EVALUATION OF HYSTERECTOMY AS BOTH EXPOSURE AND OUTCOME: ASSOCIATION WITH EPITHELIAL OVARIAN CANCER AND PREDICTION OF PREMENOPAUSAL HYSTERECTOMY WITH OVARIAN CONSERVATION

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A dissertation submitted to the faculty at the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Epidemiology in the Gillings School of Global Public Health.

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ABSTRACT

Rachel Palmieri Weber: Evaluation of hysterectomy as both exposure and outcome: Association with epithelial ovarian cancer and prediction of premenopausal hysterectomy with ovarian conservation (Under the direction of Andrew F. Olshan)

Hysterectomy is the most common non-obstetric, surgical procedure among women in the United States. Older studies have generally reported that hysterectomy is inversely associated with risk of epithelial ovarian cancer (EOC). One of the goals of this dissertation was to investigate this relationship, focusing on subtypes of EOC. Since a majority of ovarian-conserving hysterectomies occur prior to menopause, we also sought to develop a predictive model for premenopausal hysterectomy with ovarian conservation as a way of identifying traits that may help identify women for clinical monitoring and potentially earlier and less invasive treatment than hysterectomy for their conditions.

We estimated study-specific odds ratios by conditional logistic regression for almost 30,000 EOC cases and controls from 15 studies in the Ovarian Cancer Association Consortium and pooled them using random-effects models. Hysterectomy was associated with an average relative increase of 19% in the odds for invasive EOC. The average odds ratios were above the null for all subtypes of EOC, except invasive clear cell. Hysterectomies prior to age 40 or 30 years or more in the past were also positively associated with invasive serous EOC.

We also fit unconditional logistic regression models including fourteen demographic, behavioral, and reproductive and medical history variables to nearly one thousand premenopausal women with and without hysterectomy enrolled in the *Prospective Research on Ovarian Function* Study. The predictive model exhibited very good discriminatory power and was well-calibrated. Family history of benign gynecologic conditions did not contribute to the prediction model and in fact, resulted in a net worsening of classification of hysterectomy.

Our results, from the largest set of EOC cases and controls to date, do not support the long-held belief that hysterectomy is protective against EOC. Considerable heterogeneity of results, potential biases in previous and/or current studies, and/or a changing association between hysterectomy and EOC may explain our results and should be explored further. Though we were able to predict premenopausal hysterectomy with ovarian conservation with very good discriminatory power, additional model development should be pursued in larger and more diverse populations of premenopausal women as we try to better understand premenopausal women who undergo hysterectomy.

For my husband, David G. Weber

I finally finished.

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AUC	Area under the curve
BMI	Body mass index
BSO	Bilateral salpingo-oophorectomy
CI	Confidence interval
DAG	Directed acyclic graph
EOC	Epithelial ovarian cancer
EPV	Events per variable
ERT	Estrogen replacement therapy
FSH	Follicle stimulating hormone
GOF	Goodness-of-fit
HGSC	High-grade serous carcinoma
HRT	Hormonal replacement therapy
IU/L	International units per liter
LGSC	Low-grade serous carcinoma
LMP	Low malignant potential
LOH	Loss of heterozygosity
MSAS	Minimally sufficient adjustment set
NRI	Net reclassification index
OC	Oral contraceptive
OCAC	Ovarian Cancer Association Consortium
OEP	Opposite effects proportion
OR	Odds ratio
PI	Prediction interval
PROOF	Prospective Research on Ovarian Function
ROC	Receiver operating characteristic

RR	Risk ratio
RSME	Root mean square error
SE	Standard error
UK	United Kingdom
US	United States

CHAPTER 1. INTRODUCTION

1.1 Motivation for dissertation

The Ovarian Cancer Association Consortium (OCAC) was originally formed in 2005 to combine data from multiple, established studies of ovarian cancer, primarily of case-control design, in an effort to validate ovarian cancer susceptibility polymorphisms. These large-scale, collaborative efforts include centralized high-throughput genotyping and functional biology experiments, data management, and analysis. Because of the wealth of data on known and suspected epidemiological risk factors for ovarian cancer that is available from numerous member studies, the OCAC is also in the position to evaluate known and suspected epidemiological risk factors for histopathological subtypes of ovarian cancer. These subtype-specific analyses were previously difficult to do because of small sample sizes within any one given study and within specific ovarian cancer subtypes.

Though the long-established literature has reported an inverse association between hysterectomy and epithelial ovarian cancer, epidemiologists within the OCAC were not finding a higher prevalence of hysterectomy among controls in their respective studies as they analyzed other exposures of interest. Additionally, there were no evaluations of the association between hysterectomy and histopathological subtypes of epithelial ovarian due to the rarity of ovarian cancer overall and even smaller frequencies of certain subtypes. To that end, we proposed an evaluation of hysterectomy in 15 case-control studies in the OCAC that would be the largest analysis of individual-level data, to date, of the association between hysterectomy and subtypes of epithelial ovarian cancer.

Because our findings were not consistent with the long-held belief that hysterectomy and ovarian cancer were inversely associated, we sought data that could help us better describe premenopausal hysterectomies with ovarian conservation (the majority of hysterectomies in the OCAC analysis). To that end, we proposed the development of a multivariate prediction model for

hysterectomy with ovarian conservation among premenopausal women in *Prospective Research on Ovarian Function (PROOF): A Study of Hormonal Changes in Premenopausal Women*.

1.2 Dissertation layout

This dissertation includes six chapters. This introductory chapter orients the reader to the motivation behind the doctoral work and organization of the dissertation. Chapter 2 details the review of the literature on hysterectomy, ovarian cancer, and the associations between them. Chapter 3 outlines the data sources and analytic plan for each of the two sets of specific aims that focus first, on hysterectomy as an exposure and second, on hysterectomy as an outcome. Because hysterectomy is the exposure for one set of specific aims, and the outcome for another set of specific aims, the analytic plans are presented separately and correspond with the results in Chapters 4 and 5, respectively. Chapters 4 and 5 present those results in the format of two separate research manuscripts. Chapter 6 recapitulates the specific aims and findings, outlines the strengths and limitations of the analyses, briefly discusses the public health significance of the work, and provides suggestions for future research related to hysterectomy.

CHAPTER 2. REVIEW OF THE LITERATURE

2.1 Hysterectomy

2.1.1 Epidemiology of hysterectomy

Hysterectomy is the most common non-obstetric, surgical procedure among women in the United States (US).[1] Approximately 600,000 hysterectomies are performed each year in the US and more than one-third of women will have a hysterectomy by age 60.[2,3] The hysterectomy rate in the US peaked in 1975, when more than 725,000 were performed, declined through the 1980s, leveled off in the 1990s, and decreased only slightly each year between 1997 and 2005;[2-6] the incidence of age-adjusted hysterectomy was 6 per 1000 women in 1997 and by 2005, 5 per 1000 women.[2]

Hysterectomy rates and prevalence appear to be lower across the globe than they are in the US. In Australia, 20% of women have a hysterectomy by age 50. The prevalence of hysterectomy is between 10% and 22% based on Australian surveys[7] and 30,000 hysterectomies are performed annually.[8] The prevalence of hysterectomy is less in the United Kingdom (UK) than in the US or Australia and has not changed since 1996.[9] Rates are even lower in Denmark. The incidence rate of hysterectomy in 1988 in Denmark was 193 per 100,000 women and dropped to 170 per 100,000 women by 1998. Gimbel et al. compared those rates to those of England (292 per 100,000) and the US (425 per 100,000) during the same time period, noting the overall lower incidence in Denmark.[10]

The overall average age at hysterectomy in the US is 46 years old,[3] but the surgical approach varies by age. In 2003, approximately two-thirds of hysterectomies for benign gynecologic conditions were performed abdominally, with a mean age of 44.5 years at hysterectomy. Vaginal hysterectomies, which account for approximately 20% of hysterectomies, were performed at an average age of 48.2 years and laparoscopic hysterectomies (12% of hysterectomies) were performed at a younger mean age of 43.6 years.[11] Hysterectomies performed for uterine leiomyomas (fibroids) were more

frequently abdominal while those for uterine prolapse were more frequently vaginal;[11] this coincides with the reported mean ages at hysterectomy since younger women are more likely to undergo hysterectomy for fibroids or endometriosis and older women are more likely to undergo hysterectomy for prolapse or cancer.[5] Concomitant bilateral salpingo-oophorectomy (BSO) was undertaken in roughly half of hysterectomies in the US in the late 1990s, up from 25% in 1965; however, this rate varies by age.[1,5] The American College of Obstetricians and Gynecologists recommended in 1999[12] and 2008[13] that BSO be avoided in premenopausal women because negative outcomes associated with premature loss of ovarian function outweigh the potential benefits of avoiding future ovarian pathology including ovarian cancer.[14] Thus, during the years 2000-2004, only 37% of hysterectomies among women aged 15-44 years included concomitant BSO versus 78% of hysterectomies among women aged 50-54 years.[3,15,16]

Hysterectomy rates vary by a number of factors, including geographic region, demographics, and reproductive characteristics. Women in the US South undergo hysterectomy at higher rates (5.92 per 1000 women-years) than women in the US Northeast (3.33 per 1000 women-years).[1,11] Though not all studies have shown different hysterectomy rates among racial/ethnic groups,[17,18] many have reported higher rates and/or higher prevalence of hysterectomy among African American women, who more often than not undergo abdominal hysterectomy.[1,17,19-21] Women undergoing hysterectomy are also more likely to have had fewer years of education, lower income, be parous, or have had their first birth at an early age.[22-24]

2.1.2 Indications for hysterectomy

Most hysterectomies (90%) are performed for benign gynecologic conditions such as fibroids; menstrual disorders such as excessive bleeding; uterine prolapse; and endometriosis[8,11,16,25,26] but the reasons differ by race/ethnicity. In an analysis of hospital discharge data from the 2003 Nationwide Inpatient Sample, representing a 20% stratified sample of US hospitals, the most frequent indications for hysterectomy were fibroids (33%), menstrual disorders (21%), uterine prolapse (16%), and endometriosis (14%) among white women; among African American women, hysterectomies

were predominantly performed to treat fibroids (70%) rather than menstrual disorders (12%), uterine prolapse (4%), or endometriosis (6%).[11]

