable; PR = progesterone receptor.

Table 2. Association between parity and breastfeeding, and breast cancer classified by hormone receptor status and menopausal status, Breast Cancer Family Registry

	ER + PR + [a] N = 2174 OR (95% CI)	ER + PR − [a] N = 341 OR (95% CI)	ER − PR + [a] N = 179 OR (95% CI)	ER − PR − [a] N = 791 OR (95% CI)	ER − PR − *vs* ER + PR + OR (95% CI)
Parity (number of live births)					
Nulliparous	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
1–2	**0.80 (0.65–0.99)**	0.93 (0.64–1.35)	1.20 (0.71–2.02)	1.33 (1.00–1.76)	**1.62 (1.24–2.13)**
⩾3	0.93 (0.73–1.17)	0.97 (0.64–1.49)	1.50 (0.85–2.65)	**1.59 (1.15–2.18)**	**1.66 (1.23–2.25)**
Breastfeeding duration (months)					
Never	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
<12	1.04 (0.87–1.23)	0.84 (0.61–1.16)	0.66 (0.41–1.05)	**0.72 (0.57–0.91)**	**0.70 (0.56–0.88)**
⩾12	**0.80 (0.66–0.98)**	**0.69 (0.48–0.99)**	**0.57 (0.35–0.94)**	**0.52 (0.40–0.68)**	**0.64 (0.50–0.84)**
Parity and breastfeeding (BF)					
Nulliparous	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
1–2 live births, never BF	0.80 (0.63–1.00)	0.92 (0.62–1.38)	1.49 (0.86–2.60)	1.30 (0.96–1.75)	**1.59 (1.19–2.13)**
⩾3 live births, never BF	0.90 (0.68–1.19)	0.95 (0.58–1.54)	1.01 (0.49–2.07)	**1.57 (1.10–2.24)**	**1.69 (1.20–2.38)**
1–2 live births, ever BF	**0.78 (0.64–0.93)**	0.73 (0.52–1.05)	0.63 (0.38–1.05)	0.88 (0.68–1.14)	1.12 (0.87–1.45)
⩾3 live births, ever BF	**0.82 (0.67–0.99)**	0.72 (0.50–1.04)	1.00 (0.64–1.56)	0.93 (0.71–1.22)	1.09 (0.84–1.42)
Premenopausal women					
Parity (number of live births)					
Nulliparous	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
1–2	0.86 (0.65–1.15)	1.14 (0.78–2.54)	1.27 (0.66–2.42)	**1.50 (1.04–2.17)**	**1.73 (1.21–2.48)**
⩾3	0.96 (0.69–1.33)	1.12 (0.57–2.21)	1.62 (0.81–3.26)	**1.68 (1.10–2.56)**	**1.70 (1.14–2.55)**
Breastfeeding duration (months)					
Never	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
<12	1.05 (0.81–1.35)	0.86 (0.51–1.46)	0.75 (0.42–1.35)	0.74 (0.54–1.02)	**0.70 (0.51–0.96)**
⩾12	0.76 (0.58–1.01)	0.88 (0.50–1.54)	0.68 (0.36–1.19)	**0.61 (0.43–0.87)**	0.80 (0.56–1.13)
Parity and breastfeeding (BF)					
Nulliparous	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
1–2 live births, never BF	0.80 (0.59–1.09)	1.43 (0.76–2.67)	1.62 (0.83–3.18)	**1.56 (1.06–2.32)**	**1.94 (1.32–2.85)**
⩾3 live births, never BF	1.05 (0.70–1.58)	1.08 (0.45–2.62)	1.04 (0.41–2.62)	1.49 (0.87–2.55)	1.35 (0.89–2.29)
1–2 live births, ever BF	0.84 (0.67–1.06)	1.23 (0.75–2.01)	0.79 (0.44–1.41)	1.03 (0.75–1.40)	1.21 (0.89–1.64)
⩾3 live births, ever BF	0.80 (0.63–1.01)	0.99 (0.58–1.68)	1.20 (0.72–2.00)	1.13 (0.81–1.56)	1.37 (0.99–1.89)

Table 2. (Continued)

	ER+PR+[a] N=2174 OR (95% CI)	ER+PR−[a] N=341 OR (95% CI)	ER−PR+[a] N=179 OR (95% CI)	ER−PR−[a] N=791 OR (95% CI)	ER−PR− *vs* ER+PR+ OR (95% CI)
Postmenopausal women					
Parity (number of live births)					
Nulliparous	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
1–2	**0.66 (0.47–0.93)**	**0.54 (0.33–0.91)**	0.62 (0.25–1.54)	0.84 (0.52–1.33)	1.26 (0.81–1.97)
⩾3	0.84 (0.58–1.21)	0.77 (0.44–1.34)	0.82 (0.30–2.28)	1.11 (0.68–1.85)	1.30 (0.80–2.11)
Breastfeeding duration (months)					
Never	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
<12	1.08 (0.83–1.39)	0.83 (0.54–1.26)	0.56 (0.24–1.30)	0.75 (0.53–1.07)	**0.70 (0.49–0.99)**
⩾12	0.91 (0.67–1.27)	**0.49 (0.29–0.83)**	0.37 (0.13–1.03)	**0.34 (0.21–0.54)**	**0.37 (0.23–0.58)**
Parity and breastfeeding (BF)					
Nulliparous	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
1–2 live births, never BF	0.70 (0.48–1.00)	**0.58 (0.34–0.98)**	0.73 (0.29–1.85)	0.80 (0.39–1.30)	1.13 (0.72–1.81)
⩾3 live births, never BF	0.75 (0.50–1.14)	0.70 (0.38–1.28)	0.61 (0.19–1.67)	1.12 (0.66–1.92)	1.46 (0.88–2.44)
1–2 live births, ever BF	**0.65 (0.46–0.91)**	**0.38 (0.22–0.65)**	**0.24 (0.08–0.72)**	**0.57 (0.35–0.93)**	0.88 (0.55–1.41)
⩾3 live births, ever BF	0.86 (0.62–1.20)	**0.51 (0.31–0.85)**	0.44 (0.17–1.10)	**0.54 (0.83–0.88)**	**0.60 (0.38–0.97)**

