Mammographic Density as a Risk Factor for Ovarian Cancer: A Pilot Study

by

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Graduate Department of Medical Biophysics

University of Toronto

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ABSTRACT

MAMMOGRAPHIC DENSITY AS A RISK FACTOR FOR OVARIAN CANCER:
A PILOT STUDY

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Ovarian cancer and breast cancer share many of the same risk factors. The strongest known risk factor for breast cancer is mammographic density, the radiological appearance of breast tissue on a mammogram. Even though breast and ovarian cancer share many of the same risk factors, mammographic density has never been examined in relation to ovarian cancer. The present thesis describes a pilot study that was conducted to determine the feasibility of a study looking to address the issue of mammographic density as a risk factor for ovarian cancer. It was found that a larger study was feasible and should consist of approximately 700 case-control pairs recruited from cancer centres across Ontario, with cases matched to sisters or first-degree cousins. It was also found that the use of sister controls for cases did not lead to overmatching on mammographic density, and sisters are a suitable control group.
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<tr>
<td>BCFR</td>
<td>Breast Cancer Familial Registry</td>
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<td>BMI</td>
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<td>HRT</td>
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<td>MD</td>
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<tr>
<td>OC</td>
<td>Oral contraceptives</td>
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<td>OR</td>
<td>Odds ratio</td>
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<td>RR</td>
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<td>SD</td>
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<td>SHBG</td>
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<td>SIR</td>
<td>Standard incidence rate</td>
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1.1 Background on Ovarian Cancer

Ovarian cancer is the fifth most common cancer among women. Ovarian cancer is known for its poor survival rate and higher mortality. One of the reasons attributed to this low survival is the fact that early stage ovarian cancer has few early warning signs. By the time a woman starts exhibiting more serious symptoms that would cause her to seek medical attention, the disease has often already progressed to the later stages, which have the worst survival outcomes and are more difficult to treat. The majority of ovarian cancers are diagnosed in Stages III or IV when the cancer has spread beyond the ovaries and pelvis. In addition to the few warning signs and later stage diagnoses, there are also no standard screening methods to assist with early stage diagnosis of ovarian cancer.

By identifying women at high risk for this disease through the discovery of novel risk factors associated with ovarian cancer, it may be possible to diagnose ovarian cancer earlier. This could help improve the poor survival statistics and high mortality currently associated with ovarian cancer. Firstly, the development of an ovarian cancer risk prediction model similar to those used for breast cancer would be beneficial in identifying those women at the highest risk of ovarian cancer. A set of standardized screening techniques can then be applied to these high risk women, such as a CA125 serum test or a diagnostic ultrasound, both of which have not been proven successful when used to screen the general population, but whose accuracy and positive predictive value may be improved when applied to high risk women.
1.2 Organization of Thesis

The purpose of the current thesis is to set the stage for the identification of a possible novel risk factor for ovarian cancer, mammographic density, which is one of the strongest known risk factors for breast cancer.

First, a literature review on the common risk factors shared between breast and ovarian cancer will be conducted with evidence provided that these diseases share many of the same risk factors which could indicate a common aetiology. The following chapter will then present the results of a pilot study conducted to address potential research issues that may arise when designing a large study looking at MD as a potential risk factor for ovarian cancer. One of these research issues, the possibility of overmatching on MD through the use of controls, is investigated in detail, with the results presented in Chapter 4. The final chapter provides a summary of the research conducted as well as discusses the future directions for research looking into MD and ovarian cancer.
CHAPTER 2:

Comparison of Risk Factors between Ovarian Cancer and Breast Cancer
2.1 Introduction

Breast cancer and ovarian cancer are both common cancers diagnosed among women. They have been the subject of many epidemiological and genetic research studies, and share many of the same risk factors. This chapter will provide a review of the literature for the most common risk factors for breast and ovarian cancer and determine which risk factors are shared between these two cancers.

2.1.1 Search Strategy and Selection Criteria. PubMed was searched using the terms: “ovarian cancer” and “breast cancer”. For each individual section of the article, each cancer term was combined (using “and”) with the heading listed for each section, in addition to the more general terms of “reproductive risk factor”, “hormonal risk factor”, and “hormone use”. There were several terms that were searched that could be considered under an alternate name than listed on the section headings, including the following: “obesity”, “total menstrual cycles”, “early life BMI”, “lactation”, “non-breast second malignancy”, “second cancer”, and “Hiroshima”. References from the articles selected were also searched. There were no restrictions on language or date of the publication, and all articles included up to June 2009 were eligible.

Due to space constraints, not all articles retrieved in the search could be reviewed or referenced. However, for each search, randomized control trials and meta-analysis were given preference, and when population studies were referenced, cohort studies were given higher priority than case-control studies. This hierarchy is based on the methodological strength of each type of article or study. Randomization is preferred as randomization should equal out any differences between the treatment and control
group, and provides some assurance that the effect seen is the result of the treatment, and not the result of underlying group differences. Meta-analysis are preferred to single studies as they combine the results of cohort and case-control studies and thereby pool sample sizes and results, and the influence of any extreme results based on small studies is reduced. Cohorts are preferred to case-control, as cohort studies do not have the problems inherent in case-controls studies, specifically with recall bias of information and self-selection of cases into the ‘case’ category. In a cohort, usually all women are healthy when they enter the study or population and only when an event happens (i.e. develop cancer) will they be counted as a case. In theory, all women in the cohort have equal chances of becoming a case. When case-control studies have been highlighted below, it is because they were either all that was available to reference on a given topic or because they provided a special consideration to the topic being discussed. In addition, preference was given to cohort or case-control studies when the total number of cases was greater than 1000 participants, as larger samples have more power and have less of a chance of a spurious result. Research focussing on Caucasian women or high-incidence countries were selected for, as ovarian cancer rates are higher in Caucasian women and industrialized countries, and therefore, the results would apply to a greater number of ovarian cancer patients.

2.2 Strong Influences on Breast or Ovarian Cancer

2.2.1. Incidence Rates (Age). The log-log adjusted age-specific incidence rates\(^1\) for colon, breast and ovarian cancer are shown in Figure 2-1. Most epithelial cancers’ age-specific incidence rates appear like the graph depicted for colon cancer, a straight
line indicating that as the population ages, the incidence of colon cancer increases exponentially. However, when looking at the graphs for ovarian cancer and breast cancer, they are different in shape from other epithelial cancers. The breast and ovarian cancer graphs show a rapid increase in incidence up until the approximate age of menopause, and then an inflection in the curve and a slower rate of increase thereafter. The similarities in these graphs suggest that both these diseases may be influenced by similar factors and share some aspects of their biology.

Figure 2-1. Age-specific incidence rates for three epithelial cancers.
Figure adapted from (1).

Pike et al. (2004)\(^1\) created a formula to explain the effect seen in the above age-specific incidence rates:

\[
I(t) = a[d(t)]^k
\]
This formula shows the relationship between incidence, \( I(t) \), and breast tissue age, \([d(t)]\), where \( a \) is a constant and \( k \) is usually between 4 and 5\(^1\). It was hypothesized that for hormone-dependant tissues such as the breast and the ovary, the ‘effective tissue age’ is equal to the cumulative number of mitotic cell divisions brought about by the effect of ovarian hormones. This formula also helps explain the slower increase of incidence of breast and ovarian cancer after the approximate age of menopause. The authors hypothesize that the effect of menopause on incidence is a slowing of the rate of mitosis, and thereby a reduction in the risk of spontaneous and environmentally caused mutations.

### 2.2.2 BRCA1/2 Mutation

Women with a mutation on the BRCA1 or BRCA2 gene face an elevated risk of developing breast and ovarian cancer. These mutations are hereditary. Among women with the BRCA1 mutation, it has been found that approximately 68\(^2\) to 74\(^3\) will eventually develop breast cancer in their lifetime, and 28\(^3\) to 36\(^2\) will develop ovarian cancer. This is in stark contrast to the lifetime risk of breast and ovarian cancer found in noncarriers of the mutation, approximately 6.8\(^2\) for breast cancer, and 1.8\(^2\) for ovarian cancer\(^2\). For the BRCA2 mutation, there is a lower penetrance of both breast and ovarian cancer associated with the gene, however, these women still face extremely high risk of breast (45% lifetime penetrance) and ovarian (11% lifetime penetrance) cancer\(^4\).

There is some variability in the population prevalence estimates for these mutations, however, generally 6\(^5\) of all breast cancers and 6-13\(^6-7\) of all ovarian
cancers can be attributed to these two genetic mutations. The BRCA1/2 genes mark a genetic predisposition shared by breast and ovarian cancer.

2.2.3 Radiation Effects. Exposure to radiation has been associated with an increase in breast cancer\textsuperscript{8-9}. This effect can be observed among the survivors of the atomic bomb dropped in Hiroshima and Nagasaki. The most common type of study looking at the effects of radiation on cancer are cohort studies, where everyone exposed to the radiation form the cohort. Cancer cases are ascertained from this exposed group, and then the cancer rates of the exposed group are then compared to the non-exposed population. There is a higher incidence of breast cancer among women exposed to the bomb, and an inverse association between age at time of bombing (<20 years old) and breast cancer risk has been reported. The increase in risk is also dose-dependant, with those women receiving the highest doses at increased risk of breast cancer\textsuperscript{8}. Therefore, the highest incidence of breast cancer is among women who were exposed to larger doses of radiation who were under the age of 20 when the bomb hit.

Ovarian cancer has also been shown to have an age-dependant and dose-dependant association with radiation from the Atomic bomb among Hiroshima and Nagasaki bomb survivors\textsuperscript{9}. It was found that those at highest risk of ovarian cancer were those women who were under the age of 20 at the time of the bomb, who received the highest dose of radiation\textsuperscript{9}.

Both breast and ovarian cancer risk has been shown to increase in response to the effect of radiation. Furthermore, the effects of radiation are age- and dose-dependant, with women under the age of 20 when exposed and closest to where the bomb dropped
at the highest risk of both cancers. However, there were only 194 ovarian cases included in the ovarian cancer study (versus 807 breast cancer cases in the report mentioned above), and so while the age-dose effect was found among ovarian cancer patients, it is less reliable than the effect shown for breast cancer, as there were only one quarter of the participants.

2.2.4 Family History of Breast and/or Ovarian Cancer. A family history of breast cancer has been associated with an increase in risk of breast cancer. In a Canadian prospective cohort study, it was found that among women testing negative for BRCA1/2 who have a family history of breast cancer, have a four times greater risk of developing breast cancer in comparison to women without a positive family history\textsuperscript{10}. A family history of ovarian cancer has also been associated with an increased risk of breast cancer, however, with more mixed results. A study was conducted looking at the incidence of cancer among family members of women registered in the Gilda Radner Familial Ovarian Cancer Registry who reported at least 3 family members being diagnosed with ovarian cancer and it was found that there was a 2.5 fold increase in risk of breast cancer among these family members, compared to the general population\textsuperscript{11}. This study was also a cohort study with a small sample size of only 143, and therefore less reliable, however the results found are interesting as the study dealt with a specific group of women who were at increased risk of developing the disease due to extensive ovarian cancer family histories. Breast cancer risk has also been assessed for women with a family history of only ovarian cancer who tested negative for BRCA1/2 mutation, but no association was found\textsuperscript{12}.
Among women reporting a family history of breast and/or ovarian cancer among one or more of their first-degree relatives, there is an over two-fold increase in risk of developing ovarian cancer (RR=2.6, 95% CI: 2.0-3.5)\textsuperscript{13}. While this was a case-control study, the total number of ovarian cases was 1031, which is a considerable sample size for a low incidence disease like ovarian cancer. When examining a family history of just breast cancer among ovarian cases, it has been found that having at least one first-degree relative diagnosed with breast cancer is associated with a 40% increase in risk of developing ovarian cancer\textsuperscript{14}, however, this effect has not been replicated elsewhere\textsuperscript{12}. In a large cohort study, with 49,975 women with a detailed family history, it was found that the risk of ovarian cancer has been shown to increase as the number of relatives affected with breast cancer increased, with a relative risk of 1.8 (95% CI: 1.1-2.8) for women who have at least two affected first-degree relatives with breast cancer\textsuperscript{14}.

A positive family history of breast and/or ovarian cancer has been associated with an increase in risk of developing either cancer. This effect has been shown even among women testing negative for the BRCA1/2 mutation, which may indicate an underlying genetic breast/ovarian cancer susceptibility that is heritable.

**2.2.5 Hormone Replacement Therapy.** Hormone replacement therapy (HRT) is prescribed to women who are going through menopause. It was believed that taking estrogens during menopause, a time period when a woman is at increased risk of a variety of diseases and ailments, would prevent heart disease, stroke, osteoporosis, dementia and the unpleasant side effects of menopause such as hot flashes and insomnia. Looking at risk associated with HRT is complicated by the fact that the
preparations of HRT have taken many forms over the years. Basically, there are two forms of HRT, unopposed estrogen replacement therapy (ERT) and estrogen/progestin replacement therapy (EPRT), and the risks associated with each preparation vary. From the 1940s until the 1970s, women used ERT, and after that time, women with an intact uterus were prescribed EPRT, due to concerns over increased rates of endometrial cancer\textsuperscript{15}. The complete results of the research discussed below are shown in Table 2-1.

Much research has gone into HRT and risk of cancer, however, they were all observational in nature, until the Women’s Health Initiative released their report in 2002. This was the first randomized clinical trial looking at the risks and benefits of HRT use. This trial has several strengths in its design, the main benefit being the randomized assignment of treatment versus control groups. The WHI researchers discovered that women who used the combination EPRT had an elevated risk of breast cancer, with a significant excess of 26\% more breast cancer cases in the treatment group compared to the controls\textsuperscript{16}. This is consistent with the published results of other epidemiological studies looking at combined EPRT and breast cancer risk\textsuperscript{17}, and with the results of a large meta-analysis\textsuperscript{18} which looked at breast cancer risk in general HRT users. The WHI also had another arm to their study, which looked at the effect of ERT only therapy on risk\textsuperscript{19}. The results of this trial were surprising, as there was a 23\% reduction in reported breast cancers among women in the ERT treatment group. While most epidemiological studies comparing the two HRT treatment options do show a greater increase in risk of developing breast cancer among combined EPRT therapy, they still report a modest increase in risk for the ERT only preparation\textsuperscript{20-21}, or at
minimum, no increase in risk at all\textsuperscript{22}. Interestingly, among women with a high body mass index (BMI), HRT has been shown to influence the increase in risk of breast cancer associated with obesity\textsuperscript{23}. A large cohort study was conducted with over 95,000 female nurses, and found that postmenopausal women on HRT who are in the highest weight categories show no association between high BMI and breast cancer risk, however, those not on HRT do show an increase in risk\textsuperscript{23-26} compared to slimmer women.

There is less research on the effect of HRT on ovarian cancer risk; from the results that are published, mostly from case-control studies, the conclusions regarding risk are less clear compared to the breast cancer results\textsuperscript{27-30}. A meta-analysis was conducted using 42 studies with a combined total of over 12,000 ovarian cancer cases, and an increased risk of ovarian cancer was found among women who used ERT for durations longer than 5 years, and who had discontinued use within the past three years (RR= 1.8, 95\% CI, 0.8-3.7)\textsuperscript{28}. Among women who used ERT for the same duration of time, but discontinued use greater than three years prior, there was no longer any association between HRT and risk of ovarian cancer (RR=1.2, 95\% CI= 0.6-2.7)\textsuperscript{28}. This reduction in risk for past versus current users of HRT has been replicated in the Million Women Study, which follows a cohort of 948,576 UK women, and had a reported 2273 incident ovarian cancers. They found that compared to never users, past users of HRT had no increase in risk of ovarian cancer, with a relative risk of 0.98 (95\% CI: 0.88-1.11)\textsuperscript{31}. Others have found an association among ERT use and ovarian cancer only for durations of use of 10 years or longer (RR=1.89, 95\% CI: 1.22 to 2.95)\textsuperscript{29}. There have been few
studies that found an association between the progestin preparation of HRT and ovarian cancer\textsuperscript{31-32}, however, most report no association\textsuperscript{27-30}.

From the wealth of literature on HRT use and cancer, it can be concluded that there is an increase in risk among ‘ever’ users of HRT and both breast and ovarian cancer. However, while the data show that there does seem to be an increased risk among women who use HRT compared to non-users, the answer as to the degree of risk associated with HRT and breast and ovarian cancer may have more to do with the preparation of the therapy and whether progestin was involved. There is an increase of risk of breast cancer for users of the EPRT combination therapy, with little increase of breast cancer in users of ERT. The reverse appears to be true for risk of ovarian cancer, with ERT users at increased risk.