Uterine fibroids are very common in women and typically appear during a woman's reproductive years.[27,28] Although they are often symptomatic, fibroids can cause considerable morbidity related to pelvic pain, excessive bleeding, and other symptoms. It has been estimated that fibroids cost the US billions of dollars per year[29] and that worldwide, they account for approximately 40% of all hysterectomies.[8] Fibroids are positively associated with age during the reproductive years, African American race/ethnicity, earlier menarche, obesity, diabetes, hypertension, and polycystic ovarian syndrome.[27,28,30-33] African American women are also more likely to experience more numerous and larger fibroids at earlier ages, which may partially explain why they are more likely to undergo hysterectomy than white women.[20,34]

The true prevalence of endometriosis, the presence of endometrial tissue outside of the uterus, is unknown due to a lack of non-invasive diagnostic tests but is thought to be around 10%.[35-37] It has been estimated from surgical studies that the prevalence of endometriosis among fertile women undergoing tubal ligation is 3-6% while it is much higher (30-50%) among women with infertility or pelvic pain.[38] Women with endometriosis typically present with symptoms of dysmenorrhea; chronic pelvic pain; infertility; bladder and bowel pressure/discomfort; and dyspareunia.[39,40] A higher prevalence of endometriosis has been associated with earlier age at menarche; shorter menstrual cycles; nulliparity; lower body mass index (BMI) levels; alcohol and caffeine use; prior pelvic surgery; infertility; and family history of endometriosis[38,39,41,42] however, the etiology remains unclear.

2.1.3 Role of family history in indications for hysterectomy

Women with fibroids who have a family history of fibroids may be more likely than women without a family history to present with more severe symptoms, and thus be more likely to undergo hysterectomy[43,44] and at an earlier age.[45] In fact, twin studies indicate that as many as 50-70% of fibroids have a heritable component[44,45] and another study has shown associations with fumarate

hydratase gene mutations, which are seen in rare familial syndromes.[46] It may be that an apparent increase in risk for fibroids among women with a family history could be attributed to women with a family history being more likely to inquire about symptoms when consulting with their doctor. Similarly, one recent study reported no association between family history of fibroids and a fibroids diagnosis when cases were limited to women who reported their family history prior to their own diagnosis.[47]

Though researchers have suggested that "controlling for family history of uterine leiomyomas as a proxy for elevated genetic risk could reduce the racial difference in uterine leiomyoma disease outcomes,"[43] it is clear that the role of family history in fibroids diagnosis requires additional research. Broadly defined, family history is a proxy for both inherited factors and shared family environment; it is not simply a proxy for genetic risk.

A study of twins in Australia reported that the incidence of endometriosis among monozygotic twins was twice the incidence among dizygotic twins;[48] early studies of family history of endometriosis among mothers and sisters have reported up to a 6-fold increase in prevalence.[49] A more recent study reported an increase in risk of endometriosis with family history among first-degree relatives, though of a lower magnitude than in previous studies (odds ratio (OR) =2.03; 95% confidence interval (CI): 0.54, 8.25).[40] Interestingly, the investigators did not find differences in the characteristics of the cases, with or without family history, or their symptoms; earlier reports have suggested that the severity of endometriosis is higher among women with a family history.[49] As with fibroids, the role of family history of endometriosis with respect to risk, severity of symptoms, and outcome requires additional study.

2.2 Epithelial ovarian cancer

2.2.1 Epidemiology of ovarian cancer

In the US, ovarian cancer is the leading cause of death among cancers of the female reproductive tract and is the fifth leading cause of cancer death among women after lung, breast, colorectal and pancreatic cancers. In 2015, there will be an estimated 21,290 new cases of ovarian

cancer and 14,180 deaths due to ovarian cancer.[50] Worldwide, the incidence of and death due to ovarian cancer is approximately equal between developed and developing countries.[51] Ovarian cancer is a highly fatal disease, in part because reliable screening methods do not exist and most patients present with advanced disease. In the US, the five-year survival rate for all stages of disease is 45%; patients with localized disease have a five-year survival rate of 92% but only 19% of ovarian cancers are diagnosed at this stage. Patients with regional and distant ovarian cancer have five-year survival rates of 72% and 27%, respectively.[50]

2.2.2 Heterogeneity of epithelial ovarian cancer

Ovarian cancer is an etiologic heterogeneous collection of tumors. Sex cord-stromal and germ cell tumors are rare, accounting for 5-10% and <5%, respectively, of all ovarian tumors. Sex cord-stromal tumors arise in the connective tissue holding the ovary together and germ cell tumors originate in the cells that are destined to form eggs within the ovaries.[52] Epithelial ovarian cancer (EOC), occurring within the cells of the ovarian surface, accounts for over 90% of all ovarian cancers and is the focus of our research.

Low malignant potential (LMP), or borderline tumors, and invasive tumors are the two main types of EOC even though LMP tumors are not considered to be cancerous. There are several histologic subtypes of both LMP tumors and invasive EOC: serous, mucinous, endometrioid, clear cell, mixed cell, and undifferentiated. Most LMP tumors are serous (50-60%) or mucinous (30%).[53,54] Serous is the most common histologic subtype of invasive EOC, accounting for approximately 50% of EOC cases. Endometrioid (~20%), mucinous (~10-12%) and clear cell (~8-10%) are much less common.[55] The mucinous subtype is thought to be over diagnosed due to misclassification of metastatic tumors from other sites;[56] some studies have reported frequencies as low as 3% for mucinous invasive EOC in their populations.[55]

Serous and mucinous EOC are thought to be very different from one another and may have different sets of risk factors. Molecularly, *k-ras* mutations are generally found in mucinous but not serous tumors; the opposite is true for *p53* mutations.[57] Mucinous tumors are thought to be associated

with younger age compared to non-mucinous tumors[58,59] and may be associated with environmental exposures such as smoking more so than reproductive exposures such as number of children or oral contraceptive (OC) use.[52,59-61] Likewise, the endometrioid and clear cell subtypes are thought to be very different from both serous and mucinous EOC, but are often lumped together, especially since both are associated with endometriosis.[53,56,62]

Most LMP tumors do not appear to progress to invasive EOC.[53,57,63] LMP serous and invasive serous EOC have some molecular differences: *p53* mutations in invasive serous, loss of heterozygosity (LOH) on the long arm of the inactivated x chromosome in LMP serous and microsatellite instability in LMP serous.[57] If LMP serous tumors progressed to invasive serous EOC, we would expect to see the same kind of molecular profiles in both types of serous EOC.[53] Mucinous EOC appears to be the only histologic subtype to follow a progression model whereby LMP tumors progress to invasive tumors.[54,56,57] The LOH profiles of both LMP and invasive mucinous EOC are very similar.[57]

2.2.3 Ovarian cancer pathogenesis

Despite the high mortality from ovarian cancer and the burden of disease, the etiology is not clearly understood. Though the mechanisms underlying them are not fully described, reproductive factors such as parity, OC use, breast-feeding, endometriosis, and tubal ligation have been established as factors associated with EOC risk.[64] Cases of ovarian cancer are primarily sporadic; however, 5-10% are familial, primarily due to germline mutations in the *BRCA1* and *BRCA2* tumor suppressor genes.[65]

In 1971, Fathalla postulated that "incessant ovulation" might play a role in the development of ovarian neoplasms due to recurrent repair and exposure of the epithelium of the ruptured follicles to estrogen-rich follicular fluid.[66] The gonadotropin hypothesis suggests that high levels of pituitary gonadotropins, within normal cycles, increase cancer risk by stimulating the ovarian surface epithelium, a proliferative process that may propagate mutations and promote carcinogenesis.[67] Other etiologic hypotheses include: inflammatory response in the ovarian epithelium due to retrograde

transportation of contaminants or endogenous carcinogens,[68,69] a pregnancy-dependent clearance of malignant cells from the ovaries,[70] and hormonal imbalances resulting in excess androgen and deficiency in progesterone.[71]

2.3 Hysterectomy and ovarian cancer

2.3.1 Association between hysterectomy and epithelial ovarian cancer

In general, the literature suggests that hysterectomy is protective against EOC. In a large, pooled case-control study of invasive EOC[72] hysterectomy was significantly protective for the hospital-based case-control studies (OR=0.66, 95% CI: 0.50, 0.86); the result was slightly attenuated for the population-based case-control studies (OR=0.88, 95% CI: 0.72, 1.1). Harris et al.[73] also reported a decreased risk of LMP EOC (OR=0.87, 95% CI: 0.58, 1.3). Similar estimates have been reported in other case-control studies[58,61,74-81] and in cohort studies.[82-85]

Several studies have looked at the effect of hysterectomy alone and hysterectomy with unilateral oophorectomy on EOC risk. One study reported that a non-significant decreased risk of EOC was limited to women who had hysterectomy without unilateral oophorectomy.[86] Four studies report no substantial differences in the association with EOC between the hysterectomy alone and hysterectomy with unilateral oophorectomy groups; [75,85,87,88] however, these studies included very small numbers of participants exposed to hysterectomy.