Abbreviations: BMI = body mass index; ER = oestrogen receptor; HT = hormone therapy; OC = oral contraceptive; PR = progesterone receptor. Odds ratios (ORs) and 95% confidence interval (CI), adjusted for age, race/ethnicity, study site, OC use, HT use, BMI, menopausal status, age at menarche, age at first birth, and education. ORs in **bold** are statistically significant.
[a]Compared with population-based controls (*N* = 2683).

Table 3. Association between oral contraceptive use and breast cancer classified by hormone receptor status and menopausal status, Breast Cancer Family Registry

	ER+PR+[a] N=2174 OR (95% CI)	ER+PR−[a] N=341 OR (95% CI)	ER−PR+[a] N=179 OR (95% CI)	ER−PR−[a] N=791 OR (95% CI)	ER−PR− *vs* ER+PR+ OR (95% CI)
OC use					
Never	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
⩽5 years	0.97 (0.82–1.15)	**0.73 (0.54–0.99)**	0.67 (0.44–1.04)	1.16 (0.92–1.47)	1.18 (0.94–1.49)
>5 years	**0.83 (0.69–0.98)**	0.74 (0.55–1.01)	0.79 (0.52–1.20)	1.13 (0.89–1.44)	**1.35 (1.07–1.70)**
Year of first OC use					
Never	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
Before 1975	1.06 (0.91–1.25)	0.80 (0.59–1.07)	1.12 (0.73–1.73)	**1.32 (1.04–1.67)**	**1.28 (1.03–1.60)**
1975 or later	**0.59 (0.48–0.73)**	**0.52 (0.36–0.76)**	**0.34 (0.21–0.56)**	0.82 (0.63–1.08)	**1.36 (1.06–1.75)**
OC use (premenopausal)					
Never	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
⩽5 years	0.97 (0.76–1.22)	0.65 (0.41–1.05)	0.62 (0.37–1.04)	1.00 (0.73–1.38)	1.05 (0.78–1.41)
>5 years	**0.75 (0.59–0.94)**	0.83 (0.52–1.31)	0.67 (0.41–1.11)	0.98 (0.72–1.33)	1.31 (0.97–1.77)
OC use (postmenopausal)					
Never	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
⩽5 years	0.89 (0.69–1.14)	0.77 (0.51–1.15)	0.58 (0.25–1.32)	1.38 (0.95–1.99)	**1.50 (1.05–2.15)**
>5 years	0.89 (0.68–1.16)	**0.63 (0.41–0.98)**	0.76 (0.35–1.67)	1.23 (0.83–1.81)	1.36 (0.96–1.98)

Abbreviations: BMI = body mass index; ER = oestrogen receptor; HT = hormone therapy; OC = oral contraceptive; PR = progesterone receptor. Odds ratios (ORs) and 95% confidence interval (CI) adjusted for age, race/ethnicity, study site, parity, breastfeeding, HT use, BMI, menopausal status, age at menarche, age at first birth, and education. ORs in **bold** are statistically significant.
[a]Compared with population-based controls (*N* = 2683); Premenopausal refers to cases diagnosed premenopausally, postmenopausal refers to cases diagnosed postmenopausally. All OC use occurred before menopause.

triple-negative cancer (OR = 2.33, 95% CI 1.22–4.45, triple negative *vs* Luminal A, for parous, no breastfeeding) compared with nulliparous women. Parous women who breastfed had no increased risk of triple-negative cancer (OR = 1.22, 95% CI 0.67–2.22, *vs* Luminal A). Oral contraceptive use of >5 years, compared with never use, was positively associated with triple-negative cancer (OR = 1.63, 95% CI 0.97–2.76), as was OC use that began in 1975 or later (OR = 2.02, 95% CI 1.11–3.68).