\textbf{2.2.6 Oral Contraceptives.} Oral contraceptive (OC) use has generally been associated with an increased risk of developing breast cancer. In an analysis that combined over 54 epidemiological studies\textsuperscript{33}, with a pooled total of 53,297 breast cancer cases, ‘ever’ users of OCs showed a small, but significant, increase in risk (RR=1.07 [SD=0.02], p<0.0001) compared to ‘never’ users. Also, risk increased the longer a woman had used OCs (see Figure 2-2). Since the publication of this paper, other studies have also replicated this finding\textsuperscript{34-36}. However, it appears that this increase in risk does not persist over time, with a trend observed of decreasing risk over increasing duration of time since last use. After 10 years since last use, there is no longer an observed association in relative risk between breast cancer and OCs (RR=1.01 [0.02], p > 0.05)\textsuperscript{33}. Another meta-analysis, using only pre-menopausal women under 50 years of age,
including only more recent data collected from 1980 onwards, found an association between use of OCs prior to FFTP and breast cancer risk (RR=1.44, 95% CI: 1.28-1.62)\(^\text{36}\). The authors hypothesize that this is because the breast is undifferentiated prior to pregnancy, and the hormones contained in OCs increase cell division, and thereby, cancer risk. While there are a large number of epidemiological studies and meta-analyses showing a modest increase in risk of breast cancer due to OCs, there are still others that have found no association at all\(^\text{37}\). Most meta-analyses have included these null studies, and have still found significant increases in risk\(^\text{33,36}\). The answer as to the degree of breast cancer risk faced by OC users is variable; the entire body of research done on the topic does seem to point to a small to modest increase in breast cancer risk.

Use of OCs has consistently been associated with a decreased risk of ovarian cancer, with virtually all studies finding an inverse association between the ‘ever’ use of OCs and ovarian cancer\(^\text{38}\). A large collaborative study combining 45 epidemiological studies, comprising over 23,257 cases and 87,303 controls, was conducted\(^\text{38}\). All 45 studies showed a protective effect of OC use, representing most of the published literature on OC use and ovarian cancer, with only 4 eligible studies not providing their data. In the reanalysis, the authors concluded that the ‘ever’ use of OCs reduces risk of ovarian cancer by 27% (RR=0.73, 95% CI: 0.70-0.76). Furthermore, it appears that the longer a woman uses OCs the more protection against ovarian cancer is conferred (see Figure 1-2). Among OC users, it is estimated that for every 5 years of OC use, there is a 20% reduction in relative risk. Among women in the highest category of use (≥ 15 years), the risk of ovarian cancer is halved. In addition, the protection conferred by OC
use persisted even for women who had stopped taking OCs 30 years earlier. There are important public health implications that can be derived from this research, with the authors stating, based on current trends in OC use and ovarian cancer rates, that about 200,000 incident cases of ovarian cancer and 100,000 deaths worldwide have been prevented due to OCs. With the use of OCs being a modifiable risk factor that an individual is able to control, the results of research into OCs and ovarian cancer has the potential to reduce ovarian cancer risk and the number of deaths due to ovarian cancer.
Figure 2-2. Relative risk of breast (A) and ovarian cancer (B) by duration of use of oral contraceptives. Risk of breast cancer increases with greater durations of OC use. Relative risk of ovarian cancer decreases the longer a woman uses OCs. Figure (A) adapted from (33); (B) adapted from (38).
Oral contraceptives (OCs) are the one risk factor where ovarian and breast cancers are completely discordant (see Table 2-1). It has been shown that there is a small increase in risk of breast cancer due to ‘ever’ use of OC’s. This increase in risk does not persist over time, with the elevated risk disappearing over 10 years after discontinuation of use. On the other hand, use of OCs is a risk factor that time and time again has been associated with a reduced risk of ovarian cancer, with this protection still being present after 30 years since discontinuation of use. Due to the high mortality associated with ovarian cancer, it has been estimated that OCs have potentially prevented the deaths of over 100,000 women worldwide.
<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Cancer</th>
<th>Study Type</th>
<th>Menopausal status</th>
<th>n</th>
<th>Comparison</th>
<th>Odds Ratio (95% CI) unless otherwise stated</th>
<th>Adjustments</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral contraceptive use</td>
<td>Breast</td>
<td>Meta-analysis</td>
<td>Both</td>
<td>53,297 cases/100,329 controls</td>
<td>Never vs ever</td>
<td>1.07 (SD 0.17)</td>
<td>Study, age at diagnosis, parity and age at first live birth.</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Meta-analysis</td>
<td>Premenopausal</td>
<td>NR</td>
<td>Never vs ever</td>
<td>1.19 (1.09, 1.29)</td>
<td>NR</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>Ovarian</td>
<td>Meta-analysis</td>
<td>Both</td>
<td>23,257 cases/87,303 controls</td>
<td>Never vs ever</td>
<td>0.73, (0.70, 0.76)</td>
<td>Study, age, parity and hysterectomy</td>
<td>38</td>
</tr>
<tr>
<td>Hormone replacement therapy use</td>
<td>Breast</td>
<td>Randomized controlled trial</td>
<td>Postmenopausal</td>
<td>8506 treatment group/8102 placebo</td>
<td>Treatment vs placebo</td>
<td>HR: 1.26 (1.0, 1.59) (EPRT only)</td>
<td>NA</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Meta-analysis</td>
<td>Postmenopausal</td>
<td>17,949 cases/35,916 controls</td>
<td>Never vs ever</td>
<td>1.14 (SE:0.03) (EPRT only)</td>
<td>Study, age at diagnosis, time since menopause, BMI, parity and age at first live birth</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Ovarian</td>
<td>Cohort</td>
<td>Postmenopausal</td>
<td>2273 cases from 948,576</td>
<td>Never vs current user</td>
<td>1.20 (1.09, 1.32) (ERT only)</td>
<td>Age, hysterectomy, region of residence, socioeconomic group, time since menopause, parity, BMI, alcohol consumption, OC use</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Case-control</td>
<td>Postmenopausal</td>
<td>812 cases/1313 controls</td>
<td>Never vs ever</td>
<td>1.1 (0.9,1.7) (ERT only)</td>
<td>Age, county of residence, year of diagnosis/reference date, number of full-term pregnancies, and duration of OC use</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Meta-analysis</td>
<td>Postmenopausal</td>
<td></td>
<td>12,273 cases</td>
<td>Never vs ever</td>
<td>EPT:1.28 (1.18,1.40) EPRT: 1.11 (1.02,1.21)</td>
<td>NA</td>
<td>28</td>
</tr>
</tbody>
</table>
2.2.7. Previous Cancer Diagnosis. Please see Table 2-2 for a summary of the results presented below. There are few research studies investigating whether a previous diagnosis of a primary malignant ovarian cancer is a risk factor for a secondary primary diagnosis of breast cancer. What few studies have looked into this have found evidence that there is an increase in breast cancer after an ovarian cancer diagnosis\textsuperscript{39-41}. A large cohort study of over 32,000 ovarian cancer patients found that there was an estimated 18% extra breast cancer second malignancies among primary ovarian cancer cases (SIR: 1.18, 95% CI: 1.07, 1.30)\textsuperscript{40}. The absence of research on secondary tumours in ovarian cancer patients may be due to the fact that ovarian cancer is a high mortality disease, with a 5-year survival rate for ovarian patients at only 40\%\textsuperscript{42}.

There is more research on second malignancies among breast cancer survivors, which could be attributed to the fact that there are more breast cancer survivors, as the incidence of breast cancer is higher, as is the 5-year survival rate, which is around 87\%\textsuperscript{42}. From these studies, it is shown that a prior diagnosis of primary breast cancer is, in itself, a risk factor for ovarian cancer\textsuperscript{43-46}. A recent cohort study, with over 152,600 breast cancer survivors and 712 ovarian cancer second malignancies, showed a significant 28\% increase in risk of a subsequent ovarian cancer (95% CI: 1.19-1.38) for women diagnosed before the age of 50\textsuperscript{43}. Another smaller cohort study, with 9919 breast cancer cases, found a 70\% increase in risk for a subsequent ovarian cancer diagnosis (95% CI: 1.3-2.4)\textsuperscript{46}. However, this analysis is only based on 43 observed ovarian tumours, so the number of second malignancies is small and the results of this smaller study are less reliable.
While there is not a great quantity of research on ovarian cancer second malignancies, from the studies published, it can be concluded that there does seem to be an excess of breast cancer cases among ovarian cancer survivors. The reverse holds true for ovarian cancer. The fact that ovarian cancer is a risk factor for breast cancer and that breast cancer itself is a risk factor for ovarian cancer suggests a common aetiology. That among the women diagnosed with a second ovarian malignancy, they may have a familial or genetic predisposition to develop both breast cancer and ovarian cancer.
<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Cancer</th>
<th>Study Type</th>
<th>n</th>
<th>Comparison</th>
<th>Standard Incidence Ratio (95% CI)</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous ovarian cancer diagnosis</td>
<td>Breast</td>
<td>Cohort</td>
<td>404 secondary breast cancer cases from 32,251 primary ovarian cancer cases</td>
<td>Observed/Expected</td>
<td>1.18 (1.07,1.30)</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cohort</td>
<td>89 secondary breast cancer cases from 5060 primary ovarian cancer cases</td>
<td>Observed/Expected</td>
<td>1.41 (1.14,1.75)</td>
<td>41</td>
</tr>
<tr>
<td>Previous breast cancer diagnosis</td>
<td>Ovarian</td>
<td>Cohort</td>
<td>712 secondary ovarian cancer cases from 152,586 primary breast cancer cases</td>
<td>Observed/Expected</td>
<td>1.28 (1.19-1.38)</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cohort</td>
<td>181 secondary ovarian cancer cases from 31,818 primary breast cancer cases</td>
<td>Observed/Expected</td>
<td>1.4 (1.2,1.6)</td>
<td>44</td>
</tr>
<tr>
<td>(multiple countries)</td>
<td></td>
<td>Cohort</td>
<td>2277 secondary ovarian cancer cases from 525,527 primary breast cancer cases</td>
<td>Observed/Expected</td>
<td>Pre-meno: 2.84 (2.61,3.09)</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Peri-meno: 1.64 (1.52,1.77)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Post-meno: 1.12 (1.06,1.19)</td>
<td></td>
</tr>
</tbody>
</table>
2.2.8 Mammographic Density. The strongest known risk factor for breast cancer is mammographic density (MD), which is the radiological appearance of breast tissue on a mammogram. The appearance of breast tissue on a mammogram differs among women and reflects breast tissue variation\(^{47}\). The differences in breast tissue composition are due to stroma and epithelial tissue, which appear light on a mammogram, and fatty tissue, which appear dark\(^{48}\). Women in the highest density category face a 4-6 times greater relative risk of developing breast cancer in comparison to the women in the lowest density category\(^{49}\). MD has been shown to vary according to height, weight\(^{50,51}\), parity\(^{52,53}\), menopausal status\(^{54}\) and hormone use\(^{55-56}\), which are also all risk factors for breast cancer and ovarian cancer. As far as we are aware, breast density has never been looked at in relation to ovarian cancer.

2.3 Weaker Influences on Breast and Ovarian Cancer

2.3.1 Parity. A summary of the literature used to investigate the association between parity and breast and ovarian cancer is provided in Table 2-3. Parity is associated with a reduced risk of breast cancer\(^{1,57-58}\). A large meta-analysis was undertaken that combined 8 cohort and case-control Nordic studies, and had a combined total of 5,568 breast cancer cases. They found that compared to nulliparous women, having one or more live births confers a 30% reduction in risk of developing breast cancer (RR=1.30; 95% CI: 1.20-1.41)\(^{57}\). In fact, it has been found that each live birth confers a 10% reduction in risk of breast cancer\(^{59}\). The mechanism behind this effect has been hypothesized to be due to increased breast differentiation brought about during pregnancy\(^{60}\). This differentiation is thought to reduce the total epithelial tissue
in the breast, which is susceptible to carcinogenesis as it is the tissue from which cancer arises\textsuperscript{58}.

Parity has also been associated with a reduced risk of ovarian cancer\textsuperscript{1,61-63}. A large nested case-control study was conducted that had 3486 ovarian cancer cases, a sizable number for an ovarian cancer study, found that nulliparous women faced an estimated 31\% increase in relative risk compared to parous women\textsuperscript{61}. Among parous women, there has also been an observed 20\% reduction in risk of ovarian cancer for each live birth\textsuperscript{61}.

Parity has consistently been shown to be a protective risk factor for both ovarian and breast cancer. Women who have had children are at a reduced risk of developing both ovarian and breast cancer in comparison to nulliparous women, and each live birth reduces the risk associated with both cancers further.

\textbf{2.3.2 Age at First Birth.} It has been found that a later age at first birth increases risk of breast cancer\textsuperscript{58,59}, with relative risk increasing by as much as 51\% (RR=1.51, 95\% CI: 1.35-1.80) for women who delayed childbirth until after 31\textsuperscript{59} compared to women who gave birth before the age of 20, adjusted for parity. There were over 12,000 cases in this nested case-control study, providing some reliability to the results. The effect of age at first birth appears to be greatest for women who were diagnosed before the age of 40, with a 10\% increase in risk for every year childbirth is delayed\textsuperscript{64}, as found in a smaller nested case-control study.

For ovarian cancer, when an association is found with age at first birth, it is actually for a reduction in risk of ovarian cancer for every 5 years of delayed parity\textsuperscript{61,65-}. 
controlling for age, parity, and OC use. Most of the studies in the literature addressing this question are case-control, with smaller numbers of cases. From these single-study results, the effect of age at first birth on ovarian risk is less straightforward, as many studies have not found any association\textsuperscript{67,68}, and other studies actually finding an increase in risk for delayed age at first birth\textsuperscript{62,69}. However, the majority of studies do find a decrease in risk of ovarian cancer for delayed age at first birth, but more research is needed before any conclusions can be drawn.

Table 2-3 summarizes the results above. An older age at first birth has been associated with an increase in the risk of breast cancer. Whereas for ovarian cancer, when there is an association shown, it is usually opposite the direction shown in the breast cancer literature, with a decrease in risk of ovarian cancer for a delayed first childbirth.
<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Cancer</th>
<th>Study Type</th>
<th>n</th>
<th>Comparison</th>
<th>Odds Ratio (95% CI)</th>
<th>Adjustments</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parity</td>
<td>Breast</td>
<td>Meta-analysis</td>
<td>5868 cases</td>
<td>Parous vs nulliparous</td>
<td>1.30 (1.20-1.41)</td>
<td>Age</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nested case-control in cohort</td>
<td>12,782 cases/matched to 5 controls</td>
<td>Nulliparous vs 3 live births</td>
<td>0.76 (0.71, 0.81)</td>
<td>Age</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Population based case-control</td>
<td>1508 cases/1556 controls</td>
<td>Nulliparous vs parous</td>
<td>0.78 (0.63, 0.98)</td>
<td>Age</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>Ovarian</td>
<td>Nested case-control in cohort</td>
<td>3486 cases/19,980 controls</td>
<td>One live birth vs nulliparous</td>
<td>1.31 (1.18, 1.46)</td>
<td>Age</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Collaborative analysis of 12 case-control studies</td>
<td>2197 cases/8893 controls</td>
<td>Nulliparous vs parous</td>
<td>Hospital based: 0.76 (0.63, 0.93) Population based: 0.47 (0.4, 0.56)</td>
<td>Age, study, OC use</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pooled analysis of 3 case-control studies</td>
<td>1140 cases/2724 controls</td>
<td>Nulliparous vs 4 live births</td>
<td>0.60 (0.4, 0.8)</td>
<td>Age, study, socio-cultural indicators, age at menopause, OC use, # of abortions, age at first birth.</td>
<td>62</td>
</tr>
<tr>
<td>Age at first birth</td>
<td>Breast</td>
<td>Meta-analysis</td>
<td>5866 cases</td>
<td>&lt;20 vs &gt;35</td>
<td>1.40 (1.15, 1.70)</td>
<td>Age, parity</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nested case-control in cohort</td>
<td>12,782 cases/matched to 5 controls</td>
<td>&lt;18 vs &gt;31</td>
<td>1.51 (1.35, 1.80)</td>
<td>Parity</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Population based case-control</td>
<td>1508 cases/1556 controls</td>
<td>&lt;22 vs &gt;28 (among parous)</td>
<td>1.36 (1.10, 1.69)</td>
<td>Age</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>Ovarian</td>
<td>Nested case-control in cohort</td>
<td>3486 cases/19,980 controls</td>
<td>&lt;20 vs ≥35 (Epithelial cancer)</td>
<td>0.62 (0.45, 0.87)</td>
<td>% of births</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Case-control</td>
<td>655 cases/3899 controls</td>
<td>&lt;20 vs ≥35</td>
<td>0.49 (0.25, 0.96)</td>
<td>Age, parity, BMI, OC use, HRT use, age at menopause</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Case-control</td>
<td>233 cases/900 controls</td>
<td>&lt;20 vs ≥ 30</td>
<td>0.97 (0.6, 1.7)</td>
<td>Age at diagnosis, stage of residence, and parity</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pooled analysis of 4 case-control studies</td>
<td>623 cases/3432</td>
<td>&lt;19 vs ≥ 25</td>
<td>1.4 (1.1, 1.8)</td>
<td>Age, study, parity, OC use, race, tubal ligation and family history of breast/ovarian cancer</td>
<td>69</td>
</tr>
</tbody>
</table>
2.3.3. Breastfeeding. Table 2-4 summarizes the results found in the studies referenced below. To examine the effect of breast cancer and breastfeeding, a large meta-analysis was conducted that combined the results of 47 epidemiological studies, and comprised over 80% of the worldwide literature reported to date\(^70\). The authors were able to show a statistically significant decrease in relative risk of breast cancer of 4.3% for each year a woman breastfeeds. The authors hypothesize that some of the inconsistency in the literature may in part be due to the fact that the decrease in risk due to breastfeeding is relatively low for each year of lactation, and that by chance, some studies would not detect a significant association. In North America and Europe, where the rates of breastfeeding are lower and duration of lactation is less, this explanation is especially relevant. A smaller case-control study (622 matched pairs) was conducted in Turkey, a developing country\(^71\). The results of this study are very informative because, in Turkey, women have more traditional roles and many women breastfeed for long periods of time, longer than in the developed countries where most studies are set. The authors found that longer durations of lactation (>48 months) were associated with a statistically significant 65% reduction in relative risk among postmenopausal women, while not finding an association between ever lactating and breast cancer\(^71\).