Two studies reported an increased risk of LMP mucinous tumors. Jordan et al.[60] reported that women with hysterectomy were twice as likely to have an LMP mucinous tumor than women without hysterectomy (OR=2.0, 95% CI: 1.3, 3.2); they found no association between hysterectomy and invasive mucinous tumors. Another case-control study also found an increased risk of LMP mucinous tumors among women with hysterectomy (OR=1.18, 95% CI: 0.53, 2.60).[61] Other studies found no differences in association among the different histologic subgroups.[58,61,80]

Only two studies statistically evaluated effect measure modification by endometriosis or parity. In a pooled case-control study, hysterectomy was protective against EOC among women with endometriosis (OR=0.69, 95% CI: 0.38, 1.24) but not women without endometriosis (OR=1.02, 95%

CI: 0.68, 1.23, p for interaction=0.24).[89] It is important to note that these findings were for EOC overall, rather than for the endometrioid or clear cell subtypes alone. The endometrioid and clear cell subtypes of invasive EOC are both associated with endometriosis[56] so there is the possibility that the effect of hysterectomy on EOC risk among women with endometriosis might be different according to the different histologic subtypes. Hysterectomy was protective against EOC for both nulliparous women (OR=0.29, 95% CI: 0.10, 0.81) and parous women (OR=0.56, 95% CI: 0.39, 0.80, p for interaction=0.46) in another study.[81]

2.3.2 Timing of hysterectomy and epithelial ovarian cancer

A "healthy screenee" effect may explain the reported inverse association between hysterectomy and EOC. Some women who are undergoing surgery for hysterectomy have their ovaries visualized and checked for abnormalities during surgery, giving the doctor a chance to detect ovarian cancer or possible pre-cursors.[90] If this were the case, only hysterectomies performed close in time to the diagnosis of EOC would show a protective effect. One study showed support for this "healthy screenee" hypothesis. Weiss and Harlow[90] report protective odds ratios for hysterectomies less than five years prior to diagnosis or interview. However, hysterectomy appeared to be deleterious at six to ten years (OR=1.4, 95% CI: 0.83, 2.4) and at greater than ten years (OR=1.6, 95% CI: 1.1, 2.2).

The inconsistencies in the literature on this topic are likely due to insufficient power to detect associations due to very small sample size; however, most studies do not support the hypothesis.[72,76-78,81,83,86,88] One study reported that risk of EOC decreased as time since surgery increased and that the decrease in risk was highest 25 years prior to diagnosis.[77] Another study reported a similar decrease in risk over time (Risk Ratio (RR)=0.95, 95% CI: 0.91, 1.00 for each year from surgery).[88] Though Risch et al. reported a very small decrease in risk per year since hysterectomy (RR=0.996, 95% CI: 0.97, 1.03), the protective effect of hysterectomy remained after 20 years.[81] Whittemore et al. also reported a sustained protective effect, with even a decrease in risk over time for women in the population-based case-control studies that were pooled (RR=0.99, 95% CI: 0.71, 1.40 for 2-9 years

since hysterectomy; RR=0.86, 95% CI: 0.63, 1.20 for 10-19 years; and RR=0.79, 95% CI: 0.53, 1.20 for 20+ years).[72] Finally, in a large case-control study, women who reported hysterectomy more than 15 years prior to diagnosis/interview were at the lowest risk of invasive EOC (OR=0.6, 95% CI: 0.4, 0.9).[76]

2.3.3 Age at hysterectomy and epithelial ovarian cancer

Whittemore et al.[91] asserted that hysterectomy might impair ovarian function by comprising blood flow to the ovaries, thereby resulting in anovulation. If this were the case, hysterectomy during a woman's reproductive years would confer more protection than hysterectomy after menopause. Though the age at hysterectomy cut-point varied among the analyses (e.g., 40, 45, 55), most published reports suggest no difference between hysterectomy performed during the reproductive years and the post-menopausal years.[76,78,81,82,86,88] There were, however, two reports that lend support to the hypothesis that hysterectomy in the reproductive years offers more protection against ovarian cancer. In a historical cohort study in Canada, there were significantly fewer ovarian cancers observed among women who had had a hysterectomy between the ages of 25 and 44 (Observed/Expected=0.55, $p<0.001$).[83] Additionally, Whittemore and colleagues reported that women who had hysterectomy before age 40 were 42% less likely to have EOC (OR=0.58, 95% CI: 0.40, 0.86) than women who had not had a hysterectomy. Women who had hysterectomy after the age of 40 were only 27% less likely to have EOC than women who had not had a hysterectomy (OR=0.73, 95% CI: 0.51, 1.00). These pooled results were from six hospital-based case-control studies; the pooled results from six population-based case-control studies were attenuated and were more similar across age at hysterectomy strata.[72]

2.4 Conclusions

Currently, there are no known multivariate prediction models for premenopausal hysterectomy with ovarian conservation, which account for a large proportion of all hysterectomies performed. While we generally know that women with uterine fibroids, endometriosis, and other menstrual-related conditions choose to undergo hysterectomy for treatment of their common, benign

gynecologic condition(s), we need to have a better understanding of other factors that may be related to their decision. Additional traits, including family history of common, benign gynecologic conditions, may help identify women for clinical monitoring, and potentially less invasive treatment. Additionally, information about women who have hysterectomies may inform imputation models for missing hysterectomy status and future studies with respect to enrolling appropriate participants for studies of hysterectomies.

Related to the need for a better understanding of premenopausal hysterectomy is a need for more careful analyses of the association between hysterectomy and ovarian cancer. Both the exposure (hysterectomy) and outcome (EOC) have not been identified in a standardized way throughout the literature. While some studies specify EOC, and go further to distinguish between invasive and LMP tumors, other studies simply defined their outcome as ovarian cancer. Non-epithelial ovarian cancers are associated with different risk factors and should be analyzed separately. Additionally, while some studies analyze invasive and LMP tumors separately (or combine them after checking that the exclusion of LMP tumors didn't appreciably change the results), other studies simply combine the two types of tumors. Since there is some evidence that hysterectomy may have a different association with LMP EOC [60,61], than with invasive EOC, it is important to describe any differences between them.

The hysterectomy and ovarian cancer literature is outdated; the largest sample analyzed[72] is more than 25 years old and only one study [60] has published data on women from beyond the year 1999. Because hysterectomy rates have been changing in the past few decades, as have rates of concomitant oophorectomy, it is important to update the literature with current studies and larger sample sizes.

CHAPTER 3. METHODS

3.1 Association between hysterectomy and epithelial ovarian cancer

3.1.1 Specific aims

The specific aims of the study for the association between hysterectomy and epithelial ovarian cancer are as follows:

1) Specific aim 1: Determine the association between hysterectomy and epithelial ovarian cancer.
2) Specific aim 2: Determine the association between timing of hysterectomy (with regard to the time at diagnosis among cases and time at interview/reference point among controls) and epithelial ovarian cancer.
3) Specific aim 3: Determine the association between age at hysterectomy and epithelial ovarian cancer.

3.1.2 Overview of methods

We will conduct a pooled analysis of epithelial (EOC) cases and controls from 15 studies in the Ovarian Cancer Association Consortium (OCAC) to evaluate the association between hysterectomy, time since hysterectomy, and age at hysterectomy and subtypes of EOC. Study-specific ORs will be calculated by conditional logistic regression conditioned on age and race/ethnicity and adjusted for by a minimally sufficient adjustment set (MSAS) of covariates identified by an evaluation of a directed acyclic graph (DAG) representing the relationships among the exposure of interest (hysterectomy), the outcome (EOC), known and suspected risk factors for EOC, and factors associated with hysterectomy. The study-specific estimates will be pooled using random-effects models; 95% prediction intervals (PI) will be calculated when between-study variance is greater than zero.

3.1.3 Study populations and data sources

The OCAC was formed to create an opportunity for researchers to evaluate genetic associations with ovarian cancer with increased power due to larger sample size. While a major aim of the OCAC is to follow up on promising genetic associations while addressing the issues of multiple comparisons and false discoveries that are inherent to studies using high-throughput genotyping technologies,[92-99] investigators are keen to take advantage of the rich epidemiologic data that is available. To date, there have been over 60 peer-reviewed manuscripts published that highlight the work of the OCAC to identify and validate genetic associations with EOC[100-104] and to evaluate known and suspected risk factors for subtypes of EOC.[62,105-108]

Fifteen OCAC case-control studies agreed to participate in our proposed analyses of hysterectomy and EOC. One study is from Australia (AUS); one is from the UK (UKO); four are from Europe (GER, MAL, NTH, POL); and nine are from the United States (CON, DOV, HAW, HOP, NCO, NEC, STA, UCI, USC).

EOC cases were identified predominantly through cancer registries and hospital tumor or pathology boards; these cases are considered to be population-based. All but two of the studies (NTH, UKO) contributed both low malignant potential (LMP) and invasive EOC tumors to the OCAC database. Two of the studies (DOV and USC) received pathology information from their respective Surveillance, Epidemiology, and End Results cancer registries. A majority of the study sites centrally reviewed pathology information from patients' pathology reports and a subset of studies confirmed pathology, tumor behavior, and histology by examination of histopathological slides (CON, HAW, MAL, NCO, STA, and UCI). All but three studies (NTH, UCI, UKO) enrolled incident cases of ovarian cancer and collected epidemiologic data within one year of the ovarian cancer diagnosis, on average.

Controls were predominantly recruited through random digit dialing, population rosters, and neighborhoods; these controls are generally considered to be population-based. Controls from the NTH study were recruited from the Nijmegen Biomedical Study, a population-based survey based on

an age-stratified random sample of the population of Nijmegen. Controls from the UKO study were postmenopausal women participating in the United Kingdom Collaborative Trial of Ovarian Cancer Screening. All controls had to be at risk for ovarian cancer at the time of enrollment and therefore, had to have at least one intact ovary. Women reporting bilateral salpingo-oophorectomy (BSO) were not eligible for any of the studies. If a woman was unsure of her ovarian status (i.e., whether she had previously had a BSO), she was excluded from the following studies: CON, DOV, HAW, NEC, STA, and USC.

Participants from each of the studies completed questionnaires that ascertained demographic information on the known and suspected risk factors for ovarian cancer including: family history of breast and/or ovarian cancer; menstrual and reproductive histories; use of exogenous hormones such as oral contraceptive (OC) and hormone replacement therapy (HRT); medical and surgical histories; height and weight at various times in life; smoking history; and diet and medication use patterns. Not all studies collected information on the covariates of interest in this study: body mass index (BMI) in early adulthood and/or endometriosis were not collected by the NTH, POL, and STA studies.