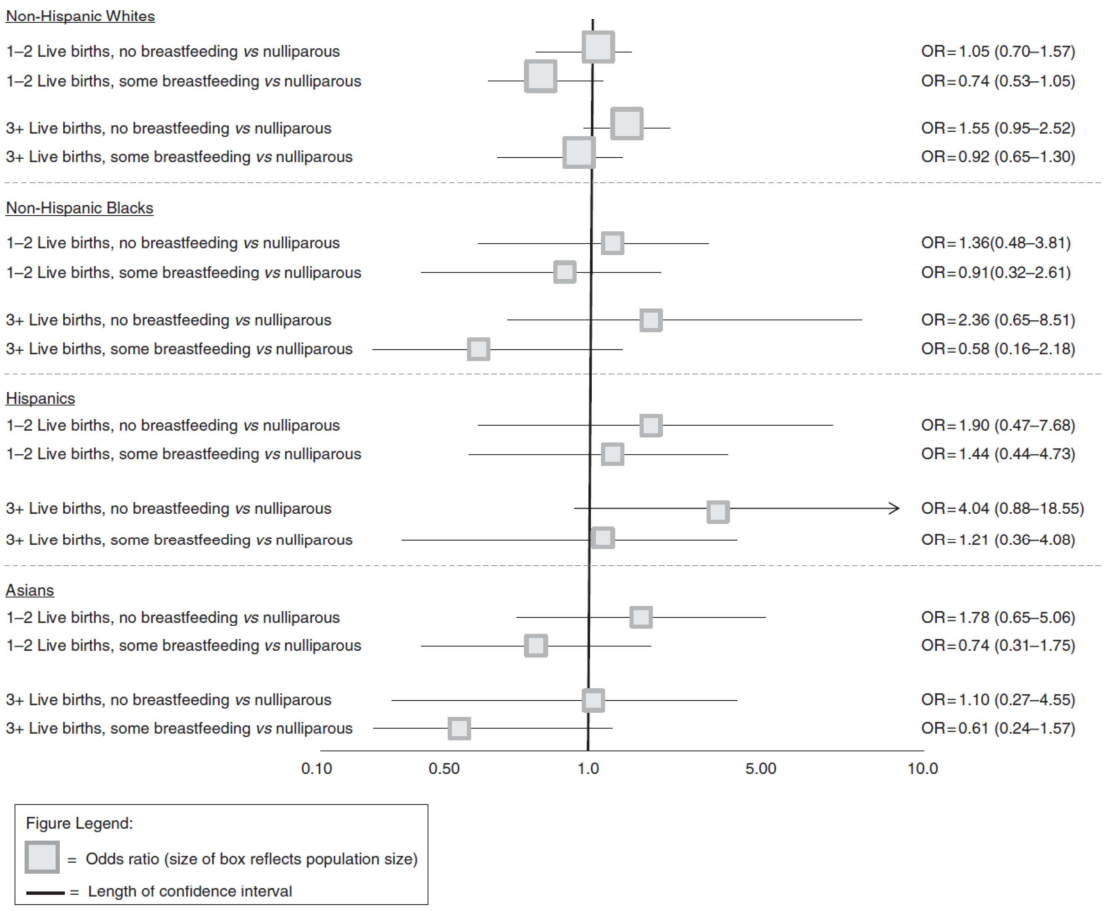


Figure 1. Comparison of odds ratios by race/ethnicity for breastfeeding and parity, Breast Cancer Family Registry, ER − PR − cases *vs* controls.

DISCUSSION

Our study sample was enriched with women at higher than population risk for breast cancer (due to oversampling of cases with early-onset breast cancer and/or a family history of breast cancer). We found that high parity was associated with an increased risk of ER − PR − cancer, compared with controls, and that breastfeeding for a total duration of ⩾12 months reduced this risk. Previous studies have found that duration of breastfeeding, coupled with parity levels, is an important factor for risk of triple-negative (ER − PR − HER2 −) breast cancer (Bauer *et al*, 2007; Kwan *et al*, 2009; Redondo *et al*, 2012). When we examined this combined variable for ER − PR − cancer, we also observed that multiparity, combined with no breastfeeding, was associated with an increased risk of ER − PR − cancer, and triple-negative cancer, but not with hormone receptor-positive cancer. We found that the association for ER − PR − cancer was similar across race/ethnicity.

In other studies examining higher risk women, the inverse association with parity was also limited to ER + /PR + cancers (Nichols *et al*, 2005; Ma *et al*, 2006b). However, in a study of very young women, aged ⩽35 years, ER status was not associated with parity (Largent *et al*, 2005). While our analysis did not find an association between parity and reduced cancer risk for hormone receptor-positive breast cancer, we did find this to be true among postmenopausal women in our study for women with 1 − 2 births. We also found a positive association between parity and ER − PR − cancer, similar to the findings of Yang *et al* (2011), in their case-only analysis, and reflecting similarities to findings among studies that examined triple-negative breast cancer (Millikan *et al*, 2007; Phipps *et al*, 2011).

Our study confirms earlier findings that breastfeeding decreases the risk of breast cancer, regardless of hormone receptor status. A recent review supported that ER or PR expression was not differentially associated with breastfeeding (Althuis *et al*, 2004), and most other studies have confirmed this finding for subtypes defined by ER/PR status (Ursin *et al*, 2005; Ma *et al*, 2006b, 2010a; Lord *et al*, 2008; Sweeney *et al*, 2008; Bao *et al*, 2011) and subtypes defined by ER/PR/HER2 status (Ma *et al*, 2010b; Xing *et al*, 2010; Gaudet *et al*, 2011). Some studies have shown, as ours did, that the inverse association with breastfeeding is stronger for ER − , ER − PR − , or triple-negative breast cancer (Largent *et al*, 2005; Millikan *et al*, 2007; Kwan *et al*, 2009; Gaudet *et al*, 2011). The Collaborative Group on Hormonal Risk Factors in Breast Cancer (2002) determined that breastfeeding is protective against breast cancer above and beyond the protection conferred by parity. Hypothesised potential protective mechanisms include the removal of oestrogens *via* breast fluid, excretion of carcinogenic agents through breast milk, delay in ovulation associated with breastfeeding, and induction of terminal differentiation of breast epithelial cells (Lipworth *et al*, 2000). It has been shown that *BRCA1* mutation carriers, who are typically diagnosed with ER − PR − cancer, were

Table 4. Association among oral contraceptive use, parity and breastfeeding and breast cancer classified by molecular status, Breast Cancer Family Registry (compared with Luminal A cases, $N = 468$)