Many studies support an inverse association in risk\(^66,17-18\) between ovarian cancer and breastfeeding. The relative risk for ever-breastfed compared to never ranges from 0.70 (95% CI: 0.5-1.0)\(^66\) to 0.86 (95% CI 0.70-1.06)\(^72\). While those results are taken from single case-control study papers, both had somewhat larger sample sizes, providing some confidence to their results. Duration of lactation also appears to have an effect on
risk, with longer durations of breastfeeding further reducing risk. One prospective cohort study using the Nurses Health Study participants, a cohort of almost 150,000 women, was able to demonstrate a 34% reduction in risk for breastfeeding for 18 months or longer (RR=0.66, 95% CI: 0.46-0.96), and a 2% reduction in relative risk for every month breastfed (RR= 0.98; 95% CI: 0.97-1.00)\textsuperscript{72}. There are few case-control studies that do not find any association between breastfeeding and risk of ovarian cancer\textsuperscript{13,73}, and one study even reported an increase in risk\textsuperscript{74}.

There has been plenty of research on breastfeeding and risk of both breast and ovarian cancer, in part, because breastfeeding is a truly modifiable risk factor, with most women being able to choose to breastfeed and the duration of time they will spend breastfeeding. If breastfeeding is a protective factor for either cancer, women can be strongly encouraged to breastfeed as a preventative cancer measure in addition to providing nourishment for their baby. Research in this area has generally supported a reduction in risk for longer durations of breastfeeding, however, for ovarian cancer, the body of research on this topic is more inconsistent.
Table 2-4. Relative risks for breast and ovarian cancer — Breastfeeding.

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Cancer</th>
<th>Study Type</th>
<th>n</th>
<th>Comparison</th>
<th>Odds Ratio (95% CI)</th>
<th>Adjustments</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breastfeeding</td>
<td>Breast</td>
<td>Meta-analysis</td>
<td>50,302 cases/96,973 controls</td>
<td>Never (0) vs ≥ 55 months</td>
<td>0.73 (0.049 FSE)</td>
<td>Study, age, parity, age at first birth, and menopausal status</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Case-control</td>
<td>622 matched pairs</td>
<td>Never (0) vs &gt;48 months</td>
<td>Postmenopausal: 0.36 (0.14, 0.93)</td>
<td>Age, marital status, menopausal status and age at menopause, BMI, age at first birth, smoking, first degree relative with breast cancer, history of BBD</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>Ovarian</td>
<td>Case-control</td>
<td>563 cases/523 controls</td>
<td>Ever vs. never</td>
<td>0.7 (0.5, 1.0)</td>
<td>Age, state, parity</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cohort</td>
<td>391 cases from 149,693 cohort</td>
<td>Never (0) vs ≥ 18 months</td>
<td>0.66 (0.46, 0.96)</td>
<td>Age, parity, duration of oral contraceptive use, tubal ligation, age at menarche</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Case-control</td>
<td>1031 cases/2411 controls</td>
<td>Never (0) vs &gt; 17 months</td>
<td>1.21 (0.85, 1.71)</td>
<td>Age, study center, education, parity, OC use, family history of ovarian and/or breast cancer</td>
<td>73</td>
</tr>
</tbody>
</table>
2.3.4 Age at Menarche. Table 2-5 summarizes the research discussed below. There has been evidence that a later age at menarche may be associated with a decreased risk of breast cancer\textsuperscript{75-77}. A multi-center case-control study was conducted that, when combined, had approximately 4000 breast cancer cases\textsuperscript{75}. This study reported a reduction in risk of 10% for every 2 year delay in menstruation (95% CI: 6-15\%) \textsuperscript{75}, while others have found the reduction in risk to only apply to premenopausal women\textsuperscript{77}. The association between age at menarche and breast cancer risk has not proven consistent, and other studies have found no effect at all\textsuperscript{71,78}.

A case-control study with over 1000 ovarian cases, found that a later age at menarche (≥ 15 years old) has also been associated with an overall reduced risk of ovarian cancer (RR=0.8, 95% CI: 0.6-1.0)\textsuperscript{13}, adjusted for age, parity and OC use. Other case-control studies that have looked at age at menarche in relation to menopausal status have found a greater reduced risk of ovarian cancer among premenopausal women who had a later age at menarche\textsuperscript{66,79}. However, others have found an overall association between later age at menarche and ovarian cancer to be either weak or non-existent\textsuperscript{68,80-81}.

Research looking into late age at menarche and risk of breast and ovarian cancer is inconsistent and weak. Most of the research in this area is case-control, and among the studies published for ovarian cancer, are based on a small sample sizes. When an association is found, for both breast and ovarian cancer, the relationship is inverse and strongest among premenopausal women.
2.3.5. Age at Menopause. The results discussed below are presented in Table 2-5. There is a consistent association between a later age at menopause and an increased relative risk of breast cancer\(^71,75-78\). Data from an international, multi-center case-control study has estimated that for every 5 year delay in menopause there is a 17\% increase in relative risk of breast cancer\(^75\). Another large, multi-centre case-control study found a 40\% increased relative risk for women who went through a later menopause (>54 years old) compared to women who stopped menstruating younger than 40 years old\(^76\).

The evidence is less consistent when looking at later menopause and ovarian cancer, but a case-control study with over 1000 ovarian cases showed that women who stopped menstruating before the age of 45 are at a statistically significantly reduced relative risk of developing ovarian cancer compared to women who stop menstruating after 53 (RR=0.6, 95\% CI: 0.5-0.9)\(^\)\(^20\). The effect has been replicated in the literature\(^65,74,80\), but others have found no association\(^63,66,68\). In a pooled analysis of 12 case-control studies, totalling 2200 cases, no association was found between ovarian cancer and an early age at menopause\(^63\).

What appears to be more important in terms of menstrual factors and risk of breast or ovarian cancer is an earlier age at menopause. A review of the literature on breast cancer has shown a general consensus that an earlier age at menopause is associated with a reduced risk of breast cancer. A reduced risk of ovarian cancer has also been shown, however, with less reproducibility than in the breast cancer literature.
Table 2-5. Relative risks for breast and ovarian cancer — Menstrual factors.

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Cancer</th>
<th>Study Type</th>
<th>Menopausal Status</th>
<th>N</th>
<th>Comparison</th>
<th>Odds Ratio (95% CI)</th>
<th>Adjustments</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at menarche</td>
<td>Breast</td>
<td>Multi-center</td>
<td>NR</td>
<td>3993 cases/11,783 controls</td>
<td>+ 2 years</td>
<td>0.90 (0.85-0.94)</td>
<td>Study center, age at interview, age at first pregnancy, parity</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>case-control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combined analysis of 4 case-control studies</td>
<td></td>
<td>NR</td>
<td>6075 cases/5492 controls</td>
<td>&lt;11 years vs &gt;15 years</td>
<td>0.90 (0.7-1.0)</td>
<td></td>
<td>Study center, age, nulliparity/age at first birth, age at menopause</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Case-control</td>
<td>Premenopausal</td>
<td>1185 cases/3227 controls</td>
<td>&lt;11 years vs &gt;15 years</td>
<td>1.4 (0.7, 2.5)</td>
<td>Age</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cohort</td>
<td>NR</td>
<td>2085 cases/70575 controls</td>
<td>&lt;12 years vs ≥16 years</td>
<td>0.81 (0.65, 1.02)</td>
<td>Age, screening center, age at menopause, family history of breast cancer, benign breast disease, height, and menopausal hormone therapy, combined age at first birth and parity</td>
<td>78</td>
</tr>
<tr>
<td>Ovarian</td>
<td></td>
<td>Case-control</td>
<td>NR</td>
<td>1031 cases/2411 controls</td>
<td>≤12 years vs ≥15 years</td>
<td>0.8 (0.60-1.0)</td>
<td>Age, study center, education, parity, OC use, and family history of ovarian cancer</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Case-control</td>
<td>Premenopausal</td>
<td>194 cases/710 controls</td>
<td>≤12 years vs ≥15 years</td>
<td>0.6 (0.3,1.1)</td>
<td>Age, education, family history, number of births, number of abortions, OC use.</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Case-control</td>
<td>Premenopausal</td>
<td>563 cases/523 controls</td>
<td>&lt;16 years vs ≥16 years</td>
<td>0.5 (0.2-1.0)</td>
<td>Age, state, parity</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pooled analysis of 3 case-control studies</td>
<td>NR</td>
<td>1140 cases/2724 controls</td>
<td>≥15 years vs &lt;12 years</td>
<td>1.0 (0.8-1.2)</td>
<td>Age, social class, parity, OC use, age at menopause</td>
<td>80</td>
</tr>
<tr>
<td>Age at menopause</td>
<td>Breast</td>
<td>Multi-center case-control</td>
<td>NR</td>
<td>3993 cases/11,783 controls</td>
<td>+ 5 years</td>
<td>1.17 (1.11, 1.22)</td>
<td>Age, study center, age at first pregnancy and parity</td>
<td>75</td>
</tr>
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</tr>
<tr>
<td>Combined analysis of 4 case-control studies</td>
<td>NR</td>
<td>6075 cases/5492 controls</td>
<td>&lt;40 vs &lt;53</td>
<td>1.4 (1.1, 1.8)</td>
<td>Study center, age, nulliparity/age at first birth, age at menarche</td>
<td>76</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cohort</td>
<td>NR</td>
<td>2085 cases/70575 controls</td>
<td>≥55 years vs &lt;45 years</td>
<td>1.29 (1.03, 1.62)</td>
<td>Age, screening center, age at menarche, family history of breast cancer, benign breast disease, height, and menopausal hormone therapy, combined age at first birth and parity</td>
<td>78</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovarian</td>
<td>Pooled analysis of 3 case-control studies</td>
<td>NR</td>
<td>1140 cases/2724 controls</td>
<td>&lt;45 years vs ≥53 years</td>
<td>1.9 (1.3, 2.6)</td>
<td>Age, social class, parity, OC use, age at menarche</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>Case-control study</td>
<td>NR</td>
<td>655 cases/3899 controls</td>
<td>49-52 years vs &lt;49 years</td>
<td>0.77 (0.61-0.96)</td>
<td>Age, parity, BMI, age at menopause, OC use, HRT use.</td>
<td>65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collaborative analysis of 12 case-controls studies</td>
<td>NR</td>
<td>2197 cases/8893 controls</td>
<td>&lt;45 years vs ≥53 years</td>
<td>Hospital based: 1.2 (0.69-1.7) Population based: 0.82 (0.54-1.3)</td>
<td>Age, study, parity and OC use.</td>
<td>63</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2.3.6. Body Mass Index. A summary of the results presented below is provided in Table 2-6. A high BMI has been associated with an increased risk of breast cancer for postmenopausal women (RR= 1.23, 95% CI: 1.00-1.59), however, for premenopausal women, there is an inverse association in risk related to high BMI (RR= 0.66, 95% CI: 0.40-1.10). This effect of menopause on breast cancer risk and BMI has been replicated in many cohort and case-control studies. The strongest association between breast cancer and a BMI of greater than 31 has been found among women not using HRT (RR=2.5, 95% CI = 1.62-3.93) compared to women with a BMI less than 22.6, and replicated in other studies. Most studies have reported an inverse association between BMI at age 18 and later risk of breast cancer (RR = 0.57, 95% CI, 0.41-0.81). This reduction in breast cancer risk due to higher BMI at age 18 has been shown for both premenopausal and postmenopausal breast cancer.

A meta-analysis was conducted on 28 cohort and case-control studies looking at the association between BMI and ovarian cancer. The authors found an overall 30% increase in risk (RR: 1.30, 95%CI: 1.12, 1.50) of ovarian cancer among women who are in the ‘obese’ BMI category. Others report the increase in risk between obesity and ovarian cancer only among pre-menopausal women under the age of 50, with relative risk ranging from 1.72 (95% CI: 1.02-2.89) to 2.19 (95% CI: 1.19-4.04). The effect of a high BMI also appears to be strongest for heavy nulliparous women (RR= 3.73, 95% CI: 1.88, 7.42). There has been inconsistent evidence for association of BMI in early life and ovarian risk, with various studies showing a modest effect of early life weight and ovarian risk, while others show no effect at all. A large, population-based cohort
study looking at 1.1 million Norwegian women, found that women who had a ‘very high’ BMI in adolescence had a 56% increase in risk of developing ovarian cancer later on in life compared to women with a ‘medium’ BMI (RR=1.56; 95% CI: 1.04 - 2.32)\(^9\).

For both ovarian and breast cancer, BMI has been shown to be a risk factor, with those women in the obese or severely obese categories facing elevated risk of both cancers\(^97\)-\(^100\). In the literature, recent attention has been paid to BMI in early life, with a high BMI at age 18 associated with a decreased risk of adult breast cancer. There is really no clear trend in the literature in relation to early life BMI and ovarian risk, but among the published research, there does seem to be an increase in risk associated with a high BMI in early life.
<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Cancer</th>
<th>Study Type</th>
<th>Menopausal status</th>
<th>N</th>
<th>Comparison</th>
<th>Odds Ratio (95% CI) unless otherwise stated</th>
<th>Adjustments</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>Breast</td>
<td>Cohort</td>
<td>Both</td>
<td>786 pre-menopause cases/ 1522 post-menopause cases from 94,805 cohort</td>
<td>18.25-25 kg/m² vs ≥30 kg/m²</td>
<td>Pre-Meno: 0.66 (0.40,1.10) Post-Meno: 1.23 (1.00,1.59)</td>
<td>NR</td>
<td>82</td>
</tr>
<tr>
<td>Cohort</td>
<td>Both</td>
<td>Both</td>
<td>1,879 cases from 73,542 pre-menopausal and 103,344 post-menopausal women</td>
<td>&lt;21.6 kg/m² vs ≥28.8 kg/m²</td>
<td>Pre-Meno: 0.82 (0.59,1.14) Post-Meno: (non-HRT): 1.36 (1.06,1.75)</td>
<td>Study center, age, education, smoking status, alcohol consumption, parity, age at first pregnancy, age at menarche, OC use</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td>Pooled analysis</td>
<td>Both</td>
<td>Both</td>
<td>4385 cases from 337,819 cohort</td>
<td>&lt;21 kg/m² vs ≥33 kg/m²</td>
<td>Pre-Meno: 0.58 (0.34, 1.0) Post-Meno: 1.27 (1.03-1.55)</td>
<td>Age at menarche, parity, age at first birth, HRT use, OC use, benign breast disease, family history of breast cancer, smoking status, education, fat intake, energy intake, and alcohol intake</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>Ovarian</td>
<td></td>
<td>Pooled analysis</td>
<td>NR</td>
<td>NR</td>
<td>18.5-24.9 kg/m² vs ≥30 kg/m²</td>
<td>1.30 (1.12-1.50)</td>
<td>NR</td>
<td>89</td>
</tr>
<tr>
<td>Cohort</td>
<td>NR</td>
<td>Both</td>
<td>2036 cases from 531,583 cohort</td>
<td>&lt;23 kg/m² vs ≥30 kg/m²</td>
<td>Pre-Meno: 1.72 (1.02, 2.89) Post-Meno: 1.07 (0.87,1.33)</td>
<td>Age at menarche, OC use, parity, smoking status, physical activity, and energy intake</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>Pooled analysis</td>
<td>NR</td>
<td>303 cases from 94,525 cohort</td>
<td>&lt;25 kg/m² vs ≥30 kg/m²</td>
<td>Non-HRT user: 1.83 (1.18,2.84) HRT ‘ever’ user: 0.96 (0.65,1.43)</td>
<td>Age, race/ethnicity, family history of ovarian or breast cancer, duration of OC use, HRT use, physical activity</td>
<td>97</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2.4 Biological Plausibility

Breast and ovarian cancer share similar ages of incidence and similar risk factors, and this suggests a common underlying biological mechanism. Below, we will discuss two potential biological mechanisms for which there is sufficient data to compare the two cancers and provide potential explanations as to their similar age of incidence and shared biological and hormonal risk factors.