3.1.4 Statistical methods

3.1.4.1 Exposures

The primary exposure of interest will be defined as hysterectomy that occurred more than 2 years prior to diagnosis (cases) or interview/reference data (controls). Women reporting hysterectomy within two years of diagnosis will be considered "unexposed" to avoid including cases whose hysterectomies were related to their sub-clinical ovarian cancer in the exposed group. Additionally, since we will only have ages at diagnosis and hysterectomy, we want to avoid including cases in the exposed group whose hysterectomies were part of their surgery to remove ovarian tumors. In preliminary analyses, the percentage of hysterectomies occurring with 2 years of diagnosis or interview was less than 10% for controls from all studies and for cases from most studies. Eleven percent of cases from the CON study, 41% of cases from the UKO study, and a majority of cases from the NTH and POL studies (54% and 71%, respectively) occurred within two years of diagnosis

and will be considered unexposed. To evaluate the association between age at hysterectomy and EOC, and because a majority of hysterectomies will have occurred prior to menopause or before age 50 (a common proxy for age at menopause), we will dichotomize the hysterectomy groups (<40 and ≥40 years of age). To evaluate the association between time since hysterectomy and EOC, we will group the hysterectomies into three groups: 2- <15 years, 15- <30 years, and ≥30 years).

3.1.4.2 Outcomes

To facilitate comparisons with the published literature, we will analyze all invasive EOC cases combined and all LMP tumors combined. However, because invasive EOC is a heterogeneous disease, we will define the case groups by tumor behavior and histology as follows: LMP serous, invasive serous EOC, invasive endometrioid EOC, and invasive clear cell EOC. For mucinous tumors, we will evaluate LMP mucinous tumors and invasive mucinous EOC cases combined and separately. Because research has suggested that low-grade invasive serous EOC more closely resembles LMP serous tumors, and because low- and high-grade invasive serous EOC may arise via different pathways, we will also evaluate them separately.[109,110]

3.1.4.3 Control of confounding

All study-specific estimates will be adjusted for age (<40; 40- <45; 45- <50; 50- <55; 55- <60; 60- <65; 65- <70; 70- <75; and ≥75) and race/ethnicity (non-Hispanic white, Hispanic white, black, Asian, and other). All study-specific estimates for the associations between the different hysterectomy exposures and the different LMP and invasive EOC outcomes will also be adjusted for a set of covariates, identified *a priori*, by careful consideration of causal diagrams. We constructed DAGs[111,112] to identify a MSAS for each of the different hysterectomy-outcome pairings using the DAG program v0.20 (http://epi.dife.de/dag/).[113] Because the outcome groups share many risk factors, the MSAS are similar. For the overall LMP, LMP serous, overall invasive and invasive serous models, the MSAS includes: BMI in early adulthood (<18.5; 18.5- <25; 25- <30; and ≥30); number of full-term births (0; 1; 2; 3; and ≥4); duration of OC use (0; <24, 24- <60; 60- <120; and ≥120 months); tubal ligation; and history of breast or ovarian cancer in the mother. The MSAS for the

invasive endometrioid and clear cell ovarian cancer models additionally includes self-reported endometriosis. The MSAS for the mucinous ovarian cancer models includes only age and race. Variables that were included in the DAGs but not identified as part of the MSAS were: age at menarche; breastfeeding; estrogen replacement therapy; combination hormone replacement therapy; unilateral oophorectomy; smoking; uterine leiomyomata; lifetime number of ovulatory cycles; premature ovarian failure; age at menopause; pelvic pain; abnormal uterine bleeding; and menopausal symptoms.

3.1.4.4 Study-specific estimates of association

We will estimate study-specific odds ratios (OR) and 95% confidence intervals (CI) for the associations between the different hysterectomy exposures and the different EOC outcomes using conditional logistic regression; the design variables of age and race/ethnicity will define the conditioning sets. We will produce both unadjusted estimates and estimates adjusted for the MSAS identified by the DAGs.

3.1.4.5 Pooling of data

We will obtain estimates of the mean and variance of random-effects distributions using the restricted maximum likelihood method of DerSimonian and Laird[114,115] in meta-regression models without regressors (i.e., intercept-only) to get estimates of the mean and variance of random effects distributions. A random-effects model produces estimated values of the mean (μ) and variance (τ^2) of a presumptively normal distribution of "true" values: in this case, a distribution of log odds ratios across different populations. The antilog of the distribution's estimated mean, $\hat{\mu}$, may be considered a point estimate of the average OR, which is reported along with a 95% CI to reflect the role of sampling error. We will describe the estimated variance (spread) of the random-effects distribution by computing the opposite effects proportion (OEP) and 95% PI. The OEP is our name for the area under the curve on the opposite side of the null value from the estimated mean[116]. For instance, if $\hat{\mu} > 0$, the estimate of the average odds ratio is greater than 1, and the OEP estimates the proportion of populations in which the odds ratios are below 1. The 95% PI, which is calculated (on the log OR

scale) as $\hat{\mu} \pm t_{k-2}\sqrt{\hat{\tau}^2 + \widehat{SE}(\hat{\mu})^2}$, where k is the number of studies in the meta-analysis, t_{k-2} is the 2.5th percentile of the t distribution with $k-2$ degrees of freedom, and $\widehat{SE}(\hat{\mu})^2$ is the estimated standard error of the sampling distribution for $\hat{\mu}$117-119. Technically, in hypothetically endless repetitions of the entire ensemble of k studies, 95% of the 95% PIs cover the "true" OR to be estimated in the "next" study (i.e., study number $k+1$). As a practical matter, the 95% PI is attractive because its width is determined not only by the estimated spread of the random-effects distribution, $\hat{\tau}^2$, but by the estimated degree of random error, in the form of $SE(\hat{\mu})^2$. When $\hat{\tau}^2 > 0$, the 95% PI for the next odds ratio is wider than the 95% CI for the average OR. When $\hat{\tau}^2 = 0$, the random-effects model reduces to a fixed-effect model and the two intervals become identical.

3.1.4.6 Heterogeneity of results

We will evaluate a list of study characteristics related to study validity and general characteristics of time and place, one at a time in a series of meta-regressions, to identify potential sources of heterogeneity of results. Regarding study validity, we will consider: case type (population-based/hospital- or clinic-based); prevalence of cases defined by mean time between diagnosis and interview (≤1 year/>1 year); control sampling (concurrent with cases/after cases were enrolled); and enrollment of controls unsure of oophorectomy status (explicitly not enrolled because of indeterminate eligibility/included). We will evaluate the following characteristics related to time and place: location (United States (US)/non-US); median year of diagnosis (≤2000/2000-); age-standardized prevalence of hysterectomy among controls (<15%/≥15%) as a surrogate for how common hysterectomy is in the source populations; and median year hysterectomies were performed among controls (<1980/1980-). To address multiple testing in our evaluation of these study characteristics, we will perform Monte Carlo permutation tests as described by Higgins and Thompson[120] and, *a priori*, use a multiplicity adjusted alpha of 0.05 for identifying study characteristics that explain heterogeneity.

3.2 Prediction of premenopausal hysterectomy

3.2.1 Specific aims

The specific aims of the prediction of premenopausal hysterectomy study are as follows:

1) Specific aim 1: Develop and internally validate a predictive model for hysterectomy with the intention of ovarian conservation among premenopausal women.
2) Specific aim 2: Evaluate the contribution (i.e., incremental value) of family history of common, benign gynecologic conditions among mothers and sisters to the predictive model for hysterectomy with intention of ovarian conservation among premenopausal women.

3.2.2 Overview of methods

We will use unconditional logistic regression to predict hysterectomy with the intention of ovarian conservation among pre-menopausal women enrolled in *Prospective Research on Ovarian Function (PROOF): A Study of Hormonal Changes in Premenopausal Women*. By review of the scientific literature, we have selected, *a priori*, the following variables to be included in the model: age; self-identified race; marital status; education level; duration of OC, number of full-term pregnancies; tubal ligation; uterine leiomyoma (fibroids); endometriosis; ovarian cysts; previous myomectomy; smoking status; and BMI. The model's discriminatory power (i.e., ability to classify correctly) will be evaluated using the concordance statistic (i.e., *c* statistic), which is equivalent to the receiver operating characteristic (ROC) area under the curve (AUC) in a logistic regression model. Model calibration (i.e., correspondence between the model-predicted probabilities and the observed probabilities) will be evaluated with a goodness-of-fit test and visual inspection of the observed versus predicted probabilities plots. Internal validation of the model will be performed by correcting the AUC for optimism/overfit by using bootstrap methods.

We will evaluate the incremental value of adding information regarding family history of common, benign gynecologic conditions in the mother and/or sister(s) to the prediction model by comparing the AUC from the model with that dichotomous variable to the AUC from the original model described above.

3.2.3 Study populations and data sources

PROOF: A Study of Hormonal Changes in Premenopausal Women was a cohort study of premenopausal women conducted in Durham, North Carolina from April 1, 2004 through December 2007.[34,121-123] Women between the ages of 30 and 47 years undergoing hysterectomy at Duke University Medical Center or Durham Regional Hospital, both part of the Duke University Health System and located in Durham, North Carolina, were enrolled. Women with intact uteri attending general gynecology practices in Durham, North Carolina were frequency matched to the women undergoing hysterectomy on age and race. The aims of the original study were to prospectively determine whether women undergoing hysterectomy were more likely to experience ovarian failure during the four years after hysterectomy than the women with intact uteri and to identify medical, reproductive, and lifestyle characteristics that may be associated with earlier ovarian failure.[121] This analysis will only include baseline data for the women who underwent hysterectomy, hereafter identified as cases, and the women with intact uteri, hereafter identified as controls.