	Luminal B $N = 118$ OR (95% CI)	HER2+ $N = 67$ OR (95% CI)	Triple negative $N = 139$ OR (95% CI)
OC use			
Never	1.0 (ref)	1.0 (ref)	1.0 (ref)
⩽5 years	0.82 (0.48–1.41)	1.30 (0.64–2.62)	1.19 (0.69–2.04)
>5 years	0.83 (0.48–1.43)	1.32 (0.65–2.67)	1.63 (0.97–2.76)
Timing of first OC			
Never	1.0 (ref)	1.0 (ref)	1.0 (ref)
Before 1975	0.94 (0.54–1.63)	1.10 (0.53–2.26)	1.11 (0.65–1.89)
1975 or later	0.73 (0.40–1.33)	1.65 (0.75–3.60)	**2.02 (1.11–3.68)**
Parity (number of live births)			
Nulliparous	1.0 (ref)	1.0 (ref)	1.0 (ref)
1–2	1.43 (0.72–2.85)	**3.39 (1.31–9.31)**	**2.16 (1.10–4.21)**
⩾3	1.32 (0.61–2.88)	2.88 (0.98–8.51)	**2.82 (1.37–5.83)**
Breastfeeding duration (months)			
Never	1.0 (ref)	1.0 (ref)	1.0 (ref)
<12	0.77 (0.43–1.39)	0.70 (0.36–1.39)	**0.49 (0.29–0.82)**
⩾12	1.10 (0.58–2.11)	0.77 (0.35–1.69)	0.57 (0.31–1.04)
Breastfeeding and parity			
Nulliparous	1.0 (ref)	1.0 (ref)	1.0 (ref)
Parous, never breastfed	1.38 (0.71–2.71)	**3.32 (1.26–8.73)**	**2.33 (1.22–4.45)**
Parous, ever breastfed	1.22 (0.69–2.18)	2.40 (0.98–5.5.86)	1.22 (0.67–2.22)

Abbreviation: OC = oral contraceptive. Luminal A is defined as ER and/or PR+, HER2−; Luminal B is defined as ER and/or PR+, HER2+; HER2+ is defined as ER−, PR−, HER2+; Triple negative is defined as ER−, PR−, HER2−. Odds ratios (OR) and 95% confidence interval (CI) adjusted for age, race, study site, and menopausal status. OC findings adjusted for breastfeeding and parity; parity findings adjusted for OC use and breastfeeding; breastfeeding findings adjusted for OC use and parity, combined parity–breastfeeding findings adjusted for OC use. **Bold** indicates $P<0.05$.

less likely to develop breast cancer if they breastfed for at least 1 year, compared with *BRCA1* mutation carriers who did not breastfeed; there was no association with breastfeeding among *BRCA2* mutation carriers, who usually have ER+ tumours (Jernstrom *et al*, 2004).

Overall, OC use greater than 5 years was associated with a reduced risk of hormone receptor-positive breast cancer, and was not associated with ER−PR− cancer. Earlier published studies reported positive associations between ER−PR− breast cancer and OC use (reviewed in Althuis *et al*, 2004), whereas most recent studies, including ours, have found no overall association between ER−PR− breast cancer and OC use (Ma *et al*, 2006b; Bao *et al*, 2011), although some studies have reached different conclusions (Rosenberg *et al*, 2010). We found that OC use in 1975 or later was inversely associated with ER+PR+ breast cancer, and a positive association between OC use and ER−PR− breast cancer was limited to women who initiated the use before 1975. Year of initiation of OC has been used previously (Collaborative Group on Hormonal Factors in Breast Cancer, 1996; Grabrick *et al*, 2000; Kahlenborn *et al*, 2006), but has not generally been examined in previous research on OC use and breast cancer risk by hormone receptor status. Data on OC use and breast cancer risk in *BRCA1* mutation carriers, including some from our own study sample (Milne *et al*, 2005; Haile *et al*, 2006; Iodice *et al*, 2010), have demonstrated no increased risk with OC use initiated after 1974, and examination of OC use among women with a family history of breast cancer found an increased risk of breast cancer only among women who began OC use before 1975 (Grabrick *et al*, 2000). In our study, findings were similar for any hormone-positive (ER+ and/or PR+) subtype, and only different for the ER−PR− type, indicating that any aetiology related to OC use may be through both oestrogen and progesterone-related mechanisms. It is unclear why OCs used before 1975 would be more strongly associated with ER−PR− cancer. Studies of synthetic progestins used in OCs have generally found that the proliferative actions of progestins used in OCs are mediated through the ER (Jeng *et al*, 1992; Jordan, 1993), which does not explain why ER− breast cancer is more likely to be affected, unless the ER is effectively 'turned off' by such proliferation. Typical oestrogen doses used in the 1960s were more than double the doses used in the 1980s, and progestin doses were also higher and included different types of progestins than current OCs (Grabrick *et al*, 2000).

Methodologic considerations. Distributions of parity and other risk factors for our sample where tumour characteristics were available and the entire case sample was similar (data not shown). Breast Cancer Family Registry pathologists used common laboratory procedures and conducted a centralised pathology view to categorise the majority of cases. A recent study has demonstrated that cancer registry-provided data may undercount the rarer ER/PR combinations, such as ER−PR+ and ER+PR− tumours, and that centralised pathology review should be considered as a gold standard when classifying tumours by hormone receptor status (Ma *et al*, 2009). For the analysis of molecular subtypes, the population differed from the overall study sample in that it comprised mostly racial/ethnic minority cases from Northern California and Ontario, as few non-Hispanic white families were enrolled in the BCFR after 2000 when HER2 data

became available in the cancer registries. Due to these limitations, we conducted a case-only analysis and acknowledge that our findings are preliminary, although they are in agreement with those of other studies. In the BCFR, differences have been observed between population controls and sister controls in some risk factors that are possibly associated with participation in research (Milne *et al*, 2011). Specifically, our population-based controls are more likely to have been highly educated, and have fewer births and higher average age at first birth, than those sister controls. The possibility of recall bias exists because we relied on participants' recalls of their exposures. However, the purpose of this analysis was to determine whether risk factor associations differed by subtype, using controls as a common comparison group. Because it is unlikely that cases report exposures differently based on their ER, PR, or HER2 status, it is unlikely that differences across tumour subtypes can be explained by recall bias.