2.4.1. Total Lifetime Ovulations. Calculating total lifetime ovulations has been detailed elsewhere\textsuperscript{101}, but briefly, the total number of ovulatory years is calculated using information about the regularity of a woman’s menstrual cycle (i.e. number of days between menstrual periods), the age she began menstruation and the age she started menopause\textsuperscript{101}. The number of ovulations per year can be determined using days per cycle. Number of pregnancies (9 months of delayed ovulation), time spent breastfeeding (1.5 months delayed ovulation per pregnancy) and years on oral contraceptives are all subtracted from this total, providing a total number of ovulations. All studies looking at total lifetime ovulations have all been cohort or case-control studies, with modest to large sample sizes. Table 2-7 summarizes the results discussed below.

Total number of lifetime ovulations has been implicated in breast cancer risk, with women in the highest category of ovulatory cycles facing between a 1.60 (95% CI: 1.25-2.04)\textsuperscript{102} to a 1.80 (95% CI: 1.09-2.96)\textsuperscript{101} increase in risk compared to those in the lowest category of cumulative ovulations, both reported risks from separate cohort studies, one with a small sample size of only 168 cases\textsuperscript{101} and the other having over 1700
breast cancer cases\textsuperscript{102}. As both reported similar results and estimated risks, the data do seem to point to a true increase in risk of breast cancer due to greater lifetime ovulations. The higher risk of breast cancer associated with greater total number of ovulations has been suggested to be due to prolonged exposure of breast tissue to ovarian hormones.

Pelucchi et al. (2007)\textsuperscript{103} used data from two large case-control studies looking at ovarian cancer risk factors, for a total combined ovarian cancer population of 1822 cases versus 4631 controls. It was shown that those women who were in the greatest lifetime ovulations category (≥ 481) had an 81% increase in relative risk (95% CI, 1.47-2.23) of developing ovarian cancer, compared to women in the lowest cumulative ovulatory cycle category (<357)\textsuperscript{103}. Similar results and estimated risk have been shown in other studies\textsuperscript{104-106}.

The total number of ovulatory years a women undergoes in her lifetime has been linked to increased risk for both breast and ovarian cancer, independent of age, parity, HRT use, and menopausal age. A study was conducted to determine what hormone(s) were elevated among those women in the highest category versus the lowest, and it was found that there was a significant inverse association between lifetime ovulatory cycles and sex-hormone binding globulin (SHBG)\textsuperscript{107}. SHBG binds to estradiol, which prevents it from leaving the circulatory system and entering into the cell. Only unbound estradiol is able to enter the cell, and as estradiol is related to cell division and proliferation, lesser quantities of this unbound hormone are available to influence cell tissue with increased levels of SHBG\textsuperscript{107}. 

38
<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Cancer</th>
<th>Study Type</th>
<th>Menopausal Status</th>
<th>N</th>
<th>Comparison</th>
<th>Odds Ratio (95% CI)</th>
<th>Adjustments</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total lifetime ovulations</td>
<td>Breast</td>
<td>Cohort</td>
<td>Postmenopausal</td>
<td>168 cases from 6031 eligible participants</td>
<td>≤ 415 cycles vs ≥491 cycles</td>
<td>1.80 (1.09, 2.96)</td>
<td>Age, family history of breast cancer, BMI, &gt;1 year of irregular cycles, HRT use, infertility, education, parity and age at first birth</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cohort</td>
<td>Postmenopausal</td>
<td>1718 cases from &gt;100,000 eligible participants</td>
<td>&lt;403 cycles vs ≥521 cycles</td>
<td>1.56 (1.22–1.99)</td>
<td>Age, alcohol intake, benign breast disease, infertility, family history of breast cancer, BMI, OC use, ever married, age at firstbirth/nulliparae, educational level</td>
<td>102</td>
</tr>
<tr>
<td>Ovarian</td>
<td></td>
<td>Case-control (2 combined studies).</td>
<td>Both</td>
<td>1822 cases/ 4631 controls</td>
<td>&lt;357 cycles vs ≥481</td>
<td>1.81 (1.47, 2.23)</td>
<td>Study, calendar year at interview, age, center, education, HRT use, and family history of ovarian and breast cancer in first degree relatives</td>
<td>103</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Case-control</td>
<td>Both</td>
<td>558 cases/ 601 controls</td>
<td>&lt;22.1 ovulatory years vs &gt;34.1 ovulatory years</td>
<td>1.82 (1.17, 2.85)</td>
<td>Age, ethnicity, study site, education, tubal ligation, HRT use, and ovulation variables</td>
<td>104</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Case-control</td>
<td>Both</td>
<td>668 cases/ 721 controls</td>
<td>Low vs High</td>
<td>1.88 (1.42, 2.47)</td>
<td>Age, study center, caesarean section, tubal ligation and hysterectomy</td>
<td>105</td>
</tr>
</tbody>
</table>
2.4.2. Insulin-like Growth Factor. Circulating levels of insulin-like growth factor (IGF-1), a known mitogen, has been linked to cancer risk; it is believed that IGF-1 increases cell proliferation and inhibits apoptosis\textsuperscript{108}, both of which increase risk of cancer. There is a summary of the current literature on this topic provided in Table 2-8.

Higher circulating levels of IGF-1 have been associated with an increased risk of breast cancer\textsuperscript{109-112}. The association between IGF-1 levels and breast cancer risk seem to be modified by age and/or menopausal status, with women under the age of 50 or premenopausal at time of diagnosis showing the strongest effect\textsuperscript{109}. A meta-analysis conducted on the literature, totalling 1263 breast cancer cases, showed a 65% increase in relative risk between premenopausal women in the highest quintile of IGF-1 compared to the lowest (RR=1.65, 95% CI: 1.26-2.08)\textsuperscript{109}, this effect being replicated in other studies since\textsuperscript{110-112}.

Research investigating whether IGF-1 plays a role in ovarian cancer is now also emerging. There are currently only three small nested case-controls studies addressing this research question. One case-control study noted a 2.4 times increase in risk between in highest and lowest tertiles of IGF-1 serum levels (95% CI= 0.9-6.4)\textsuperscript{113}. Another case-control study also found an almost 5 times increase in risk for women diagnosed under the age of 55 (RR = 4.97; 95% CI = 1.22-20.2)\textsuperscript{114}. However, the final study, a nested case-control study with participants taken from the Nurses Health Study, found no association at all\textsuperscript{115}.

IGF-1 has been associated with an increased risk of breast cancer\textsuperscript{109-112}, and more recently, with an increased risk of ovarian cancer\textsuperscript{113-114}. The evidence supporting the
association with premenopausal breast cancer risk is fairly strong, however, research into IGF-1 and ovarian cancer presents more varied results. More research needs to be done before any conclusions can be drawn about the association of elevated IGF-1 levels and ovarian cancer risk. When an association is found between IGF-1 and ovarian cancer, it is among the same group that shows an increase in risk for breast cancer, those diagnosed before the age of 55 or premenopausal women.
<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Cancer</th>
<th>Study Type</th>
<th>n</th>
<th>Comparison</th>
<th>Odds Ratio (95% CI)</th>
<th>Adjustments</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF-1</td>
<td>Breast</td>
<td>Meta-analysis</td>
<td>591 pre-meno cases/1193 controls and 672 post-meno cases/1131 controls</td>
<td>75th vs 25th percentile</td>
<td>Pre-meno: 1.93 (1.38,2.69) Post-meno: 0.95 (0.62,1.33)</td>
<td>Maximally adjusted ORs</td>
<td>109</td>
</tr>
<tr>
<td>Nested case-control</td>
<td>800 cases/ 1129 controls</td>
<td>Tertile1 vs tertile 3</td>
<td>Pre-meno: 1.6 (1.0,2.5)</td>
<td></td>
<td>Adjusted for matching variables (year of birth, menopausal status, recent HRT use, month and time of day of blood collection, and fasting status at blood draw.)</td>
<td>111</td>
<td></td>
</tr>
<tr>
<td>Ovarian</td>
<td>Nested case-control</td>
<td>214 cases/ 388 controls</td>
<td>Tertile 1 vs tertile 3</td>
<td>≥55 years: 2.4 (0.9,6.4)</td>
<td></td>
<td>Study centre, menopausal status, age, time of the day of blood collection, phase of menstrual cycle (for premenopausal women), time between blood drawn, last consumption of food/drinks, BMI, previous HRT use, previous OC use, fertility problems, parity</td>
<td>113</td>
</tr>
<tr>
<td>Nested case-control</td>
<td>132 cases/263 controls</td>
<td>Tertile 1 vs tertile 3</td>
<td>&lt;55years: 4.98 (1.21,20.6)</td>
<td></td>
<td>Full-term pregnancy, BMI, smoking, level of IGFBI-3.</td>
<td>114</td>
<td></td>
</tr>
<tr>
<td>Nested case-control</td>
<td>222/599</td>
<td>Quartile 1 vs quartile 4</td>
<td>&lt;55years: 0.70 (0.25,1.97)</td>
<td></td>
<td>Parity, duration of OC use, hysterectomy, tubal ligation, HRT use, physical activity, age at menopause, age at menarche, and matching factors including age at blood draw, fasting status, time of blood draw, month of blood draw, menopausal status at baseline and diagnosis, and recent HRT use before blood draw.</td>
<td>115</td>
<td></td>
</tr>
</tbody>
</table>
2.5 Conclusion

Breast and ovarian cancer share many hormonal and biological risk factors, and some have a stronger influence on risk than others. Both cancers appear to be influenced by ovarian function and the hormones released during menstruation, in addition to a shared genetic predisposition and clustered familial history. Breast and ovarian cancer have been shown to be strongly influenced by age, BRCA1/2 mutation, radiation, a family history of breast and/or ovarian cancer, HRT use, use of oral contraceptives and a previous cancer diagnosis. All of these strong risk factors increase risk, with the exception of oral contraceptives, which increases risk of breast cancer and decreases risk of ovarian cancer. One strong risk factor for breast cancer, mammographic density, has never been looked at in relation to ovarian cancer risk.

Both cancers are also more weakly influenced by nulliparity, age at menarche, delayed menopause, breastfeeding and obesity. For these risk factors, increases in risk are also seen, however, the body of research addressing these risk factors are more varied and the results less consistent, especially in the ovarian cancer literature.

The fact that breast and ovarian cancer share so many biological, reproductive and hormonal risk factors point to a common aetiology shared between these two cancers. There are two risk factors shared between breast and ovarian cancer that provide biologically plausible explanations as to why breast and ovarian cancer share many of the same risk factors. Increased number of total lifetime ovulations has been associated with both breast and ovarian cancer, and women in the highest category of ovulations have been shown to have higher circulating levels of SHBG. Circulating
levels of IGF-1 have also been associated with increased risk of breast and ovarian cancer, although less consistently so for ovarian cancer.

This chapter has shown that breast and ovarian cancer share many of the same biological and hormonal risk factors. From the evidence provided, we believe they share a common aetiology that should be investigated further.
CHAPTER 3:

Mammographic Density as a Risk Factor for Ovarian Cancer:

A Pilot Study.
3.1 Introduction

Ovarian cancer is the fifth most common cancer among women, with 2500 Canadian women being diagnosed every year, and 1700 reported deaths\textsuperscript{42}. Epithelial ovarian cancer accounts for 90\% of the observed tumours, with germ cell and stromal tumours accounting for the remaining 10\%.\textsuperscript{116} The five-year survival rate for ovarian cancer is only 40\%\textsuperscript{42}. One of the reasons for this low survival rate is that ovarian cancer has few early warning signs, and the warning signs that do present themselves are symptoms of other, more common illnesses\textsuperscript{116}. By the time a woman starts exhibiting more serious symptoms that would cause her to seek medical attention, the disease has often already progressed to the later stages, which have the worst survival outcomes. Approximately 60\% of all ovarian cancers are diagnosed in Stages III or IV (cancer has spread to outside pelvis), with only 25\% being diagnosed in Stage I (cancer has not spread beyond ovaries)\textsuperscript{11}. In addition to the few warning signs and later stage diagnoses, there are also no standard screening methods to assist with early stage diagnosis of ovarian cancer\textsuperscript{116}.

3.1.1 Rationale. As discussed in the previous chapter, oral contraceptive use, parity, breastfeeding, and an earlier age at menopause are associated with a decreased risk of ovarian cancer. Obesity, greater cumulative lifetime ovulatory cycles, use of hormone replacement therapy, a family history of breast and/or ovarian cancer and a BRCA1/2 mutation have all been associated with an increased risk of ovarian cancer\textsuperscript{63,117-118}. These same risk factors have also been associated with breast cancer, as summarized previously. Mammographic density (MD), which is the strongest known
risk factor for breast cancer, has been shown to vary due to these same common risk factors\textsuperscript{54,119-120}. This demonstrates that MD is influenced by ovarian activity and hormones, as most of the risk factors shared between ovarian and breast cancer are hormonal and reproductive in nature. However, even though MD is one of the strongest known risk factors for breast cancer, and breast cancer and ovarian cancer share so many of the same risk factors, to the best of my knowledge, no one has ever examined whether MD is a risk factor for ovarian cancer.

\textbf{3.1.2 Hypothesis.} We generally hypothesize that higher mammographic density may be associated with an increased risk of ovarian cancer. This study is a pilot study seeking to determine whether a larger study looking at MD as a potential risk factor for ovarian cancer is feasible. In the future we would like to investigate whether ovarian cancer patients have a greater mean MD than a group of matched controls. However, there are several research issues that arise when looking at a novel risk factor such as MD and ovarian cancer, and this pilot study hopes to address and resolve these issues to help with the design of a larger study that would be better suited to answer the hypothesis.

\textbf{3.1.3 Estimation of case-control difference.} The first issue that would be addressed is that of sample size. For sample size calculations, an estimated size of the average case-control difference, if any, would be beneficial to have. As MD in relation to ovarian cancer has never been looked at before, we would like to ensure that we have enough participants to find an effect, if one exists. To do so, we would prefer to have an estimate that relates to ovarian cancer patients directly, versus using the standard case-
control difference for MD in the breast cancer literature. As the effect could be smaller with ovarian cancer patients, by using the breast cancer case-control difference we would be underestimating our sample size and potentially not finding a true difference.

3.1.4. Availability of mammograms for cases. We would also need to determine whether women diagnosed with ovarian cancer even have mammograms. With breast cancer patients, the majority of women would have had at least one mammogram at least for the breast cancer diagnosis. However, with ovarian cancer patients, we simply do not know. MD would not be an informative risk factor for ovarian cancer if the women at risk do not actually go for mammograms.

3.1.5 Control group selection. The selection of a suitable control group is also an important part of the larger study design we would like to address. MD has been shown to be influenced by ethnicity and family history of breast cancer. When conducting a larger study, our aim would be to use sisters of cases as the control group, as we would be able to control for both ethnicity and family history. This pilot study will address whether enough cases have sisters with mammograms to serve as their controls. As ovarian cancer is a relatively low incidence disease, we do not want to have to turn away cases based on the fact that they do not have a sister to serve as their control. We will be asking the cases in this pilot about sister availability. However, the use of sister controls may pose another problem besides availability. It has been shown that MD is a highly heritable trait, with genes accounting for an estimated 70% of the variance in MD. We run the risk of overmatching on MD between our cases and
controls as they share approximately 50% of their genetic information. We have conducted another study to address this issue of overmatching separately (Chapter 4).

3.2. Methods

3.2.1 General Method. Ovarian cancer cases were identified from a hospital-based cancer registry, as being ‘Alive, living with ovarian cancer’ by an analyst working for the registry. A list of identified cases and their contact information were provided to the study investigator, and these cases were recruited via mail. Controls were previously recruited for another study seeking to determine whether breast density is a heritable trait. Original mammograms were collected and digitized for all participants, with the cranio-caudal image selected. An epidemiological questionnaire detailing hormonal exposure and reproductive history were completed for both cases and controls. Images were read for density and the density results for the case-control pairs were compared and analyzed.