Cases were women, aged 30 to 47 years, who were scheduled to undergo hysterectomy for benign gynecologic conditions. Prior to their scheduled hysterectomy, potentially eligible women received a letter from their physician describing the study and alerting them that study personnel would be contacting them to request their participation. In addition to being aged 30 to 47 years and not pregnant, women had to be premenopausal as evidenced by at least one menstrual period in the previous 3 months, had to have at least one intact ovary prior to and following the hysterectomy, had to have no personal history of cancer (except nonmelanoma skin cancer), and had to be able to complete the interview in English. Premenopausal status was confirmed by a pre-operative blood level of follicle-stimulating hormone (FSH) less than 40 international units per liter (IU/L). Seventy-seven percent of the women identified as scheduled to undergo a hysterectomy remained eligible for the study and 72% of those women completed the baseline interview and provided blood samples. Of the 501 potential cases who completed the baseline interview, 4 were excluded because they did not

undergo hysterectomy, 6 were excluded due to pre-operative FSH levels ≥ 40 IU/L, and 41 were excluded because they underwent a bilateral oophorectomy at the time of their hysterectomy. An additional 7 women from the control group were also included as cases because they ended up undergoing hysterectomy within one year after enrollment. Though 47 cases did not complete follow up in the prospective aspect of the original study, they are included in the proposed analysis of baseline data; the final number of cases in this baseline analysis will be 457.

Controls were recruited using brochures and advertisements that were placed in gynecology and family medicine clinics/practices in the Duke University Health System in Durham, North Carolina. Controls were subject to the same eligibility criteria as the cases. Of the 523 potential controls who completed the baseline interview, 15 were excluded due to pre-operative FSH levels ≥ 40 IU/L and 2 were excluded due to no blood sample (i.e., inability to confirm premenopausal status by FSH level). Though 32 controls did not complete follow up in the prospective aspect of the original study, they are included in the proposed analysis of baseline data; the final number of controls in this baseline analysis will be 506. The controls who remained eligible for the study after the exclusions described above had a higher educational level and were less likely to smoke than the controls who were excluded but were similar otherwise.

Participants enrolled in the study completed a baseline interview visit during which they signed a consent form, completed an extensive questionnaire that was administered by a study interviewer, provided a blood specimen, and had anthropometric measurements taken. For the cases, the baseline interview occurred prior to their hysterectomies, most at the time of their pre-operative visits. The questionnaire ascertained information on the following: demographics; menstrual cycle history; reproductive history; medical and gynecologic condition history; family history; and lifestyle characteristics related to alcohol, smoking, diet, and physical activity.

3.2.4 Statistical methods

3.2.4.1 Specific aim 1

3.2.4.1.1 Predictors

Steyerberg et al.[124] caution against basing the structure of prediction models solely on the data under study, especially when the dataset is small, and suggest that previously published or clinically practical parameterizations of variables are preferred over classifications that best fit the data. Based on a review of the scientific literature, we selected the following predictors, *a priori*, for inclusion in the prediction model for hysterectomy with intent of ovarian conservation among premenopausal women: age; self-identified race; marital status; education level; duration of OC use, number of full-term pregnancies; tubal ligation; fibroids; endometriosis; ovarian cysts, previous myomectomy; smoking status; and BMI.

To avoid overfitting the model, the parameterizations of the predictors have also been prespecified and are described in detail in Table 1. Briefly, dichotomous categorical variables (i.e., tubal ligation; fibroids; endometriosis; ovarian cysts; and previous myomectomy) will remain as such in the model. Nominal categorical variables (i.e., race; marital status; education level; and smoking status) will remain as such in the model with similar categories being grouped together to decrease the degrees of freedom being used in the model. Age will remain a linear predictor in the model. It has been reported that dichotomization of predictors results in a loss of discriminative ability[124,125] and as such, the remaining continuous variables (i.e., duration of OC use, number of full-term pregnancies, and BMI) will be included as more flexible restricted cubic splines with 3 or 4 knots.[126] Tests for interaction generally require larger sample sizes to provide adequate statistical power. Additionally, there are no known important interactions to consider *a priori*, based on review of the literature. As such, we will only include main effects in the proposed prediction model.

Overall, we propose the inclusion of 13 predictors in the prediction model, with 21 degrees of freedom. With 457 women having undergone hysterectomy, the number of events per variable (EPV) is 35 and the number of events per degree of freedom is 21. In simulation studies, no major problems

occurred when the EPV was greater than or equal to 10.[127] Subsequent investigators have questioned that guideline, especially in the presence of high regression coefficients and strong correlations between predictors,[128] while others argue that there are circumstances in which model performance is acceptable even with less than 10 EPV.[129]

3.2.4.1.2 Missing data

In complete case analyses, any participant missing data on at least one predictor included in a regression model would be dropped from analyses, resulting in an inefficient analysis and potentially biased estimates, depending on the type of missing data. Using an indicator for missing data is also not recommended.[130,131] First, we will assess the frequency of missing data for each of the variables included in the prediction models by case/control status. If the extent of missingness in the variables is low overall (i.e., <5%), and does not appear to be differential with respect to outcome, we will proceed with a complete case analysis. Because we anticipate a moderate percentage of participants to be missing data on family history of common, benign gynecologic conditions, we will review the distributions of the variables in the prediction model by data status (i.e., non-missing, missing) for family history. Additionally, we will perform a sensitivity analysis to evaluate the scope of impact the participants missing data might confer on the model's performance by fitting the logistic regression for the full model an additional two times: firstly, we will recode the missing family history observations as "no" family history and secondly, we will recode the observations as "yes" family history.

3.2.4.1.3 Model specification and estimation

I will use unconditional logistic regression to predict hysterectomy with the intention of ovarian conservation coded as yes (cases) or no (controls), among pre-menopausal women enrolled in the PROOF Study; coefficients will be estimated with maximum likelihood methods. The model will include all predictors as described above and in Table 1, with the exception of the dichotomous variable for family history of common benign gynecologic conditions in the mother or sister(s), which is reserved for specific aim 2.

Though the practice is common, several studies have concluded that stepwise selection methods for inclusion (or exclusion) of predictors in a model are deficient, often resulting in overestimation of regression coefficients and loss of predictive power.[124,132,133] In a simulation study, Steyerberg et al.[124] evaluated a model that contained 8 true and 9 noise predictors that were randomly associated with the outcome. Compared to a model containing only the true predictors (AUC=0.802), the model with noise predictors was only marginally less discriminative (AUC=0.785). When stepwise selection methods were applied to the full model of 17 predictors (α=0.05), all 9 noise predictors were eliminated from the model, but so were 5 of the 8 true predictors, resulting in an AUC of 0.749. Because of these reports, and the relatively small size of the PROOF dataset, we will not apply any selection methods to the prediction model in order to identify a final set of statistically significant predictors.

3.2.4.1.4 Model performance

The prediction model's performance will be evaluated by both discrimination and calibration.[134,135] Discrimination, the ability of the model to correctly classify women with and without hysterectomy, is most commonly assessed using the concordance statistic (*c* statistic), a rank-order statistic for predictions against actual outcomes. For a binary outcome, the *c* statistic is equivalent to the AUC; the true positive rate (i.e., sensitivity) is plotted on the y-axis against the false positive rate (i.e., 1-specificity) on the x-axis. It is interpreted as the probability that a patient with a higher predicted probability has the outcome when two patients, one with and one without the outcome, are considered. We will present both the ROC curve and the AUC value (i.e., *c* statistic).

Though calibration is often very good in model development and critical in external validation,[133] we will evaluate it for the prediction models without and with family history of common, benign gynecologic conditions . Calibration is the agreement between the observed and predicted outcomes and will be assessed with the Hosmer-Lemeshow Goodness of Fit (GOF) test, a chi-squared test based on the grouping of similar participants into n strata (often 10) on the basis of their probabilities. These strata will be visualized by plotting the observed proportion of cases on the

y-axis against the predicted probability of hysterectomy on the x-axis. We will also estimate the intercept and slope of the calibration line in a logistic regression by regressing the observed outcome on the predicted probabilities; perfect calibration, whereby the observed distributions of outcomes and predicted probabilities are in complete agreement, is indicated by an intercept of 0 and a slope of 1.[136]

3.2.4.1.5 Model validation

Internal validation, the validity of the model in the underlying population that the data came from, may address issues of stability in predictor selection (which we will not employ) and quality of the predictions. The apparent performance of a prediction model is always better in the training/development set than it is in a validation set, even when the two datasets come from the same population; the magnitude of this "optimism" can be used to correct performance metrics such as the AUC.

Internal validation is commonly performed using a split-sample approach: a (usually random) percentage of the study population is included in the training/development stage of prediction and the remaining percentage of the study population is used for validation of the model. In simulation studies, this method resulted in underestimation of the model's performance, unstable estimates, and a high root mean squared error (RMSE); large sample sizes are also required to make this method reasonable. In cross-validation, development occurs in a percentage of the population, validation occurs in the remaining percentage, and vice versa; this procedure is repeated numerous times and the average is taken as an estimate of performance. In simulation studies, this method was preferred over split-sample validation but it did not accurately estimate all of the performance measures. Bootstrapping methods performed similarly to cross-validation methods in the simulation studies, resulting in stable and nearly unbiased estimates of performance; bootstrapping also resulted in the lowest RSME values across methods.[137]

Investigators reported that 50 to 100 bootstrap samples with replacement were sufficient in their simulations but advised a higher number in practice;[137] we will use 200 bootstrap samples to internally validate the prediction model for hysterectomy with intention of ovarian conservation

among premenopausal women in each of the bootstrap datasets. The training/development sample will include 100% of the participants outlined above from the PROOF study. The bootstrap samples will also be drawn from 100% of the participants, resulting in bootstrap samples the same size as the training/development set; on average 63.2% of the participants will be included in at least one bootstrap sample.[138] The bootstrap is used to estimate optimism, the decrease in performance between the bootstrap sample and performance in the original sample. The optimism-corrected performance is then calculated by subtracting the optimism from the apparent performance of the model in the original training/development sample.[134] The observed optimism will be used to correct the AUC. An independent study population has not been identified for this prediction model and thus, no external validation will be carried out. It has been suggested that even internal validation with bootstrapping methods is not sufficient in small datasets[139] so external validation will need to eventually be carried out if this model is to be considered for clinical practice or other settings.