Summary. Overall, we found that multiparity is associated with an increased risk of ER − PR − cancer, but this risk was reduced by breastfeeding, such that multiparous women with a history of breastfeeding were no longer at increased risk. In the United States, initiation of breastfeeding has increased steadily since the 1970s and the average duration of breastfeeding is also increasing (U.S. Department of Health and Human Services, 2011). Recent trends examining SEER incidence data suggest that rates of ER − PR − breast cancer are decreasing and will likely continue to decrease in the coming years (Anderson *et al*, 2011). Despite these trends, however, there remain large differences in both ER − PR − breast cancer incidence and breast feeding prevalences across racial and ethnic groups, suggesting that increasing breast feeding in all women is essential to breast cancer prevention.

ACKNOWLEDGEMENTS

This study was supported through the National Cancer Institute, National Institutes of Health under RFA-CA-06-503, UM1 CA164920, Cancer Care Ontario (U01 CA69467), Cancer Prevention Institute of California (U01 CA694117), University of Melbourne (U01 CA69638) and through cooperative agreements with members of the Breast Cancer Family Registry (BCFR) and Principal Investigators. The Australian Breast Cancer Family Study was also supported by the National Health and Medical Research Council of Australia, the New South Wales Cancer Council and the Victorian Health Promotion Foundation. The recruitment of controls by the Northern California Cancer Center was supported in part by National Institutes of Health Grant U01CA 71966. The recruitment of controls in Ontario was supported by the Canadian Breast Cancer Research Initiative. Funding for MEW was supported by a training grant from the National Cancer Institute, National Institutes of Health (T32 CA009529) and a training grant from the National Center for Advancing Translational Sciences, National Institutes of Health (TL1 TR00008), formerly the National Center for Research Resources (TL1 RR024158). The content of this manuscript does not necessarily reflect the views or policies of the National Cancer Institute or any of the collaborating centers in the BCFR, nor does mention of trade names, commercial products, or organisations imply endorsement by the US Government or the BCFR.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Althuis MD, Fergenbaum JH, Garcia-Closas M, Brinton LA, Madigan MP, Sherman ME (2004) Etiology of hormone receptor-defined breast cancer: a systematic review of the literature. *Cancer Epidemiol Biomarkers Prev* **13**: 1558–1568.

Anderson WF, Katki HA, Rosenberg PS (2011) Incidence of breast cancer in the United States: current and future trends. *J Natl Cancer Inst* **103**: 1397–1402.

Bao PP, Shu XO, Gao YT, Zheng Y, Cai H, Deming SL, Ruan ZX, Su Y, Gu K, Lu W, Zheng W (2011) Association of hormone-related characteristics and breast cancer risk by estrogen receptor/progesterone receptor status in the shanghai breast cancer study. *Am J Epidemiol* **174**: 661–671.

Bauer KR, Brown M, Cress RD, Parise CA, Caggiano V (2007) Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype: a population-based study from the California cancer Registry. *Cancer* **109**: 1721–1728.

Brinton LA, Sherman ME, Carreon JD, Anderson WF (2008) Recent trends in breast cancer among younger women in the United States. *J Natl Cancer Inst* **100**: 1643–1648.

Britton JA, Gammon MD, Schoenberg JB, Stanford JL, Coates RJ, Swanson CA, Potischman N, Malone KE, Brogan DJ, Daling JR, Brinton LA (2002) Risk of breast cancer classified by joint estrogen receptor and progesterone receptor status among women 20-44 years of age. *Am J Epidemiol* **156**: 507–516.

Carey LA, Perou CM, Livasy CA, Dressler LG, Cowan D, Conway K, Karaca G, Troester MA, Tse CK, Edmiston S, Deming SL, Geradts J, Cheang MC, Nielsen TO, Moorman PG, Earp HS, Millikan RC (2006) Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. *JAMA* **295**: 2492–2502.

Clarke CA, Keegan TH, Yang J, Press DJ, Kurian AW, Patel AH, Lacey JV Jr (2012) Age-specific incidence of breast cancer subtypes: understanding the black-white crossover. *J Natl Cancer Inst* **104**: 1094–1101.

Colditz GA, Rosner BA, Chen WY, Holmes MD, Hankinson SE (2004) Risk factors for breast cancer according to estrogen and progesterone receptor status. *J Natl Cancer Inst* **96**: 218–228.

Collaborative Group on Hormonal Factors in Breast Cancer (1996) Breast cancer and hormonal contraceptives: collaborative reanalysis of individual data on 53 297 women with breast cancer and 100 239 women without breast cancer from 54 epidemiological studies. *Lancet* **347**: 1713–1727.

Collaborative Group on Hormonal Factors in Breast Cancer (2002) Breast cancer and breastfeeding: collaborative reanalysis of individual data from 47 epidemiological studies in 30 countries, including 50 302 women with breast cancer and 96 973 women without the disease. *Lancet* **360**: 187–195.