3.2.2. Subject Selection and Data Collection. Ovarian cancer patients were recruited from the Familial Ovarian Cancer Center at Princess Margaret Hospital in Toronto, Ontario, Canada. Patients classified as having invasive ovarian cancer with a current status of ‘Alive, living with ovarian cancer’ were identified as eligible for the study and mailed a package consisting of a mammogram release form and a short epidemiological questionnaire (see Appendix 1). Figure 3-1 shows the results of the ovarian cancer patient’s recruitment. In total, 183 participants were mailed, of which 79 (43%) consented. Of the consented individuals, 15 were excluded due to not ever having had a mammogram and an additional 12 were excluded because of
mammogram problems (i.e. only having digital images, not having a pre-diagnostic mammogram, or not being able to locate the mammogram film record). This left a total of 52 cases with digitized mammograms. The reasons for nonparticipation of the other 104 cases were due to refusals (5 cases), ‘no contact’ or returned mail (11 cases), death (13) and ‘non-response’ or not responding to participation requests (75). Once the cases consented to participate in our study, we obtained their tumour histology information, and further excluded 8 cases with borderline ovarian cancer. To supplement these lost ovarian cancer cases, we added 6 ovarian cancer patients who participated in another study looking at the heritability of MD. These additional cases were originally recruited from the Breast Cancer Familial Registry with the same method used to recruit the ovarian cases and the selected controls. The additional cases had completed epidemiological questionnaires and had digitized mammograms.
Figure 3-1. Results of the recruitment process for ovarian cancer patients.
Only pre-diagnostic images were selected for cases, as the effects of the treatment they received for ovarian cancer may affect the natural density levels in the breast. The epidemiological questionnaire consisted of questions related to the participant’s reproductive history and hormone exposure. There was also a section that inquired about the availability of a sister as a potential control. The questionnaire was completed by the participant and mailed back to the investigators.

Controls were previously recruited from the Weekend to End Breast Cancer walk in Toronto, Ontario, Canada for a different study. They were eligible for the current study if they had never been diagnosed with ovarian cancer and had a mammogram. The controls had mammograms digitized previously and a completed epidemiological questionnaire (Appendix 2), which was also administered through the mail. Controls were matched to cases on age at mammogram (within 3 years) and menopausal status at time of mammogram (premenopausal versus postmenopausal).

3.2.3. Mammographic Density Measurement. The different variations in MD and an example of the computer-assisted measurement method are shown in Figure 3-2. All mammograms were digitized following the same quality control procedure and using a Lumisys model 85 digitizer at a pixel size of 260 µm and 12 bits precision, and measured by one observer (N.F.B.). There was one read that consisted of 110 images. The case-control pairs were randomly distributed throughout the read. Reader reliability was assessed within the read using a 10% random selection of images. The observer showed a reliability of 0.98 within the read. Mammogram evaluation is a several step process, which has been detailed elsewhere, but will be briefly described


here. The images are uploaded into the Cumulus 3 program, which uses thresholding and pixel counts to determine mammographic density. A read assistant (L.L.) first masks all identifying information located on the image to ensure the observer performing the read is blinded to case status. The ‘blinded’ observer then first marks the outer and inner edge of the breast, which is calculated as ‘total breast area’. Through the use of the thresholding tool, the light area is determined by the observer, which provides a measurement for ‘dense area’. The final measurement is the percentage of total area that is dense, or percent mammographic density, which is the variable used in the following analysis. The read assistant then verifies the outer and inner edge markings made by the read observer and ensures that the outer breast edge was drawn correctly and removes any areas outside the breast from the pixel count.
Figure 3-2. Variations in MD and the measurement of MD using a computer-assisted method. The image on the left shows the different variations seen in mammographic density, from 0% dense (A) to the highest category of density, >75% density (F). The image on the right illustrates how density is measured in Cumulus. Figures adapted from (120).
3.2.4. **Statistical Methods.** The data analyzed consisted of 50 matched case-control pairs, for a total of 100 individuals. Data analysis was carried out using SAS analog software package (version 9.3 for Windows). Relationship between percent density, dense area and total breast area for the pairs was assessed using a paired t-test (p ≤ 0.05). Conditional logistic regression was used to estimate risk of ovarian cancer for MD as a continuous variable, and also for MD as a categorical variable. There were three equal categories of percent density; 0 to <16.45%, 16.46% to 34.69%, and ≥ 34.70%, which were defined by the controls (N=50).

3.2.5. **Description of Covariates Used.** In the logistic regression analysis, we used the responses from the epidemiological questionnaire as the source of our covariate information. BMI was controlled for as a continuous variable. Parity was a yes/no variable, being determined by at least one live birth, and not at least one pregnancy. Use of oral contraceptives and use of hormone replacement therapy were also yes/no variables, with duration of use not taken into account. Therefore, we will have a small number of women reporting use of either of these preparations who have used them for under one year before discontinuing use altogether.

3.3. **Results**

3.3.1. **Characteristics of Subjects.** Table 3-1 shows the pertinent demographic, hormonal and reproductive factors that have been associated with MD variation for the cases and controls. The cases did not differ from the controls on age at mammogram or menopausal status, as we matched on these variables. As expected from the literature, while not statistically significant, in general; the ovarian patients used oral
contraceptives less, were less parous, and among the parous women, had fewer children. They did not appear to differ on breastfeeding (ever versus never) or hormone replacement therapy use, and the cases had a smaller mean BMI, all of which is less consistent with what is found in the literature.

Table 3-1. Descriptive Characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Mean (Std Dev)</th>
<th>Case – Control</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case</td>
<td>Control</td>
<td>Mean (Std Dev)</td>
<td>p-value</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N= 50</td>
<td>N = 50</td>
<td>N=50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at mammogram</td>
<td>56.2 (8.9)</td>
<td>56.0 (8.8)</td>
<td>0.1 (8.8)</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>Age at diagnosis</td>
<td>56.3 (9.3)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>25.2 (4.7)</td>
<td>26.2 (5.1)</td>
<td>-0.9 (4.9)</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td>OC use</td>
<td>64%</td>
<td>76%</td>
<td></td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td>78%</td>
<td>88%</td>
<td></td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>Number of Live Births</td>
<td>1.7 (1.3)</td>
<td>2.1 (1.2)</td>
<td>-0.4 (1.3)</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>Breastfed (Parous only)</td>
<td>76%</td>
<td>77%</td>
<td></td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>Menopausal Status</td>
<td>52%</td>
<td>54%</td>
<td></td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>HRT Use</td>
<td>44%</td>
<td>40%</td>
<td></td>
<td>0.68</td>
<td></td>
</tr>
</tbody>
</table>

1 BMI missing for one participant.
3.3.2. Case-Control Comparison of Percent MD and Estimated Sample Size.

For the following analysis, p-values are reported, however, none of our findings were significant, as we expected for this pilot study. Figure 3-3 shows the distributions of percent MD by case-control status. The cases did have a higher mean percent MD (Mean=29.9, SD=17.1) than the controls (Mean=27.5, SD=15.9). As the shapes of the distributions were relatively normal, a paired t-test was performed on the mean percent MD difference. There was a mean case-control difference of 2.34% (SD=21.2, p=0.44). The average case-control difference normally seen in the breast cancer literature is approximately 5%\textsuperscript{125}, so this difference is roughly half of the effect seen with breast cancer patients.

Figure 3-3. Distribution of percent MD by case-control status.
Total dense area and total breast area were also examined. The cases had less dense area (Mean=35.9 cm$^2$) and a smaller total breast area (Mean=132.9) than the controls (Mean dense area=38.2 cm$^2$, Mean total area=149.1 cm$^2$). While these results were not statistically significant, these measures may reflect the differences in BMI between the two groups, and we will therefore just focus on percent MD for the rest of our analysis.

Table 3-2 shows the different sample sizes required for different statistical power levels. The paired mean case-control difference and standard deviation mentioned above were used for the calculations. Based on an achieved case-control difference of 2.34% (SD=21.2) to achieve a power level of 0.8, we would need approximately 700 case-control pairs$^{126}$.

**Table 3-2. Sample size calculations.**

<table>
<thead>
<tr>
<th>Paired T-test</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(0.05 significance, two-tailed)</td>
<td></td>
</tr>
<tr>
<td>Case-control difference</td>
<td>2.34%</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>21.2</td>
</tr>
<tr>
<td><strong>Power</strong></td>
<td><strong>Sample Size</strong></td>
</tr>
<tr>
<td>0.5</td>
<td>317</td>
</tr>
<tr>
<td>0.6</td>
<td>404</td>
</tr>
<tr>
<td>0.7</td>
<td>509</td>
</tr>
<tr>
<td>0.8</td>
<td>646</td>
</tr>
<tr>
<td>0.9</td>
<td>864</td>
</tr>
</tbody>
</table>
3.3.3. **Availability of a Mammogram for Cases.** Out of the 79 ovarian cancer cases who consented to our study, 64 (81%) had available mammograms. Only 15 women did not have any mammogram film record at all.

3.3.4. **Availability of Sister Controls.** From the responses to the epidemiological questionnaire, we found that approximately 75% of the cases had sisters, and of those with sisters, 84.4% had a mammogram. However 17% of those women who had sisters with a mammogram would not allow us to contact their sister. This was for various reasons, including: their sister did not speak English or their sister lived out of the country. In total, from our sample, if we had used sisters of cases as our control group, only 53% of our cases would have had a control.

3.3.5. **Risk of ovarian cancer associated with variation in MD.** Table 3-3 shows the odds ratio for risk of ovarian cancer, using MD as a continuous variable. From this analysis, once age, BMI, parity, OC use and HRT use were controlled for, there was relative risk of 1.011 (95% CI: 0.98, 1.04). Therefore, for every 1% increase in mammographic density there is a 1.1% increase in the relative risk for ovarian cancer. This same analysis was applied to a published dataset125, of 1114 cases and 1114 controls, looking at percent MD and risk of breast cancer, and a relative risk of 1.020 (95% CI: 1.014, 1.025) was found. The risk of ovarian cancer is about half the risk found in breast cancer studies, which is what we would expect as they have half the effect size.
**Table 3-3. Percent MD and risk of ovarian cancer.**

<table>
<thead>
<tr>
<th></th>
<th>PD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong># of Subjects</strong></td>
<td></td>
</tr>
<tr>
<td>Ovarian Cases</td>
<td>50</td>
</tr>
<tr>
<td>Controls</td>
<td>50</td>
</tr>
<tr>
<td><strong>OR (95% CI)</strong></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>1.011 (0.98, 1.04)</td>
</tr>
<tr>
<td>Age, BMI, OC Use, Parity, HRT</td>
<td>1.011 (0.98, 1.04)</td>
</tr>
</tbody>
</table>

Risk of ovarian cancer was then looked at in relation to MD as a categorical variable, as shown in Table 3-4. The category parameters were determined by arranging the percent MD values for the controls in increasing order and then dividing them into three equal groups. These category parameters were then applied to the ovarian cases to provide the frequency table shown at the top of Table 3-4.
<table>
<thead>
<tr>
<th>Number of subjects</th>
<th>Percent Density</th>
<th>P-test for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovarian Cases</td>
<td>0 to &lt;16.45</td>
<td>16.46 to &lt;34.69</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>24</td>
</tr>
<tr>
<td>Controls</td>
<td>16</td>
<td>17</td>
</tr>
</tbody>
</table>

**OR b (95% CI b)**

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted</th>
<th>Age, BMI c, OC Use d, Parity, HRT e</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2.76 (0.78, 9.74)</td>
<td>2.38 (0.54, 10.55)</td>
</tr>
<tr>
<td></td>
<td>1.81 (0.51, 6.39)</td>
<td>1.81 (0.41, 7.96)</td>
</tr>
<tr>
<td></td>
<td>0.67</td>
<td>0.72</td>
</tr>
</tbody>
</table>

* Conditional logistic regression analysis of cases matched with controls. b OR=Odds Ratio. CI=Confidence interval. c Body mass index. d OC=Oral Contraceptive. e HRT = Hormone replacement therapy.
It was found that women in the highest tertile of percent MD had an 81% increase in relative risk of developing ovarian cancer (95% CI: 0.41, 7.96), in comparison to the women in the lowest density category, controlling for age, BMI, parity, OC use and HRT use. The trend was not linear, as the middle tertile had the greatest relative risk of 2.38 (95%CI: 0.54, 10.55).

Comparing these results to that of the breast cancer dataset, using the same tertile parameters, women in the highest tertile had 2.27 times the relative risk (95% CI: 1.77, 2.92) of developing breast cancer in comparison to women in the lowest density tertile.

### 3.3.6. Risk of Ovarian Cancer Associated with Other Risk Factors

In order to provide some validation of our data, we then looked at the risk of ovarian cancer with the other epidemiological variables we collected from our cases. We can then cross-reference the values that we obtain with the values in the literature to determine how representative our samples of ovarian cancer cases are to the much larger ovarian cancer general population. All variables were analyzed according to the variable description provided in the methods sections.

Figure 3-4 shows the relative risk of ovarian cancer associated with each of the variables we collected, with the reported relative risk after controlling for the other variables shown in the figure. There was no effect of BMI as a continuous variable on risk of ovarian cancer (RR=0.99, 95% CI: 0.90, 1.09), however, we did not have enough women (both case and control) to test for obesity as a risk factor for ovarian cancer. OC use was associated with a reduced relative risk of ovarian cancer (RR=0.71, 95% CI: 0.28,
1.84), which is consistent with the literature, and the risk estimate we obtained is also within the range that is generally found in case-control studies looking at this variable. Also consistent with the published literature, parity was associated with a reduced risk of ovarian cancer (RR=0.57, 95% CI: 0.19, 0.70). We did find an elevated risk of ovarian cancer among women who reported having ‘ever’ used HRT (RR=1.33, 95% CI: 0.99, 4.87), however, we did not have any information on the preparation used.

Figure 3-4. **Risk of ovarian cancer by established risk factors.** All risk factors have been controlled for the other factors shown in the graph.
3.4. Discussion

The purpose of this pilot study was to determine whether a larger study investigating this novel research question was feasible. As expected, due to our small sample size, none of the results we presented above were statistically significant. Our main aims were to address specific research issues and determine the best course of action for a larger study. From this pilot study, we were able to answer each research question and will detail the solutions below.

The first issue that we were able to address was that of effect size and thereby, the sample size needed for a larger study looking at ovarian cancer and percent MD. We found a case-control difference of 2.34%, with a standard deviation of 21.2. From the data we are able to conclude that in order to find a true effect, if one does exist, we would need a sample of approximately 700 ovarian cases matched with an equal number of controls. Based on current ovarian cancer diagnosis trends in Ontario of 1000 women every year, and the consent rate of 43% achieved in this pilot study, this goal of 700 cases can be reached in approximately 2-3 years with active recruitment of ovarian patients from Ontario hospitals.

We also wanted to determine whether ovarian cancer patients had available mammograms; a necessary condition for studying MD. From our recruitment results, we are able to conclude that the majority of ovarian cancer patients (81%) do have available mammograms.

Finally, we wanted to look into whether sisters of cases would serve as suitable controls in a case-control study. We found that approximately 53% of our study sample
would have an available sister with a mammogram that we could contact to serve as their control. However, if we relied on a study design that would depend solely on sister controls, we would have to exclude half of our cases simply because they do not have a sister with a mammogram. In a larger study, we would need a supplementary source of controls for those women who do not have a sister control available, such as first-degree cousins. The issue of overmatching on MD through the use of sister controls was addressed in the next chapter (see Chapter 4).

3.4.1. Limitations of Study Design. The current pilot study was not intended to produce definitive results or directly assess MD as a risk factor for ovarian cancer. However, there were some limitations to our study design. The first is the fact that there was a definite survival bias in our sample. The majority of our patients were diagnosed in Stage I (34%) or Stage II (25%), which are much higher proportions than are normally diagnosed in the early stages of ovarian cancer. Normally, only about 40% of ovarian patients are diagnosed in Stages I and II combined, with the majority being diagnosed in Stages III and IV. The problem here rests on our recruitment strategy. We approached anyone who had been diagnosed with ovarian cancer that was currently still alive, regardless of how long ago they had been diagnosed. As such, we had women in our sample that had been cancer free for over 5 years, and as ovarian cancer is a high mortality disease with a 5-year survival rate of only 40%, these women are probably not representative of the ovarian cancer population as a whole. It is possible we had a significant proportion of women who did not have a very aggressive form of ovarian cancer in our study sample, and we can only speculate the effect this
would have on the percent MD results of the current study. In a larger study, we feel it is important to only include incident cases of ovarian cancer in order to get a more representative sample of aggressive versus less severe forms of ovarian cancer.