3.2.4.2 Specific aim 2

All of the methods described above will be repeated for a prediction model that includes one additional predictor variable with 1 degree of freedom. The variable regarding family history of common, benign gynecologic conditions in the biological mother or sisters will be a dichotomous summary variable (yes/no) derived from multiple yes/no interview questions regarding endometriosis, fibroids, and ovarian cysts in first-degree relatives (the biological mother or sisters). Daughters are not considered because the premenopausal women included in this study will, on average, not have daughters old enough to have had the opportunity to be diagnosed with these common, benign gynecologic conditions.

Whether the addition of family history information substantially improves prediction will be tested by evaluating whether the difference between the two models' AUC equals zero;[140] the change in the AUC will likely depend on the strength of the model developed in Aim 1.[141] Additionally, we will present a reclassification table,[142] stratified by observed outcome[143] which will show how many subjects were reclassified by adding the family history variable to the prediction model. To further

describe the reclassification, we will calculate the percent of patients reclassified and the percent of reclassified patients that were reclassified correctly[144] as well as the Net Reclassification Improvement (NRI).[143,145] The NRI is similar to the percent of patients reclassified, but it takes into account movements in the correct direction. In other words, the NRI reflects the movement of cases upward and the movement of controls downward, calculated in the following way:

$$NRI = [Pr(up|cases) - Pr(down|cases)] - [Pr(up|controls) - Pr(down|controls)]$$

Alternatively, it can be rearranged to be the sum of the relative improvement for cases and relative improvement for controls. It has been reported that the NRI depends mainly on the effect size of the added predictor rather than the strength of the baseline model.[141] Though this newly developed prediction model (as well as the model developed in Aim 1) is not meant to be used for clinical decision making, the measures by which we evaluate reclassification may provide insight into future models that are intended for clinical use; moving from a prediction model to a prediction rule will require external validation in independent studies.[146]

Table 1. Variables included in the prediction models for hysterectomy with the intention of ovarian conservation among pre-menopausal women in the *Prospective Research on Ovarian Function (PROOF) Study*

Predictor	Self-Report Options / Related Questions from Interview	Parameterization in Prediction Models	Degree(s) of Freedom
Age	Age in years	Linear	1
Race	Nominal categorical: white; black/African-American; American Indian/Eskimo; Asian/Pacific Islander; other	Nominal categorical: white; black/African-American; other	2
Marital status	Nominal categorical: single, never married; married; living with significant other; divorced/separated; widowed	Nominal categorical: single, never married; married/living with significant other; divorced/separated/widowed	2
Education level	Nominal categorical: 8^{th} grade or less; 9^{th} to 11^{th} grade; high school grad/GED; post high school trade or tech school; 1-3 years college; college grad; graduate/professional school	Nominal categorical: high school graduate or lower; some college or post-high school schooling; college graduate or higher	2
Duration of OC use	Continuous time in years based on number of years and months reported for total OC use	Restricted cubic spline with 4 knots at 1 year; 3 years; 5 years; and 10 years	3
Number of full-term pregnancies	Count variable for number of full-term pregnancies	Restricted cubic spline with 3 knots at 1, 2, and 3 pregnancies	2
Tubal ligation	Dichotomous categorical: yes, no	Dichotomous categorical: yes, no	1
Uterine leiomyomas (Fibroids)	Dichotomous categorical: yes, no	Dichotomous categorical: yes, no	1
Endometriosis	Dichotomous categorical: yes, no	Dichotomous categorical: yes, no	1
Ovarian cysts	Dichotomous categorical: yes, no	Dichotomous categorical: yes, no	1
Previous myomectomy	Dichotomous categorical: yes, no	Dichotomous categorical: yes, no	1
Smoking status	Derived from two questions: (Have you smoked at least 100 cigarettes in your entire life?) and (Do you smoke cigarettes now?)	Nominal categorical: never; former; current	2
BMI	Continuous BMI at baseline calculated as kg/m^2 (derived from self-reported height in feet and inches and weight in pounds)	Restricted cubic spline with 3 knots at 18.5 (lower limit of "normal"), 25 (lower limit of "overweight"), and 30 (lower limit of "obese")	2
Family history of common benign gynecologic disorders in mother and/or sister(s)	Derived from a number of yes/no questions in the interview regarding endometriosis, fibroids, and ovarian cysts in the biological mother and/or sister(s).	Dichotomous categorical: yes, no	1

CHAPTER 4. A POOLED ANALYSIS OF HYSTERECTOMY AND SUBTYPES OF EPITHELIAL OVARIAN CANCER

4.1 Abstract

Epidemiologic studies have generally reported inverse associations between hysterectomy and ovarian cancer. However, recent reports indicate that ovarian cancer is a heterogeneous disease with histologic subtype-specific risk factors and the association with hysterectomy may be changing. We conducted a pooled analysis of 12,499 epithelial ovarian cancer (EOC) cases and 16,887 controls from 15 studies in the Ovarian Cancer Association Consortium (OCAC) to evaluate the association between hysterectomy and subtypes of EOC. Study-specific odds ratios (OR) were calculated by conditional logistic regression conditioned on age and race/ethnicity and pooled using random-effects models; 95% prediction intervals (PI) were calculated when between-study variance was >0. Hysterectomy was associated with an average relative increase of 19% in the odds (average OR =1.19, 95% PI: 1.05, 1.36) for invasive EOC. The average ORs for all low malignant potential (LMP) tumors and subtypes of LMP serous, invasive serous, invasive endometrioid, and mucinous EOC were above the null. There was an inverse association between hysterectomy and invasive clear cell EOC (average OR=0.75, 95% PI: 0.28, 2.00). Hysterectomies prior to age 40 (average OR=1.36, 95% PI: 1.15, 1.61) or ≥30 years in the past (average OR=1.85, 95% PI: 0.88, 3.90) were positively associated with invasive serous EOC. Considerable heterogeneity of results, potential biases in previous and/or current studies, and/or a changing association between hysterectomy and EOC may explain why these results do not support the long-held belief that hysterectomy is protective against ovarian cancer.

4.2 Introduction

Older studies have generally reported that hysterectomy, with or without unilateral oophorectomy, is inversely associated with, and interpreted as causally reducing, risk of EOC.[147] However, recent research showing that EOC is a heterogeneous disease, with risk factors differing by histologic subtypes,[58,61,62,105,107,108,148-151] prompts a new examination of this hypothesis among different histological types of EOC. Additionally, examination of a recent meta-analysis[147] suggests that the relationship between hysterectomy and EOC may be changing, though the overall conclusion was that hysterectomy is associated with a decrease in risk.

Hysterectomy is common, with ~600,000 hysterectomies performed annually in the US. More than one-third of women will have a hysterectomy by age 60.[2,3] The hysterectomy rate in the US peaked in 1975, when more than 725,000 were performed, declined through the 1980s, leveled off in the 1990s, and decreased approximately 1.9% per year between 1997 and 2005.[2-6] Hysterectomy rates in Australia, Europe, and the United Kingdom have also been declining in recent years and are lower than in the US.[9,10,152-157] Most hysterectomies are performed for benign gynecologic conditions such as uterine leiomyoma, excessive bleeding, uterine prolapse, and endometriosis.[8,25] Given that it is a common procedure, having a better understanding of the relationship between hysterectomy and EOC is important. To that end, we conducted a pooled analysis of hysterectomy and EOC subtypes among nearly 30,000 women from 15 recent case-control studies in the Ovarian Cancer Association Consortium.

4.3 Materials and methods

4.3.1 Data sources

This analysis included 15 case-control studies in the OCAC, including one from Australia,[158] five from Europe,[101,102,159-164] and nine from the US.[165-178] All studies, except two (NTH, UKO), were population-based and conducted between 1992 and 2010 (Table 2). The NTH study[101,102] was limited to cases alive 2-19 years after diagnosis, with controls selected from a separate cross-sectional study conducted in the middle of the case incidence period. The UKO study[159] included cases from

gynecological oncology centers and controls from an ovarian cancer screening trial. All studies had ethics board approval and obtained written informed consent from all participants.

Self-reported data for hysterectomy and other epidemiologic variables from each study were submitted to the OCAC data coordination center at Duke University where common coding schemes were applied. Non-epithelial ovarian cancer cases and epithelial cases with missing tumor behavior (i.e., LMP or invasive) or histology were excluded from the analysis (n=278). Additionally, women with missing ages at diagnosis (cases), interview/reference date (controls) or hysterectomy were excluded (n=669). Our analytic dataset included 2,654 women with LMP ovarian tumors, 9,845 women with invasive EOC, and 16,887 controls.

4.3.2 Statistical methods

The primary exposure was defined as hysterectomy that occurred >2 years prior to diagnosis (cases) or interview/reference date (controls). Women reporting hysterectomy within two years of diagnosis were considered 'unexposed' to avoid including cases whose hysterectomies were related to their (sub-clinical) ovarian cancer in the exposed group. We also analyzed age at hysterectomy (<40 and ≥40 years) and time since hysterectomy (2- <15 years, 15- <30 years, and ≥30 years).

For primary analyses, we analyzed five case groups: LMP serous; invasive serous; invasive endometrioid; invasive clear cell; and mucinous (LMP and invasive combined). We also evaluated cases with LMP and invasive mucinous tumors separately. Similarly, we evaluated low-grade (grade=1/well-differentiated) and high-grade (grade=2+/moderately, poorly, or undifferentiated) invasive serous cancers separately (LGSC and HGSC, respectively).[109,110] Results were similar for both LGSC/HGSC and LMP/invasive mucinous (Appendix Table 1). To facilitate comparisons with the published literature, we also analyzed all invasive EOC cases combined and all LMP tumors combined.