Gaudet MM, Press MF, Haile RW, Lynch CF, Glaser SL, Schildkraut J, Gammon MD, Douglas Thompson W, Bernstein JL (2011) Risk factors by molecular subtypes of breast cancer across a population-based study of women 56 years or younger. *Breast Cancer Res Treat* **130**: 587–597.

Grabrick DM, Hartmann LC, Cerhan JR, Vierkant RA, Therneau TM, Vachon CM, Olson JE, Couch FJ, Anderson KE, Pankratz VS, Sellers TA (2000) Risk of breast cancer with oral contraceptive use in women with a family history of breast cancer. *JAMA* **284**: 1791–1798.

Haile RW, Thomas DC, Mcguire V, Felberg A, John EM, Milne RL, Hopper JL, Jenkins MA, Levine AJ, Daly MM, Buys SS, Senie RT, Andrulis IL, Knight JA, Godwin AK, Southey M, Mccredie MR, Giles GG, Andrews L, Tucker K, Miron A, Apicella C, Tesoriero A, Bane A, Pike MC, Whittemore AS (2006) BRCA1 and BRCA2 mutation carriers, oral contraceptive use, and breast cancer before age 50. *Cancer Epidemiol Biomarkers Prev* **15**: 1863–1870.

Iodice S, Barile M, Rotmensz N, Feroce I, Bonanni B, Radice P, Bernard L, Maisonneuve P, Gandini S (2010) Oral contraceptive use and breast or ovarian cancer risk in BRCA1/2 carriers: a meta-analysis. *Eur J Cancer* **46**: 2275–2284.

Jeng MH, Parker CJ, Jordan VC (1992) Estrogenic potential of progestins in oral contraceptives to stimulate human breast cancer cell proliferation. *Cancer Res* **52**: 6539–6546.

Jernstrom H, Lubinski J, Lynch HT, Ghadirian P, Neuhausen S, Isaacs C, Weber BL, Horsman D, Rosen B, Foulkes WD, Friedman E, Gershoni-Baruch R, Ainsworth P, Daly M, Garber J, Olsson H, Sun P,

Narod SA (2004) Breast-feeding and the risk of breast cancer in BRCA1 and BRCA2 mutation carriers. *J Natl Cancer Inst* **96**: 1094–1098.

John EM, Hopper JL, Beck JC, Knight JA, Neuhausen SL, Senie RT, Ziogas A, Andrulis IL, Anton-Culver H, Boyd N, Buys SS, Daly MB, O'malley FP, Santella RM, Southey MC, Venne VL, Venter DJ, West DW, Whittemore AS, Seminara D (2004) The Breast Cancer Family Registry: an infrastructure for cooperative multinational, interdisciplinary and translational studies of the genetic epidemiology of breast cancer. *Breast Cancer Res* **6**: R375–R389.

Jordan VC (1993) Growth factor regulation by tamoxifen is demonstrated in patients with breast cancer. *Cancer* **72**: 1–2.

Kahlenborn C, Modugno F, Potter DM, Severs WB (2006) Oral contraceptive use as a risk factor for premenopausal breast cancer: a meta-analysis. *Mayo Clin Proc* **81**: 1290–1302.

Knight JA, John EM, Milne RL, Dite GS, Balbuena R, Shi EJ, Giles GG, Ziogas A, Andrulis IL, Whittemore AS, Hopper JL (2006) An inverse association between ovarian cysts and breast cancer in the breast cancer family registry. *Int J Cancer* **118**: 197–202.

Kwan ML, Kushi LH, Weltzien E, Maring B, Kutner SE, Fulton RS, Lee MM, Ambrosone CB, Caan BJ (2009) Epidemiology of breast cancer subtypes in two prospective cohort studies of breast cancer survivors. *Breast Cancer Res* **11**: R31.

Largent JA, Ziogas A, Anton-Culver H (2005) Effect of reproductive factors on stage, grade and hormone receptor status in early-onset breast cancer. *Breast Cancer Res* **7**: R541–R554.

Lipworth L, Bailey LR, Trichopoulos D (2000) History of breast-feeding in relation to breast cancer risk: a review of the epidemiologic literature. *J Natl Cancer Inst* **92**: 302–312.

Lord SJ, Bernstein L, Johnson KA, Malone KE, Mcdonald JA, Marchbanks PA, Simon MS, Strom BL, Press MF, Folger SG, Burkman RT, Deapen D, Spirtas R, Ursin G (2008) Breast cancer risk and hormone receptor status in older women by parity, age of first birth, and breastfeeding: a case-control study. *Cancer Epidemiol Biomarkers Prev* **17**: 1723–1730.

Ma H, Bernstein L, Pike MC, Ursin G (2006a) Reproductive factors and breast cancer risk according to joint estrogen and progesterone receptor status: a meta-analysis of epidemiological studies. *Breast Cancer Res* **8**: R43.

Ma H, Bernstein L, Ross RK, Ursin G (2006b) Hormone-related risk factors for breast cancer in women under age 50 years by estrogen and progesterone receptor status: results from a case-control and a case-case comparison. *Breast Cancer Res* **8**: R39.

Ma H, Henderson KD, Sullivan-Halley J, Duan L, Marshall SF, Ursin G, Horn-Ross PL, Largent J, Deapen DM, Lacey Jr JV, Bernstein L (2010a) Pregnancy-related factors and the risk of breast carcinoma in situ and invasive breast cancer among postmenopausal women in the California Teachers Study cohort. *Breast Cancer Res* **12**: R35.