Figure 3-5 illustrates how our sample differed in ovarian cancer diagnosed histology’s in comparison to the that of all ovarian cancers diagnosed in Canada\textsuperscript{127}. From this graph, it is clear that we had more than the expected number of serous, endometriod, clear cell and sex-cord stromal tumours, and smaller numbers of mucinous, germ cell or adenocarcinomas. Again, we are not sure what effect our non-representative histology distribution would have on MD, if any effect at all, but every effort should be made to have a representative sample of all ovarian cancers. Other risk factors have been shown to vary depending on the type of ovarian tumour diagnosed\textsuperscript{38,128}. Some have found that different histology’s have different survival rates associated with them\textsuperscript{129}, so the distribution we had could also be reflective of the survival bias we detected. However, we only recruited from one clinic in downtown Toronto, so we believe that enlisting a variety of clinics from all over Ontario, our chances of having the most representative sample will be increased.
Figure 3-5. Histology distribution of OVCA sample differs from actual OVCA diagnoses. Information regarding Canadian ovarian cancer incidence adapted from (124).
3.4.2. **Conclusions.** We can conclude from the results of this pilot study that a larger study looking at whether MD is a risk factor for ovarian cancer is feasible and justified. We believe from our preliminary evidence that this is worth investigating further. In our small sample, the cases had a higher mean percent MD in comparison to the controls, and there was an excess in risk of ovarian cancer for women in the highest density category compared to the lowest. Based on the observed case-control difference, a case-control study consisting of approximately 700 incident ovarian cancer cases matched on age and menopausal status with an equal number of controls. Sister controls would be preferred, but first-degree cousins would be a viable option for those cases without sisters. Recruitment should be Ontario-wide, with all major cancer centers encouraged to participate.

The identification of a novel strong risk factor for ovarian cancer might allow the identification and selective screening of women at increased risk for the disease and lead to earlier detection and improved survival. MD may also allow the identification of women most likely to benefit from potential preventive interventions. If MD is associated with an increased risk of ovarian cancer, the information gained from this new risk factor can be combined with the current data on existing risk factors and worked into a model similar to current breast cancer risk prediction models.
CHAPTER 4:

Mammographic Density and Familial Breast Cancer: A Comparison of Related and Unrelated Controls.
4.1 Introduction

The radiological appearance of the breast varies among women and reflects breast tissue variation\textsuperscript{47}. The differences in breast tissue composition are due to stroma and epithelial tissue, which appear light on a mammogram, and fatty tissue, which appears dark\textsuperscript{130}. A greater amount of density has been strongly associated with an elevated risk of breast cancer\textsuperscript{49, 54, 119,131}, and the variations seen in breast tissue have been shown to be highly heritable\textsuperscript{47}. While MD varies according to height, weight\textsuperscript{50-51}, parity\textsuperscript{52-53}, menopausal status\textsuperscript{54} and hormone use\textsuperscript{55}, these factors explain only 20 to 30\% of the observed variance among women.

Research has shown that MD is a highly heritable trait, with heritability accounting for approximately 60\% of the variation in breast density\textsuperscript{47}. A family history of breast cancer has also been shown to influence MD\textsuperscript{122-123}. Women with a first-degree relative diagnosed with breast cancer are more likely to have dense breasts in comparison to those with no family history. Furthermore, the risk of having dense breasts increases as the number of first degree relatives diagnosed with breast cancer increases\textsuperscript{123}.

MD increases the risk of developing breast cancer, is heritable, and a family history of breast cancer increases MD. From this evidence, it can be reasoned that MD may play a role in familial breast cancer. To examine the role that family history of breast cancer may have on MD requires that a suitable control group should be identified. The use of sister’s of the cases as the control group have the advantage of matching on complex variables such as family history, ethnicity and socio-economic
status, and perhaps age, which have all been associated with MD. However, it is unclear whether using sister controls could result in over-matching, and thus attenuating the case-control difference normally seen in comparison to unrelated control groups. The purpose of this study is to examine the potential effects of using controls related to cases versus the more traditional use of unrelated controls.

4.2 Methods

4.2.1 General Method. We used images previously collected from three different epidemiological studies seeking to determine whether breast density is a heritable trait. All three studies had at least two full-sisters from a family. We formed a triplet consisting of an invasive breast cancer case, a sister control, and an unrelated control. One cranio-caudal mammogram view was used for all participants, with side of breast selected randomly. Only original films were used in analysis. Ethics approval was obtained from the University Health Network’s Research Ethics Board.

4.2.2 Study Populations. There were three sources of study participants, the first being the Breast Cancer Family Registry (BCFR). We used participants from the two North American population-based sites, in Ontario and Northern California. Information regarding study design and participant characteristics has been published elsewhere\textsuperscript{132}. Participating sites collect family history, clinical and epidemiological information on risk factors that have been linked to breast cancer. There was also follow-up of the affected individuals for vital status and recurrence of the disease. Mammograms were not collected by the registry, however, information on the location and quantity of mammograms was recorded.
The other two sources of participants were not connected to the BCFR, however, the data for both were collected in an identical fashion to the registry recruitment process, using the same epidemiological and familial history questionnaire. The first source consisted of women participating in The Weekend to End Breast Cancer, a 60 kilometre walk in Toronto in 2006 and 2007. There are approximately 5000 women participating in the fundraiser. An open call to all walkers was placed in an email sent out after the race, and the individuals were instructed to call or email if interested in participating. They were sent the same questionnaires as the BCFR to ensure comparable data. The final source of participants was from a previously conducted study involving North American twins, and the recruitment process has been described in detail elsewhere\(^47\).

4.2.3 Subject Selection and Data Collection. All participants had relevant epidemiological and family history data collected and digitized mammograms. To properly test our hypothesis, we decided on a study design that consisted of a case, a first-degree related control, and an unrelated control. First, a case was identified as having invasive breast cancer, with the mammogram prior to the cancer diagnosis digitized. When the diagnostic image was selected, the breast contra-lateral to the affected breast was digitized. Cases with bilateral disease were excluded. Therefore, for all cases, only unaffected images were included in the study. After the case was identified and the mammogram digitized, the sister control was selected. All sister controls were matched to their case-sister using age at mammogram as the matching factor. All mammograms were matched within 5 years of age to each other, and were
selected no later than up to one year from the epidemiological questionnaire administration. In situations where there was more than one unaffected sister in the family, the sister with the mammogram closest in age to that of the case was chosen. Once the case and her sister were identified, the unrelated control was selected from the North American Twin population. She was also matched pairwise based on her age at mammogram screening in relation to the case’s age at mammogram using the same criterion of matching within five years.

**4.2.4 Determining Menopausal Status.** Menopausal status was classified according to reported age at cessation of menstruation and the age at mammogram. If the age at mammogram was less than age at cessation of menstruation or the age at mammogram was greater than the age at cessation of menstruation by less than one year, then the participant was considered pre-menopausal. Only when the mammogram age was greater than twelve months compared to the age menstruation stopped, the participant was considered post-menopausal.

**4.2.5 Mammographic Density Measurement.** All mammograms were digitized following the same quality control procedure and using a Lumisys model 85 digitizer at a pixel size of 260 µm and 12 bits precision, and measured by one observer (N.F.B.). Reliability was assessed both within and between reads using a 10% random selection of images. The observer showed a reliability of 0.97 and 0.79 for within and between reads, respectively. There were 8 reads of 110 images, containing the within and between reliability images. The case-sister-control set were always in the same read, however they were randomly distributed within that read. Mammogram evaluation is
a several step process, which has been detailed elsewhere\textsuperscript{124}, but will be briefly described here. The images are uploaded into the Cumulus 3 program, which uses thresholding and pixel counts to determine mammographic density. The observer first marks the outer and inner edge of the breast, which is then calculated as ‘total breast area’. Through the use of the thresholding tool, the light area is determined by the observer, which provides a measurement for ‘dense area’. The final measurement is the percentage of total area that is dense, or percent mammographic density, which is the variable used in the following analysis.

4.2.6 Statistical Analysis. The data analyzed consisted of three matched groups of 229 individuals, for a total of 687 participants. Data analysis was carried out using SAS (version 9.3 for Windows). The relationships between percent density, dense area and total breast area for the three participant groups was assessed using paired t-tests (p ≤ 0.05) and Pearson’s Correlation Coefficient. Conditional logistic regression was used to estimate the risk of breast cancer for categories of percent density: 0 to <10%; 10% to 25%; and ≥ 50%. The covariates that were controlled for are shown in the tables below.

4.3 Results

4.3.1 Characteristics of Subjects. Table 4-1 shows the relevant demographic and lifestyle characteristics that have been shown to affect breast density. The cases, related controls and unrelated controls were similar for most of the characteristics we examined. As expected, the cases and their sister controls were more similar in height, weight and BMI in comparison to the unrelated controls. There was a significant
difference in the proportion of postmenopausal subjects ($p < 0.02$) between the cases and the sisters controls, where 45.0% of cases were post-menopausal versus 45.4% of sister controls. Family history data was also compared (not shown in table), and 48% of the cases had at least one first degree relative diagnosed with breast cancer, in comparison to the unrelated controls, with 23% having a family history of the disease. As sisters are considered a first-degree relative, 100% of the sister controls had a family history of breast cancer.
Table 4-1 Descriptive statistics for case and control subjects, and for case-control paired differences.

<table>
<thead>
<tr>
<th></th>
<th>Mean (Std Dev)</th>
<th>Case – Sister control</th>
<th>Case – Unrelated control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case N=229</td>
<td>Control N=229</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sister N=229</td>
<td>Unrelated N=229</td>
<td></td>
</tr>
<tr>
<td>Age at mammogram (years)</td>
<td>50.8 (8.1)</td>
<td>51.4 (8.1)</td>
<td>51.0 (7.6)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>69.1 (13.9)</td>
<td>68.9 b (16.2)</td>
<td>67.0 (15.8)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>163.4 (6.9)</td>
<td>163.4 c (6.2)</td>
<td>162.5 (6.8)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25.9 (4.8)</td>
<td>25.8 c (5.8)</td>
<td>25.3 (5.4)</td>
</tr>
<tr>
<td>Menopausal status (% post)</td>
<td>44.98</td>
<td>45.41</td>
<td>44.98</td>
</tr>
<tr>
<td>Parous (% yes)</td>
<td>79.91</td>
<td>84.28</td>
<td>80.79</td>
</tr>
<tr>
<td>HRT a ever used (% yes)</td>
<td>41.92</td>
<td>34.21 b</td>
<td>37.55</td>
</tr>
</tbody>
</table>

*p-value*  
-0.68 (2.0) <0.0001  
2.10 (20.3) 0.12  
-0.08 (6.8) 0.86  
0.06 (6.0) 0.88  
-0.43 0.02  
4.37 0.30

*Hormone replacement therapy. b N=228. c N=227. For analysis, the overall averages were substituted for the missing values.
4.3.2 Comparison of mammographic measures between cases and controls.

Figure 4-1 shows the mean and standard deviation of percent density, dense area and total breast area, by case-control status. The cases had a higher mean percent density (34.6%) and more dense tissue (44.51 cm²) than the related controls (Means = 30.4% and 37.4 cm², respectively). Cases also had higher percent density and more dense tissue than unrelated controls (Mean = 29.9% and 33.8 cm², respectively). Total breast area was not significantly different between the cases (Mean = 139.6 cm²) and the related controls (Mean = 143.8 cm²). However, the cases had a significantly greater breast area compared to the unrelated controls (Mean = 127.4 cm²; p=0.02).

The mean difference between the cases and the related and unrelated control groups in percent density (P < 0.008 and P < 0.007, respectively) and dense area (P < 0.006 and P < 0.0001) were both statistically significant. In addition, the difference between the case and related controls and the case and unrelated controls was calculated and tested for significance. The difference in percent density for the related group (Mean = 4.16%) did not differ significantly from the unrelated group difference (M=4.7 %); this was similar to the results for dense tissue, with no difference between the related group (M = 7.08 cm²) and the unrelated group (Mean = -4.29 cm²).

The two control groups did not differ significantly on percent density or dense area. However, the two control groups did differ significantly on total breast area, with the related control group having a greater total breast area (p < 0.01).
Figure 4-1 Distributions of percent density, dense area, and total area for cases, sister controls, and unrelated controls.
4.3.3 Correlation of mammographic density between cases, sister controls and unrelated controls. As shown in Figure 4-2, the related case-control pairs showed a moderate correlation in percent mammographic density ($r = 0.40, p <0.001$), dense area ($r = 0.33, p <0.0001$) and total breast area ($r = 0.42, p <0.0001$). As expected, the unrelated case-control pairs showed no significant correlation in percent mammographic density ($r = 0.04, p=0.58$), and total breast area ($r = 0.07, p>0.28$), but did show a small correlation in dense area ($r = 0.14, p =0.03$).
Figure 4-2 Correlations between MD measurements and control group. Correlations of percent density, dense area, and total area between cases and sister controls, and cases and unrelated controls. R is the Pearson correlation coefficient.
3.3.4 Risk of breast cancer according to category of mammographic density.

Table 4-2 shows the odds ratio for each category of percent MD. The unadjusted odds ratio in women with greater than 50 percent density was 2.70 (95% CI: 1.1, 6.5) for the sister controls and 1.87 (95% CI: 1.0, 3.6) for the unrelated controls, in comparison to the reference group (<10%). Adjustment for BMI had the strongest effect on OR estimates, which increased the odds for the sister controls to 3.76 (95% CI: 1.4, 9.9) and for the unrelated controls to 3.33 (95% CI: 1.5, 7.3). Once we adjusted for all the variables shown to have an effect on percent density (age, BMI, menopausal status, parity, and HRT use) the odds ratio associated with being in the high density group for the sister controls was 2.58 (95% CI: 0.9, 7.5) and 3.20 (95% CI: 1.4, 7.2) for the unrelated controls.
Table 4-2 Category of percent density and risk of breast cancer a.

<table>
<thead>
<tr>
<th>Percent density (%)</th>
<th>&lt;10</th>
<th>10 to &lt;25</th>
<th>25 to &lt;50</th>
<th>&gt;50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>27</td>
<td>38</td>
<td>121</td>
<td>43</td>
</tr>
<tr>
<td>Sister controls</td>
<td>39</td>
<td>54</td>
<td>98</td>
<td>38</td>
</tr>
<tr>
<td>Unrelated controls</td>
<td>46</td>
<td>51</td>
<td>92</td>
<td>40</td>
</tr>
<tr>
<td>OR b (95% CI b)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sister</td>
<td>1</td>
<td>1.24 (0.6, 2.6)</td>
<td>2.55 (1.2, 5.2)</td>
<td>2.70 (1.1, 6.5)</td>
</tr>
<tr>
<td>Unrelated</td>
<td>1</td>
<td>1.19 (0.6, 2.2)</td>
<td>2.25 (1.3, 3.9)</td>
<td>1.87 (1.0, 3.6)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sister</td>
<td>1</td>
<td>0.97 (0.4, 2.1)</td>
<td>2.06 (1.0, 4.3)</td>
<td>2.01 (0.8, 5.0)</td>
</tr>
<tr>
<td>Unrelated</td>
<td>1</td>
<td>1.20 (0.6, 2.3)</td>
<td>2.12 (1.2, 3.7)</td>
<td>1.65 (0.8, 3.3)</td>
</tr>
<tr>
<td>BMIc</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sister</td>
<td>1</td>
<td>1.39 (0.6, 3.0)</td>
<td>3.45 (1.5, 7.4)</td>
<td>3.76 (1.4, 9.9)</td>
</tr>
<tr>
<td>Unrelated</td>
<td>1</td>
<td>1.51 (0.8, 2.9)</td>
<td>3.56 (1.8, 6.8)</td>
<td>3.33 (1.5, 7.3)</td>
</tr>
<tr>
<td>Menopausal status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sister</td>
<td>1</td>
<td>1.24 (0.6, 2.6)</td>
<td>2.58 (1.3, 5.3)</td>
<td>2.75 (1.1, 6.6)</td>
</tr>
<tr>
<td>Unrelated</td>
<td>1</td>
<td>1.20 (0.6, 2.2)</td>
<td>2.36 (1.3, 4.1)</td>
<td>2.00 (1.0, 4.0)</td>
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<tr>
<td>Age, BMIc, menopausal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sister</td>
<td>1</td>
<td>1.09 (0.5, 2.5)</td>
<td>2.89 (1.2, 6.7)</td>
<td>3.09 (1.1, 8.7)</td>
</tr>
<tr>
<td>Unrelated</td>
<td>1</td>
<td>1.56 (0.8, 3.0)</td>
<td>3.63 (1.9, 7.1)</td>
<td>3.26 (1.5, 7.3)</td>
</tr>
<tr>
<td>Age, BMIc, menopausal, parous, HRTd ever used</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sister</td>
<td>1</td>
<td>1.04 (0.5, 2.4)</td>
<td>2.65 (1.1, 6.3)</td>
<td>2.58 (0.9, 7.5)</td>
</tr>
<tr>
<td>Unrelated</td>
<td>1</td>
<td>1.54 (0.8, 3.0)</td>
<td>3.57 (1.8, 7.0)</td>
<td>3.20 (1.4, 7.2)</td>
</tr>
</tbody>
</table>
Conditional logistic regression analysis of cases matched with sister controls, and cases matched with unrelated controls. OR=Odds Ratio. CI=Confidence interval. Body mass index. Hormone replacement therapy.
Correlations for height, weight and BMI were calculated between cases and sister controls and cases and unrelated controls (Figure 4-3), as adjusting for these variables reduces the risk attributable to sister controls. As expected, we found a significant positive correlation between sister controls and cases (p < 0.001) for all three variables, but not for unrelated controls and cases.
Figure 4-3 Correlations of weight, height, and body mass index between cases and sister controls, and cases and unrelated controls. Pearson correlation coefficient is shown.
4.4 Discussion

As MD has been shown to be strongly genetically determined\textsuperscript{47}, the selection of suitable controls for projects looking at MD and its influences is vital. MD has been shown to be influenced by many risk factors, such as age, menopausal status, ethnicity and family history. When designing a study, it is important to be able to control for these potentially confounding variables. Some, such as age or parity, are easier to control for, while others, such as family history, can prove to be difficult. There are few control group options, and the two that were investigated here were the use of age-matched, unrelated paired controls versus a control group consisting of sisters of breast cancer cases.