All estimates were adjusted for age (<40; 40- <45; 45- <50; 50- <55; 55- <60; 60- <65; 65- <70; 70- <75; and ≥75) and race/ethnicity (non-Hispanic white; Hispanic white; black; Asian; and other). We constructed causal diagrams (directed acyclic graphs (DAG))[111,112] to identify a minimally

sufficient adjustment set (MSAS) for each of the different hysterectomy-outcome pairings using the DAG program v0.20 (http://epi.dife.de/dag/).[113] Because the outcome groups share many risk factors, the MSAS were similar. For the overall LMP, LMP serous, overall invasive and invasive serous models, the MSAS also included: body mass index (BMI) in early adulthood (<18.5; 18.5- <25; 25- <30; and ≥30); number of full-term births (0; 1; 2; 3; and ≥4); duration of oral contraceptive (OC) use (0; <24, 24- <60; 60- <120; and ≥120 months); tubal ligation; and history of breast or ovarian cancer in the mother. The MSAS for the invasive endometrioid and clear cell ovarian cancer models additionally included self-reported endometriosis. The MSAS for the mucinous ovarian cancer models included only age and race. As reliable information on BMI in early adulthood and/or endometriosis were not collected by the NTH, POL, and STA studies, we could not adjust for these variables in their study-specific models. Variables evaluated but not included in the MSAS were: age at menarche; breastfeeding; estrogen replacement therapy; combination hormone replacement therapy; unilateral oophorectomy; smoking; fibroids; lifetime number of ovulatory cycles; premature ovarian failure; age at menopause; pelvic pain; abnormal uterine bleeding; and menopausal symptoms.

Study-specific ORs and 95% confidence intervals (CI) for the associations between hysterectomy and EOC subtypes were estimated using conditional logistic regression models with the design variables age and race/ethnicity defining the conditioning sets. For all outcome groups and most studies, there were no material differences between the unadjusted and adjusted effect estimates (results not shown); adjusted ORs are presented here.

Using the *metareg* command in Stata 9 (StataCorp LP, College Station, Texas, USA), we obtained estimates of the mean and variance of random-effects distributions using the restricted maximum likelihood method of DerSimonian and Laird[114,115] in meta-regression models: without regressors (i.e., intercept-only) to get estimates of the mean and variance and with regressors to evaluate, one at a time, a list of study characteristics. Regarding study validity, we considered: case type (population-based/hospital- or clinic-based); prevalence of cases defined by mean time between

diagnosis and interview (≤1 year/>1 year); control sampling (concurrent with cases/after cases were enrolled); and enrollment of controls unsure of oophorectomy status (explicitly not enrolled because of indeterminate eligibility/included). We evaluated the following characteristics related to time and place: location (United States (US)/non-US); median year of diagnosis (≤2000/2000-); age-standardized prevalence of hysterectomy among controls (<15%/≥15%) as a surrogate for how common hysterectomy is in the source populations; and median year hysterectomies were performed among controls (<1980/1980-) (Appendix Table 2). To address multiple testing in our evaluation of these study characteristics, we performed Monte Carlo permutation tests as described by Higgins and Thompson[120] and, *a priori*, used a multiplicity adjusted alpha of 0.05 for identifying study characteristics that explained heterogeneity.

A random-effects model produces estimated values of the mean (μ) and variance (τ^2) of a presumptively normal distribution of "true" values: in this case, a distribution of log ORs across different populations. The antilog of the distribution's estimated mean, $\hat{\mu}$, may be considered a point estimate of the average OR, which is reported with a 95% CI to reflect the role of sampling error. It remains to informatively describe the estimated variance, or spread, of the random-effects distribution. We do so in two ways. One is to compute the opposite effects proportion (OEP), which is our name for the area under the curve on the opposite side of the null value from the estimated mean.[116] For instance, if $\hat{\mu} > 0$, the estimate of the average OR is greater than 1 and the OEP estimates the proportion of populations in which the ORs are below 1. The other approach is to compute a 95% prediction interval (PI), which is calculated (on the log OR scale) as $\hat{\mu} \pm t_{k-2}\sqrt{\hat{\tau}^2 + \widehat{SE}(\hat{\mu})^2}$, where k is the number of studies in the meta-analysis, t_{k-2} is the 2.5th percentile of the t distribution with $k-2$ degrees of freedom, and $\widehat{SE}(\hat{\mu})^2$ is the estimated standard error of the sampling distribution for $\hat{\mu}$.[117-119] Technically, in hypothetically endless repetitions of the entire ensemble of k studies, 95% of the 95% PIs cover the "true" OR to be estimated in the "next" study (i.e., study number $k+1$). As a practical matter, the 95% PI is attractive because its width is

determined not only by the estimated spread of the random-effects distribution, $\hat{\tau}^2$, but by the estimated degree of random error, in the form of $SE(\hat{\mu})^2$. When $\hat{\tau}^2 > 0$, the 95% PI for the next OR is wider than the 95% CI for the average OR. When $\hat{\tau}^2 = 0$, the random-effects model reduces to a fixed-effect model and the two intervals become identical.

Analyses were conducted using SAS 9.2 (SAS Institute, Cary, North Carolina, USA) and Stata 9.

4.4 Results

Less than 1% of controls reported hysterectomy within 2 years of interview/reference date and were reclassified as 'unexposed.' Six percent of cases, a majority of which were from 3 studies (NTH, POL, UKO), reported hysterectomy within 2 years of diagnosis and were reclassified as 'unexposed' (Appendix Table 3). Sensitivity analyses of recent hysterectomies yielded similar results (Appendix Table 4; Appendix Table 5). After reclassification, the unadjusted prevalence of hysterectomy among controls ranged from 4% (POL) to 25% in the UCI study. Among all cases, the prevalence of hysterectomy ranged from 3% (POL) to 23% (NCO) (Table 3). The median ages at hysterectomy were 39 and 40 and the median years since hysterectomy were 22 and 21 for cases and controls, respectively. Among the nine studies that provided reasons for hysterectomy, the most frequently reported reasons among cases and controls, respectively, were fibroids (40%, 37%); uterine bleeding problems (25%, 26%); uterine prolapse (10%, 12%); and endometriosis (8%, 7%).

For all invasive forms of EOC combined, hysterectomy was associated with an average relative increase of 19% in the odds (average OR = 1.19, Figure 1). As the study-specific estimates were fairly close to each other, the estimated spread of the random-effects distribution was modest ($\hat{\tau}^2$=0.002, OEP<1%) and the 95% PI for the next OR (1.05, 1.36) was similar to the 95% CI for the average OR (1.08, 1.32) (Figure 1, Table 4). For LMP tumors, the point estimate was farther from the null (average OR = 1.32, Figure 2), but the estimated spread of the random-effects distribution was

considerably broader ($\hat{\tau}^2$=0.07, OEP=15%). The 95% PI for the next OR (0.69, 2.52) was therefore noticeably wider than the 95% CI for the average OR (1.02, 1.71) (Figure 2, Table 4).

Considerable heterogeneity was apparent for all but one of the EOC subtypes (Table 4). The exception was invasive serous EOC, for which the estimated average OR of 1.20 was approximately a fixed-effect estimate and the 95% PI of 1.01 to 1.43 for the next OR was very similar to the 95% CI of 1.07 to 1.35 for the average OR. The average OR was estimated to be below the null for invasive clear cell EOC and above the null for the remaining subtypes, but in each case the OEP suggested the presence of sizable proportions of populations (from 16% for LMP serous tumors to 24% for invasive clear cell EOC) with associations in the opposite direction from the average (Table 4). No study characteristic met our predesignated criterion for explaining heterogeneity in any of the meta-regression analyses for any subtype of EOC.

There was a 36% average relative increase in the odds of invasive serous EOC for women who had their hysterectomy before age 40 (average OR=1.36, 95% PI: 1.15, 1.61) but the average OR was attenuated for women who had their hysterectomy at age ≥40 (1.09, 95% CI: 0.95, 1.24) (Table 5). There was little heterogeneity within these exposure sub-groups; the difference between them was significant (*P*=0.02). Women younger than 40 years at hysterectomy were less likely to report fibroids (32%) and more likely to report endometriosis (10%) as reasons for their surgery than women aged 40 years or more at hysterectomy (45% and 5% for fibroids and endometriosis, respectively).

There was an average relative increase of 85% in the odds of invasive serous EOC for women whose hysterectomies occurred 30 years or more in the past; this was the strongest positive association among the three exposure sub-groups, which produced different estimates (*P*<0.001) (Table 6). The heterogeneous results for women who had their hysterectomy <15 years in the past were explained by variation in the prevalence of hysterectomy among controls across the study sites (*P*=0.01). The average relative odds of invasive serous EOC were increased among women from studies where <15% of the controls reported hysterectomy (OR=1.34, 95% CI: 0.95, 1.89) and were decreased among women from studies where ≥15% of the controls reported hysterectomy (OR=0.76,

95% CI: 0.58, 1.00). There was no heterogeneity of results for hysterectomies performed 15-29 years in the past.

4.5 Discussion

Contrary to earlier published results, the more recent studies included in this analysis generally showed positive associations between hysterectomy and most subtypes of EOC. The results were consistent across study sites for invasive serous EOC but there was considerable heterogeneity in the results for the other subtypes, as evidenced by OEP values of 16% (LMP serous) and 21% (invasive endometrioid). Our analyses also suggest a positive association between hysterectomy and mucinous tumors which is consistent with previous findings.[60,61] The small sample size, wide PI, and substantial heterogeneity suggest that subsequent studies need to replicate the inverse association between hysterectomy and invasive clear cell EOC that we found. In general, our sample size was relatively small among the non-serous subtypes of EOC within each study, even with more than 12,000 cases in total.

A recently published meta-analysis on the association between hysterectomy and ovarian cancer[147] reported an overall average OR of 0.74 (95% CI: 0.64, 0.85) from studies published between 1969 and 2010. However, there was significant heterogeneity among the studies (P<0.01), possibly due to heterogeneous outcome groups (i.e., benign and LMP tumors, invasive EOC), and/or inclusion of at least one study conducted among carriers of *BRCA1/2* mutations. Examination of the published forest plot suggests that the heterogeneity may also be due to the more recent studies, published after 2003. Among those, four include data from studies that are part of the present analysis (AUS,[179-181] HAW,[89] NCO,[89,182] and NEC) and show either no association or an increase in the odds of EOC with hysterectomy.