Ma H, Wang Y, Sullivan-Halley J, Weiss L, Burkman RT, Simon MS, Malone KE, Strom BL, Ursin G, Marchbanks PA, Mcdonald JA, Spirtas R, Press MF, Bernstein L (2009) Breast cancer receptor status: do results from a centralized pathology laboratory agree with SEER registry reports? *Cancer Epidemiol Biomarkers Prev* **18**: 2214–2220.

Ma H, Wang Y, Sullivan-Halley J, Weiss L, Marchbanks PA, Spirtas R, Ursin G, Burkman RT, Simon MS, Malone KE, Strom BL, Mcdonald JA, Press MF, Bernstein L (2010b) Use of four biomarkers to evaluate the risk of breast cancer subtypes in the women's contraceptive and reproductive experiences study. *Cancer Res* **70**: 575–587.

Mccredie MR, Dite GS, Southey MC, Venter DJ, Giles GG, Hopper JL (2003) Risk factors for breast cancer in young women by oestrogen receptor and progesterone receptor status. *Br J Cancer* **89**: 1661–1663.

Mctiernan A, Thomas DB, Johnson LK, Roseman D (1986) Risk factors for estrogen receptor-rich and estrogen receptor-poor breast cancers. *J Natl Cancer Inst* **77**: 849–854.

Millikan RC, Newman B, Tse CK, Moorman PG, Conway K, Smith LV, Labbok MH, Geradts J, Bensen JT, Jackson S, Nyante S, Livasy C, Carey L, Earp HS, Perou CM (2007) Epidemiology of basal-like breast cancer. *Breast Cancer Res Treat* **109**(1): 123–139.

Milne RL, John EM, Knight JA, Dite GS, Southey MC, Giles GG, Apicella C, West DW, Andrulis IL, Whittemore AS, Hopper JL (2011) The potential value of sibling controls compared with population controls for association studies of lifestyle-related risk factors: an example from the Breast Cancer Family Registry. *Int J Epidemiol* **40**(5): 1342–1354.

Milne RL, Knight JA, John EM, Dite GS, Balbuena R, Ziogas A, Andrulis IL, West DW, Li FP, Southey MC, Giles GG, Mccredie MR, Hopper JL, Whittemore AS (2005) Oral contraceptive use and risk of early-onset breast cancer in carriers and noncarriers of BRCA1 and BRCA2 mutations. *Cancer Epidemiol Biomarkers Prev* **14**: 350–356.

Nichols HB, Trentham-Dietz A, Love RR, Hampton JM, Hoang Anh PT, Allred DC, Mohsin SK, Newcomb PA (2005) Differences in breast cancer risk factors by tumor marker subtypes among premenopausal Vietnamese and Chinese women. *Cancer Epidemiol Biomarkers Prev* **14**: 41–47.

Palmer JR, Boggs DA, Wise LA, Ambrosone CB, Adams-Campbell LL, Rosenberg L (2011) Parity and lactation in relation to estrogen receptor negative breast cancer in African American women. *Cancer Epidemiol Biomarkers Prev* **20**: 1883–1891.

Phipps AI, Chlebowski RT, Prentice R, Mctiernan A, Wactawski-Wende J, Kuller LH, Adams-Campbell LL, Lane D, Stefanick ML, Vitolins M, Kabat GC, Rohan TE, Li CI (2011) Reproductive history and oral contraceptive use in relation to risk of triple-negative breast cancer. *J Natl Cancer Inst* **103**: 470–477.

Potter JD, Cerhan JR, Sellers TA, Mcgovern PG, Drinkard C, Kushi LR, Folsom AR (1995) Progesterone and estrogen receptors and mammary neoplasia in the Iowa Women's Health Study: how many kinds of breast cancer are there? *Cancer Epidemiol Biomarkers Prev* **4**: 319–326.

Redondo CM, Gago-Dominguez M, Ponte SM, Castelo ME, Jiang X, Garcia AA, Fernandez MP, Tome MA, Fraga M, Gude F, Martinez ME, Garzon VM, Carracedo A, Castelao JE (2012) Breast feeding, parity and breast cancer subtypes in a spanish cohort. *PLoS One* **7**: e40543.

Rosenberg L, Boggs DA, Wise LA, Adams-Campbell LL, Palmer JR (2010) Oral contraceptive use and estrogen/progesterone receptor-negative breast cancer among African American women. *Cancer Epidemiol Biomarkers Prev* **19**: 2073–2079.

Rosenberg LU, Einarsdottir K, Friman EI, Wedren S, Dickman PW, Hall P, Magnusson C (2006) Risk factors for hormone receptor-defined breast cancer in postmenopausal women. *Cancer Epidemiol Biomarkers Prev* **15**: 2482–2488.

Rosenberg LU, Granath F, Dickman PW, Einarsdottir K, Wedren S, Persson I, Hall P (2008) Menopausal hormone therapy in relation to breast cancer characteristics and prognosis: a cohort study. *Breast Cancer Res* **10**: R78.

Rusiecki JA, Holford TR, Zahm SH, Zheng T (2005) Breast cancer risk factors according to joint estrogen receptor and progesterone receptor status. *Cancer Detect Prev* **29**: 419–426.

Setiawan VW, Monroe KR, Wilkens LR, Kolonel LN, Pike MC, Henderson BE (2009) Breast cancer risk factors defined by estrogen and progesterone receptor status: the multiethnic cohort study. *Am J Epidemiol* **169**: 1251–1259.

Slanger TE, Chang-Claude JC, Obi N, Kropp S, Berger J, Vettorazzi E, Braendle W, Bastert G, Hentschel S, Flesch-Janys D (2009) Menopausal hormone therapy and risk of clinical breast cancer subtypes. *Cancer Epidemiol Biomarkers Prev* **18**: 1188–1196.