The use of sister controls in mammographic density studies can prove to be advantageous, as the investigator is simultaneously able to control for both family history of cancer as well as ethnicity. Both of these variables have been associated with breast density\textsuperscript{122-124,133}. With the use of sister controls, one is gaining better agreement on variables that are more difficult to match on. However, as MD is a heritable trait, by using sisters (where, on average, 50\% of their DNA is identical), one could argue that while confounding variables have now been better controlled, there is a risk that the sisters are too similar genetically to show a difference in MD seen in previous studies between cases and controls.

The results from this study suggest that sisters are suitable controls for case-control studies examining risk factors for mammographic density and breast cancer. In our comparison of cases to the related and unrelated control groups on percent MD and
dense area, there was a significant difference between the cases and both of the control groups. Also, the two control groups did not differ from each other on percent MD and dense area, and they both showed similar differences in the percent mammographic density between cases and controls as has been reported elsewhere in the literature. The related group, as expected, did show a moderate correlation in all density measures, whereas there was no correlation in percent MD among the unrelated group. This is expected, as percent MD has been shown to be a highly heritable trait.

When examining the adjusted risk estimates, there is evidence of a weak attenuation of risk for the sister controls compared to the unrelated controls, depending on what variables are being controlled for. Controlling for all variables, there is a higher odds ratio associated with the unrelated group, however, the confidence intervals overlap substantially. As there were fewer women in the highest density category of ≥75% dense tissue, we had to combine the 50% to <75% group with the highest density category, which resulted in slightly diluted risk estimates for both of the control groups. While the sister controls did have a slightly smaller adjusted risk estimates associated with percent breast density, both of the risk estimates involved fall within the generally accepted range of risk attributed to percent MD (i.e. between a 2- to 6-fold increase in risk), even with the combination of the two highest density categories together. Considering that both the sister controls and the unrelated controls did not differ on density measurements, thereby showing the same effect size, and both groups show an elevated risk for having high percent MD, we believe that sisters are suitable controls in a case control study examining percent MD. By matching controls pairwise
versus frequency matching, we were able to more precisely control for potential confounding variables.

**4.4.1 Conclusion.** The use of sister controls for mammographic density studies presents certain strengths in study design, controlling for both ethnicity and family history without compromising the case-control difference seen in MD. However, the sister controls and the cases were significantly correlated on height, weight and BMI, which after adjustment, had the largest effects on the risk estimates. The results of this study are context specific, and generalization will depend upon the research question being investigated.
CHAPTER 5:

GENERAL CONCLUSIONS
5.1 Overall Conclusions

The objectives of this thesis were threefold. Firstly, a review of the literature on the risk factors for breast and ovarian cancer was conducted, with common risk factors shared between the two cancers highlighted. Next we sought to determine the feasibility of a larger study investigating whether MD is a potential risk factor for ovarian cancer, through a small pilot study addressing several research issues. Finally, we investigated whether sisters of breast cancer cases would serve as suitable controls in MD studies.

Breast cancer and ovarian cancer share many of the same hormonal and biological risk factors, including age of incidence, BRCA1 or 2 mutation, radiation exposure, HRT use, OC use, and having a family history of breast and/or ovarian cancer. Menopause, parity, breastfeeding, age at menarche, and BMI are also associated with breast and ovarian cancer, however the association is more varied and the research less strong for these factors. That breast and ovarian cancer share many of the same risk factors suggests a common aetiology between the two cancers. Both total lifetime ovulations and circulating levels of IGF-1 were presented as potential biological plausible explanations for the increase in risk seen for both breast and ovarian cancer associated with the above risk factors.

Mammographic density is one of the strongest known risk factors for breast cancer; however, to the best of our knowledge, no one has ever looked at ovarian cancer in relation to mammographic density. A pilot study was conducted to address research issues that may arise when looking at this potential novel risk factor for ovarian cancer.
in a large study. We sought to determine what the average case-control difference (if any) would be between ovarian cancer cases and healthy controls. This would allow us to estimate the approximate sample size that would be necessary to investigate whether MD is a risk factor for ovarian cancer. We also looked into whether the majority of ovarian cancer patients have had mammograms, a necessary condition for a MD study. We then looked at the availability of sister controls for ovarian cancer patients, as they would be the preferred control group (as discussed in Chapter 3).

We found that the ovarian cancer patients included in our pilot study did have a higher mean percent MD than our controls. Using this case-control difference, we determined that a larger study would need approximately 700 case-control pairs for an effect to be found, if one does exist. Based on current ovarian cancer trends, there are approximately 1000 women diagnosed with ovarian cancer each year in Ontario. Using our response rate of 43%, we can expect to achieve the goal of 700 cases with approximately 2-3 years of recruitment. One single centre would not have enough ovarian patients and the patients recruited from one clinic may be biased towards a certain histological type of ovarian cancer, so we would hope to recruit from a wide variety of cancer clinics from across Ontario. We were also able to determine that the majority of ovarian cancer patients do have available mammograms, with 81% having had a mammogram in the past. We addressed the issue of availability of sister controls in the epidemiological questionnaire the participants were asked to fill out. We found that a study design that relied only on sisters of cases as the control group would end
up excluding almost a third of ovarian cases. A supplementary control group option would be necessary for a larger study.

In a separate chapter we then addressed the issue of whether the use of sister controls would result in overmatching on MD, our variable of interest. As MD is a highly heritable trait, this concern is especially important, as 50% of sister’s DNA is identical. Use of sister controls would allow the investigator to control for family history and ethnicity, both of which have also been shown to be associated with MD. We formed 229 case-sister control-unrelated control triplets to address the issue of overmatching. We found that the use of sister controls did not result in overmatching on MD, as sister controls showed the same case-control difference as our unrelated control group. However, there was some attenuation seen in the odds ratio for the sister controls.

5.2 Future Directions

We feel that a larger study addressing whether MD is a risk factor for ovarian cancer is feasible and justified. The study should aim to have 700 case-control pairs recruited from a variety of cancer clinics across Ontario. The use of sister controls would not result in overmatching on MD, however, a supplementary control group option should be available for those cases that do not have sisters. One potential for this supplementary control group would be first-degree cousins of cases, as there would still be some degree of control for family history and ethnicity, while still opening up the pool of potential controls.
CHAPTER 6:

REFERENCES


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and interactions in an international case-control study. *Int. J Cancer* 1990; 46: 796-800.


APPENDIX 1

Epidemiological questionnaire developed for ovarian cancer pilot study.
Mammographic Density as a Risk Factor for Ovarian Cancer

Client ID: ___________________

Date of Diagnosis: ___________________

Epi Date: ___________________

Initials: ______     Date of Birth: ___________________

Address: ________________________________________________________________

City: ___________________________     Province/State: ________________

Postal/Zip Code: ________________     Telephone: (____)_____________

Email: _________________________
Mammographic Density as a Risk Factor for Ovarian Cancer

Personal History Questionnaire

Height

1. How tall are you?

___Feet____ Inches or _______ cm

Weight

2. At the time of your diagnosis, what was your weight?

_____ lbs or _______ kgs

3. What is your current weight?

_____ lbs or _______ kgs

Medical History

4. Has a doctor ever told you that you had a disease such as cancer, leukemia or a malignant tumour?

☐ Yes → What was the type(s) of cancer? How old were you when this was 1st diagnosed?

____________________________   _________ years old
____________________________   _________ years old
____________________________   _________ years old

☐ No
☐ Don’t know
5. Has a doctor told you that you had benign breast disease, such as a non-cancerous cyst or breast lump?

☐ Yes  How old were you when this was first diagnosed? __________ years old
☐ No
☐ Don’t know

6. Has a doctor told you had cysts in one or both ovaries?

☐ Yes  How old were you when this was first diagnosed? __________ years old
☐ No
☐ Don’t know

7. Have you had a breast completely removed?

☐ Yes, the right breast  at what age was this? __________ years old
☐ Yes, the left breast  at what age was this? __________ years old
☐ No

8. Have you ever had an ovary completely removed?

☐ Yes, one ovary  at what age was this? __________ years old
☐ Yes, both ovaries  at what age was this? __________ years old
☐ No
☐ Don’t know

9. Have you ever had a breast biopsy or lumpectomy (i.e. breast tissue removed by surgery) that was diagnosed as cancer?

☐ Yes  at what age was this done? ____________ years old
☐ No
☐ Don’t know
10. Have you had a breast biopsy (i.e. breast tissue removed by surgery, not by fine needle biopsy) that was diagnosed as being benign breast disease such as a non-cancerous cyst or breast lump?

☐ Yes → at what age was this done? ___________ years old
☐ No
☐ Don’t know

Menopause and Hormone Replacement Therapy

11. How long ago was your last period?

☐ Less than 1 month
☐ 1 to 6 months before that date
☐ 7 months to less than 1 year before that date
☐ 1 year or more before that date
☐ Never had a period

12. At the time of your diagnosis, had your menstrual periods stopped for one year or more? (Please do not include times when your period stopped when you were pregnant or breast-feeding, or during serious illness or strenuous exercise).

☐ Yes
☐ No → Please go to # 15

13. How old were you when you had your last period before your periods stopped for one year or more?

__________ years old

14. Why did your periods stop?

☐ Natural menopause (periods stopped by themselves)
☐ Don’t Know
☐
Surgery or other medical treatment

What surgery or other medical treatment did you receive that made your periods stop?

*Please tick as many as apply:*

- [ ] Hysterectomy
- [ ] Both ovaries removed
- [ ] Radiation or chemotherapy
- [ ] Don’t know
- [ ] Other

Please specify: ________________________

15. Had you ever taken estrogen, progestin, or other female hormones for menopause? The preparation may have been pills, injections/shots, skin patches, vaginal creams, or vaginal suppositories. **This question does not include oral contraceptives (birth control pills).**

- [ ] Yes
- [ ] No  ➔  Please go to #20
- [ ] Don’t know  ➔  Please go to #20

16. How old were you when you **first** took estrogen, progestin, or other female hormones?

______ years old

17. Were you still having periods when you **first** took estrogen, progestin or other female hormones?

- [ ] Yes
- [ ] No
- [ ] Don't know

18. At the time of your diagnosis, were you taking estrogen, progestin, or other female hormones?

- [ ] Yes
113

How old were you when you last took estrogen, progestin, or other female hormones?

_____ years old

19. How many years, in total, did you take estrogen, progestin, or other female hormones?

_____ years

Pregnancy History

20. Have you been pregnant?

☐ Yes

☐ No  ➔ Please go to #26

21. How many times have you been pregnant? _____

22. How many live births have you had? _____

23. How old were you when your had your first live birth? _____

24. How old were you when you had your last live birth? _____

25. Did you breastfeed?

☐ Yes  ➔ For how long? Child 1: _______ Child 2: _______ Child 3: _______

☐ No

Reproductive History

26. Have you ever taken hormonal contraceptives, in the form of birth control pills, implants or injections (this does not include hormone replacement therapy)?
27. How old were you when you first started taking hormonal contraceptives?

_____ years old

28. At the time of your diagnosis, were you taking hormonal contraceptives?

☐ Yes

☐ No

→ How old were you when you last took hormonal contraceptives?

_____ years

29. For how many years in total did you take hormonal contraceptives?

_____ years

30. Have you ever taken a drug for infertility (to become pregnant), or because your periods were stopped?

☐ Yes

☐ No → Please go #35

→ Was the drug prescribed for infertility as part of GIFT (gamete inter-Fallopian transfer) or IVF (in vitro fertilization) treatment?

☐ Yes

☐ No

31. How old were you when you first started this type of drug?

_____ years
32. At the time of your diagnosis were you taking infertility drugs?
   □ Yes
   □ No

33. For how many months in total, have you taken fertility drugs?
   _____ months

34. What is (are) the name(s) of the drug(s)?
   ○ Clomid
   ○ Pergonal
   ○ Serophene
   ○ hCG
   ○ Other       Please specify __________________________
   ○ Don’t know

**Tamoxifen**

35. Have you taken tamoxifen?
   □ Yes
   □ No      →  go to end
   □ Don’t know →  go to end

36. How old were you when you first took tamoxifen? _______ years old

37. How old were you when you last took tamoxifen? _______ years old

38. In total, for how many years have you taken tamoxifen? ____________ years
APPENDIX 2

Epidemiological questionnaire used for controls.
The Sister Study:
Genetic Studies of Breast Density

Personal History Questionnaire

This questionnaire is about factors that may relate to a person’s risk of developing cancer. Although it is important to have complete data for scientific reasons (that is, we encourage you to answer all questions), we recognize that some areas may be sensitive for some people. If you come to a question that you do not want to answer, it would be helpful to “prefer not to answer” beside it and then to continue to answer the remaining questions. You may receive a call from us if certain questions are left blank.