Stanford JL, Szklo M, Boring CC, Brinton LA, Diamond EA, Greenberg RS, Hoover RN (1987) A case-control study of breast cancer stratified by estrogen receptor status. *Am J Epidemiol* **125**: 184–194.

Stead LA, Lash TL, Sobieraj JE, Chi DD, Westrup JL, Charlot M, Blanchard RA, Lee JC, King TC, Rosenberg CL (2009) Triple-negative breast cancers are increased in black women regardless of age or body mass index. *Breast Cancer Res* **11**: R18.

Sweeney C, Baumgartner KB, Byers T, Giuliano AR, Herrick JS, Murtaugh MA, Slattery ML (2008) Reproductive history in relation to breast cancer risk among Hispanic and non-Hispanic white women. *Cancer Causes Control* **19**: 391–401.

Ursin G, Bernstein L, Lord SJ, Karim R, Deapen D, Press MF, Daling JR, Norman SA, Liff JM, Marchbanks PA, Folger SG, Simon MS, Strom BL, Burkman RT, Weiss LK, Spirtas R (2005) Reproductive factors and subtypes of breast cancer defined by hormone receptor and histology. *Br J Cancer* **93**: 364–371.

U.S. Department of Health and Human Services. *The Surgeon General's Call to Action to Support Breastfeeding*. U.S. Department of Health and Human Services, Office of the Surgeon General: Washington, DC, 2011. Available at http://www.surgeongeneral.gov.

Work ME, Andrulis IL, John EM, Hopper JL, Liao Y, Zhang FF, Knight JA, West DW, Milne RL, Giles GG, Longacre TA, O'malley F, Mulligan AM, Southey MC, Hibshoosh H, Terry MB (2012) Risk factors for uncommon histologic subtypes of breast cancer using centralized pathology review in the Breast Cancer Family Registry. *Breast Cancer Res Treat* **134**(3): 1209–1220.

Xing P, Li J, Jin F (2010) A case-control study of reproductive factors associated with subtypes of breast cancer in Northeast China. *Med Oncol* **27**: 926–931.

Yang XR, Chang-Claude J, Goode EL, Couch FJ, Nevanlinna H, Milne RL, Gaudet M, Schmidt MK, Broeks A, Cox A, Fasching PA, Hein R, Spurdle AB, Blows F, Driver K, Flesch-Janys D, Heinz J, Sinn P, Vrieling A, Heikkinen T, Aittomaki K, Heikkila P, Blomqvist C, Lissowska J, Peplonska B, Chanock S, Figueroa J, Brinton L, Hall P, Czene K, Humphreys K, Darabi H, Liu J, Van 'T Veer LJ, Van Leeuwen FE, Andrulis IL, Glendon G, Knight JA, Mulligan AM, O'malley FP, Weerasooriya N, John EM, Beckmann MW, Hartmann A, Weihbrecht SB, Wachter DL, Jud SM, Loehberg CR, Baglietto L, English DR, Giles GG, Mclean CA, Severi G, Lambrechts D, Vandorpe T, Weltens C, Paridaens R, Smeets A, Neven P, Wildiers H, Wang X, Olson JE, Cafourek V, Fredericksen Z, Kosel M, Vachon C, Cramp HE, Connley D, Cross SS, Balasubramanian SP, Reed MW, Dork T, Bremer M, Meyer A, Karstens JH, Ay A, Park-Simon TW, Hillemanns P, Arias Perez JI, Menendez Rodriguez P, Zamora P, Benitez J, Ko YD, Fischer HP, Hamann U, Pesch B, Bruning T, Justenhoven C, Brauch H, Eccles DM, Tapper WJ, Gerty SM, Sawyer EJ, Tomlinson IP, Jones A, Kerin M, Miller N, Mcinerney N, Anton-Culver H, Ziogas A, Shen CY, Hsiung CN, Wu PE, Yang SL, Yu JC, Chen ST, Hsu GC, Haiman CA, Henderson BE, Le Marchand L, Kolonel LN, Lindblom A, Margolin S, Jakubowska A, Lubinski J, Huzarski T, Byrski T, Gorski B, Gronwald J, Hooning MJ, Hollestele A, van Ouweland AM, Jager A, Kriege M, Tilanus-Linthorst MM, Collee M, Wang-Gohrke S, Pylkas K, Jukkola-Vuorinen A, Mononen K, Grip M, Hirvikoski P, Wingvist R, Mannermaa A, Kosma VM, Kauppinen J, Kataja V, Auvinen P, Soini Y, Sironen R, Bojesen SE, Orsted DD, Kaur-Knudsen D, Flyger H, Nordestgaard BG, Holland H, Chenevix-Trench G, Manoukian S, Barile M, Radice P, Hankinson SE, Hunter DJ, Tamimi R, Sangrajrang S, Brennan P, McKay J, Odefrey F, Gaborieau V, Devilee P, Huijts PE, Tollenaar RA, Seynaeve C, Dite GS, Apicella C, Hopper JL, Hammet F, Tsimiklis H, Smith LD, Southey MC, Humphreys MK, Easton D, Pharoah P, Sherman ME, Garcia-Closas M (2011) Associations of breast cancer risk factors with tumor subtypes: a pooled analysis from the Breast Cancer Association Consortium studies. *J Natl Cancer Inst* **103**: 250–263.

Yoo KY, Tajima K, Miura S, Takeuchi T, Hirose K, Risch H, Dubrow R (1997) Breast cancer risk factors according to combined estrogen and progesterone receptor status: a case-control analysis. *Am J Epidemiol* **146**: 307–314.