Should you wish to talk to someone about this questionnaire, you may call 416-946-2939 or 1-866-249-3901
Background Information

1. How old are you? _________ years old

2. What is your date of birth? ___/___/___
   day month year

3. Are you:
   ○ Male
   ○ Female

4. What was the highest level of education you completed?
   ○ Less than 8 years
   ○ 8 to 11 years (without graduation)
   ○ High school graduation
   ○ Vocational or technical school
   ○ Some college or university
   ○ Bachelor’s degree
   ○ Graduate degree

5. Are you currently:
   ○ Married or living as married
   ○ Widowed
   ○ Divorced
   ○ Separated
   ○ Never married

6. Were you born in Canada?
   ○ Yes ➔ Please go to #9
   ○ No ➔ In which country were you born?
     Please specify________________________

7. In what year did you first come to live in Canada? _________________

8. In total, how many years have you lived in Canada? _________________
9. In which country were your parents and your grandparents born?
   Your mother __________________________
   Your father __________________________
   Your mother’s mother __________________
   Your mother’s father __________________
   Your father’s mother __________________
   Your father’s father __________________

10. What was the first language you learned to speak?
   ○ English → Please go to #15
   ○ French
   ○ Spanish
   ○ Chinese
   ○ Japanese
   ○ Tagalog
   ○ Vietnamese
   ○ Hindi
   ○ Other → Please specify __________________

11. Which of these choices best describes how well you speak English?
   ○ Well
   ○ Medium
   ○ Little
   ○ Not at all → Please go to #15
   ○ Don’t know

12. When you are speaking with your spouse or partner, how often do you speak English?
   ○ Always
   ○ Most of the time
   ○ About half of the time
   ○ Occasionally
   ○ Never
   ○ I do not have a spouse or partner

13. When you are speaking with your children, how often do you speak English?
   ○ Always
   ○ Most of the time
   ○ About half of the time
   ○ Occasionally
   ○ Never
   ○ I have no children

14. When you are speaking with your friends, how often do you speak English?
   ○ Always
   ○ Most of the time
   ○ About half of the time
   ○ Occasionally
   ○ Never
15. Please tick the religion into which you, your parents and your grandparents were born:

<table>
<thead>
<tr>
<th></th>
<th>You</th>
<th>Your Mother</th>
<th>Your Father</th>
<th>Your Mother's</th>
<th>Your Mother's</th>
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<td>Seventh Day Adventist</td>
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<td>Don’t know</td>
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<td>O</td>
<td>O</td>
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<td>Other</td>
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<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
</tbody>
</table>

Please specify


16. Please tick the religion which you currently practice?

- Protestant
- Catholic
- Buddhist
- Ashkenazi Jewish
- Sephardic Jewish
- Other Jewish
- Hindu
- Eastern Orthodox
- Muslim
- Mormon
- Seventh Day Adventist
- None
- Other

Please specify


17. What is your ethnic or racial background? Please tick as many as apply.

- Black
- White
- Native (e.g. Indian, Inuit)
- Filipino
- Japanese
- Chinese
- Vietnamese
- Other East Asian (e.g. Korean, Indonesian)
- South Asian (e.g. East Indian, Pakistani)
- Middle Eastern
- Hispanic
- Don’t know
- Other

Please specify


Height and Weight
18. How tall are you?
   ____ feet ____ inches or ____ cm

19. What is your current weight?
   ____ lb or ____ stone ____ lb or ____ kg

Alcohol
20. Have you ever consumed any alcoholic beverages, such as beer, wine, or spirits, at least once per week, for 6 months or longer?
   ○ No → Please go to #25
   ○ Yes

21. At what age did you first start consuming alcohol at least once per week, for 6 months or longer?
   _______ years old

22. For how many years in total have you consumed alcohol at least once per week?
   _______ years

23. When you consume(d) alcohol at least once per week, how many drinks do (did) you usually have in a week?
   beer (12 oz can or bottle) _______
   wine or wine coolers (1 medium glass) _______
   liquor (1 shot) _______

24. Are you currently consuming alcohol at least once per week?
   ○ Yes
   ○ No

   At what age did you stop consuming alcohol at least once per week?
   _______ years old

Smoking
25. Have you ever smoked at least 1 cigarette a day for 3 months or longer?
   ○ No → Please go to #30
   ○ Yes

26. At what age did you first start smoking at least 1 cigarette per day, for 3 months or longer?
   _______ years old

27. For how many years in total have you smoked at least 1 cigarette per day?
   _______ years

28. When you smoke(d), how many cigarettes do (did) you usually smoke in a day?
   _______ cigarettes per day

29. Are you currently smoking at least 1 cigarette per day?
   ○ Yes
   ○ No

   At what age did you stop smoking at least 1 cigarette per day?
   _______ years old

Medical History
30. Has a doctor ever told you that you had a disease such as cancer, leukemia or a malignant tumour?
   ○ Yes → what was the type(s) of cancer? How old were you when this was first diagnosed?
      1. __________________________  _________ years old
      2. __________________________  _________ years old
      3. __________________________  _________ years old

   ○ No
   ○ Don’t know
Males Only:
31. Has a doctor ever told you that you had **prostatic hyperplasia** (BPH or enlarged prostate)?
   - Yes → how old were you when this was **first** diagnosed? ______ years old
   - No
   - Don’t know

32. Has a doctor ever told you that you had **gynecomastia** (enlarged breasts)?
   - Yes → how old were you when this was **first** diagnosed? ______ years old
   - No
   - Don’t know

**Males: please go to #74. Females: please continue with #33.**

Females Only:
33. Has a doctor ever told you that you had **benign breast disease**, such as a **non-cancerous cyst** or **breast lump**?
   - Yes → how old were you when this was **first** diagnosed? ______ years old
   - No
   - Don’t know

34. Has a doctor ever told you that you had **cysts in one or both ovaries**?
   - Yes → how old were you when this was **first** diagnosed? ______ years old
   - No
   - Don’t know

**Surgical History**
35. Have you ever had a breast completely removed?
   - Yes, the right breast → at what age was this? ______ years old
   - Yes, the left breast → at what age was this? ______ years old
   - No

36. Have you ever had an ovary completely removed?
   *If your ovaries were removed at different times, please give your age at the most recent operation.*
   - Yes, one ovary → at what age was this? ______ years old
   - Yes, both ovaries → at what age was this? ______ years old
   - No
   - Don’t know

37. Have you ever had a breast biopsy or lumpectomy (i.e. breast tissue removed by surgery) that was diagnosed as cancer?
   - Yes → at what age was this first done? ______ years old
   - No
   - Don’t know
38. Have you ever had a breast biopsy (i.e. breast tissue removed by surgery, not by fine needle biopsy) that was diagnosed as being benign breast disease such as a non-cancerous cyst or breast lump?
   ○ Yes → at what age was this first done? _______ years old
   ○ No
   ○ Don't know

**Breast Examination**

39. Have you ever had a mammogram (x-ray examination of the breasts)?
   ○ No
   ○ Don't know
   ○ Yes → when and where did you have your last mammogram?
     Hospital/clinic: __________________________ Date: ___ / ___ / ___
     day month year

In total, how many mammograms have you had? _______ if unsure give an approximate number

**Reproductive History**

40. Have you ever had a menstrual period?
   ○ Yes → at what age did you have your first menstrual period? _______ years old
   ○ No

41. Has a doctor ever told you that you had primary amenorrhea (failure of menstrual periods to start naturally)?
   ○ Yes → how old were you when this was first diagnosed? _______ years old
   ○ No
   ○ Don't know

42. Have you ever used hormonal contraceptives, in the form of birth control pills, implants or injections?
   ○ Yes
   ○ No → Please go to #46
   ○ Don't know → Please go to #46

43. How old were you when you first started taking hormonal contraceptives?
    _______ years old

44. Are you currently taking hormonal contraceptives?
   ○ Yes
   ○ No → How old were you when you last took a hormonal contraceptive?
    _______ years old

45. In total, for about how many years have you taken hormonal contraceptives?
    _______ years
**Pregnancy History**

46. Have you ever been pregnant?
   - No ➔ Please go to #57 on page 12
   - Yes

47. How many pregnancies have you had? _____

48. How many live births have you had? _____

Please complete questions #52 to #56 for each pregnancy.

<table>
<thead>
<tr>
<th></th>
<th>1st Pregnancy</th>
<th>2nd Pregnancy</th>
<th>3rd Pregnancy</th>
<th>4th Pregnancy</th>
<th>5th Pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>52. What was the outcome of this pregnancy?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Currently Pregnant</td>
<td>Currentl Pregnant</td>
<td>Currently Pregnant</td>
<td>Currently Pregnant</td>
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</tr>
<tr>
<td></td>
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<tr>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>53. On what date did your pregnancy end?</th>
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<th>1/1/1</th>
<th>1/1/1</th>
<th>1/1/1</th>
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<tr>
<td></td>
<td>day</td>
<td>month</td>
<td>year</td>
<td>day</td>
<td>month</td>
</tr>
</tbody>
</table>

49. How old were you when you had your first live birth? _____ years old

50. How old were you when you had your last live birth? _____ years old

51. Did you ever breast feed a child for one month or more?
   - Yes
   - No
If you have had more than 5 pregnancies, please turn to pages 10 and 11. If not, please go to page 12.
Pregnancy History (continued)

If you have had more than 10 pregnancies, please use the blank page at the end of the questionnaire to answer questions #52 to #56 for each additional pregnancy.

<table>
<thead>
<tr>
<th>1st Pregnancy</th>
<th>2nd Pregnancy</th>
<th>3rd Pregnancy</th>
<th>4th Pregnancy</th>
<th>5th Pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>O Currently Pregnant</td>
<td>O Currently Pregnant</td>
<td>O Currently Pregnant</td>
<td>O Currently Pregnant</td>
<td>O Currently Pregnant</td>
</tr>
<tr>
<td>O Multiple birth</td>
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</tr>
<tr>
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<td>O Induced abortion</td>
<td>O Induced abortion</td>
</tr>
</tbody>
</table>

52. What was the outcome of this pregnancy?

53. On what date did your pregnancy end?

_____/____/____
day month year

_____/____/____
day month year

_____/____/____
day month year

_____/____/____
day month year

_____/____/____
day month year
<table>
<thead>
<tr>
<th>Question</th>
<th>Options</th>
</tr>
</thead>
</table>
| .54. How long was this pregnancy?                                        | ○ 3 months or under  
○ 4 to 6 months  
○ 7 or more months  |
| For live births/stillbirths only:                                        |                                                                          |
| .55. What was the sex of each child delivered from this pregnancy?       | ○ number of males  
○ number of females  |
| For live births only:                                                    |                                                                          |
| .56. Did you breastfeed this child?                                      | ○ No  
○ Yes  
○ Under 1 month  
○ 1 to 5 months  
○ 6 to 11 months  
○ 12 to 24 months  
○ Over 24 months  
○ Under 1 month  
○ 1 to 5 months  
○ 6 to 11 months  
○ 12 to 24 months  
○ Over 24 months  
○ Under 1 month  
○ 1 to 5 months  
○ 6 to 11 months  
○ 12 to 24 months  
○ Over 24 months  
○ Under 1 month  
○ 1 to 5 months  
○ 6 to 11 months  
○ 12 to 24 months  
○ Over 24 months  
○ Under 1 month  
○ 1 to 5 months  
○ 6 to 11 months  
○ 12 to 24 months  
○ Over 24 months  |
Menopause and Hormone Replacement Therapy

57. How long ago was your last period?
   ○ Less than 1 month ago
   ○ 1 to 6 months ago
   ○ 7 months to less than 1 year ago
   ○ 1 year or more ago
   ○ Never had a period

58. Have your menstrual periods stopped for one year or more? (Please do not include times when your periods stopped when you were pregnant or breast feeding, or during serious illness or strenuous exercise.)
   ○ Yes
   ○ No → Please go to #61

59. How old were you when you had your last period before your periods stopped for one year or more?
   _______ years old

60. Why did your periods stop?
   ○ Natural menopause (periods stopped by themselves)
   ○ Don’t know
   ○ Surgery or other medical treatment
     What surgery or other medical treatment did you receive that made your periods stop?
     Please tick as many as apply.
     ○ Hysterectomy (uterus or womb removed)
     ○ Both ovaries removed
     ○ Radiation or chemotherapy
     ○ Other
     Please specify ____________________________
     ○ Don’t know

61. Have you ever taken estrogen, progestin, or other female hormones for menopause?
The preparation may be pills, injections/shots, skin patches, vaginal creams, or vaginal suppositories. This question does not include oral contraceptive (birth control) pills.
   ○ Yes
   ○ No → Please go to #66
   ○ Don’t know → Please go to #66

62. How old were you when you first took estrogen, progestin, or other female hormones?
   _______ years old
63. Were you still having periods when you first took estrogen, progestin or other female hormones?
   ○ Yes
   ○ No

64. Are you currently taking estrogen, progestin, or other female hormones?
   ○ Yes
   ○ No → How old were you when you last took estrogen, progestin, or other female hormones?
       ______ years old

65. In total, for how many years have you taken estrogen, progestin, or other female hormones?
     ______ years

66. Have you ever taken a drug for infertility (to try to become pregnant), or because your periods stopped?
    ○ No → Please go to #70
    ○ Don’t know → Please go to #70
    ○ Yes → Was the drug prescribed for infertility as part of GIFT (gamete intra-fallopian transfer) or IVF (in vitro fertilization) treatment?
        ○ Yes
        ○ No

67. How old were you when you first started this type of drug?
     ______ years old

68. In total, for how many months have you taken this type of drug?
     ______ months

69. What is (are) the name(s) of the drug(s)?
    ○ Clomid
    ○ Pergonal
    ○ Serophene
    ○ hCG
    ○ Other
    Please specify ________
    ○ Don’t know

70. Have you ever taken tamoxifen?
    ○ Yes
    ○ No → Please go to #74
    ○ Don’t know → Please go to #74.

71. How old were you when you first took tamoxifen?
     ______ years old

72. Are you currently taking tamoxifen?
    ○ Yes
    ○ No → How old were you when you last took tamoxifen?
     ______ years old

73. In total, for how many years have you taken tamoxifen?
     ______ years

74. Are you, or have you ever been, a participant in a cancer prevention trial?
    ○ No
    ○ Yes → ○ A tamoxifen trial
           ○ A dietary trial
           ○ Other
    Please specify ________
### Radiation Exposure

For questions #75 to #78 please tick as many answers as apply:

**75.** Have you ever had any of the following types of x-ray examinations that included the chest area?

<table>
<thead>
<tr>
<th>Option</th>
<th>Number of x-ray examinations</th>
<th>Age at first x-ray examination</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-ray examinations for heart catheterization</td>
<td>...........................</td>
<td>...............................</td>
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<tr>
<td>X-ray examinations for scoliosis</td>
<td>...........................</td>
<td>...............................</td>
</tr>
<tr>
<td>Other: <em>Please specify</em></td>
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<tr>
<td>None</td>
<td>...........................</td>
<td>...............................</td>
</tr>
<tr>
<td>Don’t know</td>
<td>...........................</td>
<td>...............................</td>
</tr>
</tbody>
</table>

**76.** Have you ever had any of the following types of x-ray examinations that included the lower abdomen or pelvis?

<table>
<thead>
<tr>
<th>Option</th>
<th>Number of x-ray examinations</th>
<th>Age at first x-ray examination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barium examination of the lower bowel</td>
<td>...........................</td>
<td>...............................</td>
</tr>
<tr>
<td>CT scan or x-ray examinations of the lower spine or pelvis</td>
<td>...........................</td>
<td>...............................</td>
</tr>
<tr>
<td>Other: <em>Please specify</em></td>
<td>...........................</td>
<td>...............................</td>
</tr>
<tr>
<td>None</td>
<td>...........................</td>
<td>...............................</td>
</tr>
<tr>
<td>Don’t know</td>
<td>...........................</td>
<td>...............................</td>
</tr>
</tbody>
</table>

**77.** Have you ever been treated with radiation that included the chest area for any of the following conditions?

<table>
<thead>
<tr>
<th>Condition</th>
<th>Number of treatments</th>
<th>Age at first treatment</th>
</tr>
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<tbody>
<tr>
<td>Cancer</td>
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<td>...........................</td>
<td>...............................</td>
</tr>
<tr>
<td>Enlarged thymus gland</td>
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<td>...............................</td>
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<tr>
<td>Tuberculosis (fluoroscopic x-rays)</td>
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<td>...............................</td>
</tr>
<tr>
<td>Hemangioma</td>
<td>...........................</td>
<td>...............................</td>
</tr>
<tr>
<td>Other: <em>Please specify</em></td>
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<tr>
<td>None</td>
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<tr>
<td>Don’t know</td>
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</table>

**78.** Have you ever been treated with radiation that included the lower abdomen or pelvis for any of the following conditions?

<table>
<thead>
<tr>
<th>Condition</th>
<th>Number of treatments</th>
<th>Age at first treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer</td>
<td>...........................</td>
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<tr>
<td>Bleeding from the uterus or womb</td>
<td>...........................</td>
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<tr>
<td>Growth on the uterus or womb</td>
<td>...........................</td>
<td>...............................</td>
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<tr>
<td>Other: <em>Please specify</em></td>
<td>...........................</td>
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<tr>
<td>None</td>
<td>...........................</td>
<td>...............................</td>
</tr>
<tr>
<td>Don’t know</td>
<td>...........................</td>
<td>...............................</td>
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</tbody>
</table>
Physical Exercise

79. How often did you participate in strenuous exercise activities or sports (e.g. swimming laps, aerobics, calisthenics, running, jogging, basketball, cycling on hills, racquetball)?

*Please complete for each age group up to and including your current age.*

<table>
<thead>
<tr>
<th>Ages</th>
<th>None</th>
<th>1/2 hr</th>
<th>1 hr</th>
<th>1 1/2 hrs</th>
<th>2 hrs</th>
<th>3 hrs</th>
<th>4-6 hrs</th>
<th>7-10 hrs</th>
<th>11 or more hrs</th>
<th>1-3 months</th>
<th>4-6 months</th>
<th>7-9 months</th>
<th>10-12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>12-17</td>
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</table>

80. How often did you participate in moderate exercise activities or sports (e.g. brisk walking, golf, volleyball, cycling on level streets, recreational tennis, or softball)?

*Please complete for each age group up to and including your current age.*

<table>
<thead>
<tr>
<th>Ages</th>
<th>None</th>
<th>1/2 hr</th>
<th>1 hr</th>
<th>1 1/2 hrs</th>
<th>2 hrs</th>
<th>3 hrs</th>
<th>4-6 hrs</th>
<th>7-10 hrs</th>
<th>11 or more hrs</th>
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<tbody>
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<td>Past 3 years</td>
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Twin Question

81. Are you a twin?

- No
- Yes

*If yes, please read the following statement and answer the question.*

Non-identical twins are no more alike than ordinary brothers and sisters.
Genetically identical twins on the other hand look so much alike (that is, they have a strong resemblance to each other in height, colouring, features of the face, etc.) that people often mistake one for the other, especially during their childhood.

Do you think you and your twin are identical?

- Yes
- No
- Don’t know