Previously, a pooled case-control study of 2,197 cases with invasive EOC and 8,893 controls reported a 34% decrease in risk associated with hysterectomy among hospital-based studies but an attenuated 12% decrease in risk among population-based studies.[72] Inverse associations have also been reported in several smaller case-control studies,[58,61,75-81] as well as in some cohort

studies.[82,83,85,150] In the Nurses' Health Study, however, the prevalence of hysterectomy was slightly higher among EOC cases (14%) than among non-cases (13%); hysterectomy was only inversely associated with ovarian cancer after adjustment for a number of covariates, notably post-menopausal estrogen use (ERT).[150] ERT use often, but not always, occurs subsequent to hysterectomy and is affected by hysterectomy since an intact uterus is a contraindication for ERT use. ERT use has also been positively associated with EOC.[183,184] We hesitate to attribute our findings to increased ERT use among women with hysterectomy because ERT was also prescribed to women in previous studies where there was an inverse relationship between hysterectomy and EOC. Detailed information on the timing of and indications for each use of ERT, unavailable at the time of our analysis, is necessary to fully understand the relationship between hysterectomy and EOC, as it is impacted by ERT use.

Whittemore et al[72] suggested that hysterectomy might impair ovarian function by compromising blood flow to the ovaries, resulting in anovulation. If true, hysterectomy during the reproductive years should confer more protection than post-menopausal hysterectomy. With the exception of a pooled analysis of hospital-based case-control studies[72] and a historical cohort study in Canada,[83] published studies have shown no differences in the associations between ages at hysterectomy and ovarian cancer.[76,78,185] However, several studies, including a pooled analysis of population-based case-control studies,[72] have reported decreased risk of ovarian cancer associated with increasing time between hysterectomy and diagnosis of ovarian cancer.[81,88] In contrast, our results are inconsistent with previous publications; the average relative odds of invasive serous EOC were increased with hysterectomies occurring prior to age 40 or occurring 30 years or more before diagnosis.

With regard to age at hysterectomy, some data suggest that more recent pregnancies and OC use are associated with greater reductions in ovarian cancer risk.[186] It may be that women who undergo a pre-menopausal hysterectomy are missing the risk lowering effects of later births and/or OC use. Our estimates are adjusted for number of full-term births and duration of OC use, but additional consideration for ages at last birth and last use of OCs, which may be on the pathway

between hysterectomy and ovarian cancer, may be warranted in future studies. The same scenario holds true for women who have a tubal ligation (i.e., earlier ages at last pregnancy and OC use), which is consistently shown to have an inverse association with all subtypes of EOC.[147] In fact, 11 of our 15 studies were included in a recent pooled analysis that reported an inverse association (OR=0.71, 95% CI: 0.66, 0.77) between tubal ligation and invasive EOC.[108] However, there may be a risk lowering mechanism associated with tubal ligation that is independent of those effects.

With regard to time since hysterectomy, Weiss and Harlow[90] suggested that the "healthy screenee effect," whereby pre-malignant/malignant ovaries/tubes are discovered and removed at the time of the hysterectomy, may explain inverse associations between recent hysterectomies and EOC. However, it is unlikely to explain our findings since we reclassified hysterectomies around the time of diagnosis as 'unexposed' to avoid hysterectomies related to malignancy. Additionally, only recently has there been identification of a possible precursor to EOC (i.e., p53 signature) and it is not apparent upon visual inspection of the upper genital tract.[187] Ovarian endometriosis may also be considered a precursor to some subtypes of ovarian carcinogenesis; however, we adjusted for self-reported endometriosis in the invasive endometrioid and clear cell models.

Both hysterectomy and oophorectomy were self-reported and may be subject to misclassification. In the published literature, agreement between self-reported hysterectomy and medical records was 95% or better, with kappa statistics ranging from 0.70 to 0.93.[74,152,188-192] Thus, it seems unlikely that misreporting of hysterectomy status substantially influenced the current results. Reporting of oophorectomy at the time of hysterectomy is less accurate.[74,189,193,194] In one study,[189] agreement with medical records was 95% for women reporting hysterectomy only, 83% for hysterectomy with bilateral salpingo-oophorectomy (BSO), and 77% for hysterectomy with unilateral oophorectomy. In situations where BSO is overreported and for ovarian cancer studies which exclude women if they are unsure of whether or not they had a BSO, women will be excluded as potential controls. Since a majority of them will have had a hysterectomy, the proportion of controls with a hysterectomy will be underestimated, resulting in potentially positive associations between

hysterectomy and ovarian cancer. Though the proportions of hysterectomy among controls in the current studies vary by study/geographic region as expected[2] and are generally lower than the prevalences of all hysterectomies (including those with concomitant BSO) in the source populations,[77,84,195,196] also as expected, some women with hysterectomies may still have been excluded from the control groups, resulting in average ORs biased away from the null. When BSO is underreported, the proportion of controls with hysterectomy will be overestimated, resulting in potentially inverse associations between hysterectomy and ovarian cancer. Underreporting of oophorectomy was more likely than overreporting in some of the older studies,[74,191] which could have contributed to the inverse associations between hysterectomy and ovarian cancer seen in studies around the same time. Most of the published validity studies of oophorectomy status occurred in the more distant past, when details of medical care were not always shared with patients. It is possible that the accuracy of self-reported oophorectomy has improved and is more accurate in these studies.

Concomitant bilateral salpingo-oophorectomy (BSO) was undertaken in roughly half of hysterectomies in the United States in the late 1990s, up from 25% in 1965; however, this rate varies by age.[1,5] The American College of Obstetricians and Gynecologists recommended in 1999[12] and 2008[13] that BSO be avoided in premenopausal women because of the negative outcomes associated with premature loss of ovarian function.[14] Thus, only 37% of hysterectomies among women aged 15-44 years included concomitant BSO versus 78% of hysterectomies among women aged 50-54 years.[3,15,16] Because loss of ovarian function is generally noticeable, misclassification of BSO status among women with premenopausal hysterectomy is less likely than among women with postmenopausal hysterectomy who have already experienced symptoms of hormone depletion. Because the median age at hysterectomy among controls was 40 and 90% of reported hysterectomies in these studies occurred before the age of 50, it is unlikely that misclassification of BSO status dramatically influenced our results, at least among women who had a hysterectomy prior to menopause.

Our primary finding that hysterectomy is not inversely associated with EOC is inconsistent with the published literature and may be due to temporal changes in the association. The authors of a recent meta-analysis reported that median year of cancer diagnosis explained the significant heterogeneity of their results and concluded that there appears to be a temporal shift in the association between hysterectomy and ovarian cancer. The summary relative risk (RR) for studies with a median year of diagnosis prior to 2000 was 0.70 (95% CI: 0.65, 0.76) while the summary RR for studies with a median year of diagnosis post 2000 was 1.18 (1.06, 1.31).[197] The median year of diagnosis among our studies ranged from 1994 to 2005 (Appendix Table 2) but did not explain any heterogeneity of results for any of the subtypes of EOC. It remains unclear what would underlie a temporal change. Indications for hysterectomy have not radically changed over the past several decades and it seems unlikely that unmeasured confounding by indication could explain our results, especially since uterine fibroids, bleeding, and prolapse have not been shown to be associated with EOC. However, as alternative therapies are used to treat such conditions, symptoms may need to be more severe before hysterectomies are indicated. Theoretically, increased severity of benign gynecologic conditions could potentially be related to ovarian cancer risk. It is imperative that the relationships among hysterectomy, gynecologic conditions, and ovarian cancer are carefully considered in future studies, especially as trends in the surgical approach to hysterectomy move from abdominal to laparoscopic, the age at hysterectomy increases,[198] and if women with gynecologic conditions, specifically those requiring hysterectomy, are more likely to participate as controls in studies.

In this pooled analysis of almost 30,000 women from 15 studies, hysterectomy was positively associated with increased average odds of all histologic subtypes of EOC except invasive clear cell; however, there was considerable heterogeneity that we could not explain. Increased average odds of invasive serous EOC were associated with hysterectomy before the age of 40 or ≥30 years before diagnosis/interview. These findings are also inconsistent with the published literature and may be due to biases in the previous (or current) studies or a changing association between hysterectomy and EOC. Future research should carefully classify women according to their oophorectomy status,

account for how the ovarian cancer cases were diagnosed (e.g., at the time of hysterectomy), and consider the downstream consequences of hysterectomy (e.g., timing of the use of OCs and ERT). Medical records of all potential controls reporting hysterectomy may have to be reviewed in order to exclude women who had a concomitant BSO and to understand the circumstances surrounding their hysterectomies. Additional research into the indications for hysterectomy and their relation to ovarian cancer risk may also be warranted. Until additional research can be done, clinicians should be aware that recent evidence does not support the long-held belief that hysterectomy confers some protection against all types of EOC.

Table 2. Description of 15 case-control studies included in the analyses of hysterectomy and epithelial ovarian tumors from the Ovarian Cancer Association Consortium

Study Acronym	Study Name	Location	Period of Ascertainment	Method(s) of Data Collection[a]	Response Rates[b]	
					Cases	Controls
AUS[158]	Australian Ovarian Cancer Study & Australian Cancer Study (Ovarian Cancer)[c]	Australia	2002-2006	Questionnaire	65%	47%
CON[165]	Connecticut Ovarian Cancer Study	USA	1998-2003	Interview	69%	61%
DOV[166]	Diseases of the Ovary and their Evaluation	USA	2002-2009	Interview	74%	62%
GER[163]	German Ovarian Cancer Study	Germany	1993-1996	Questionnaire	58%	51%
HAW[167,168]	Hawaii Ovarian Cancer Case-Control Study	USA	1993-2008	Interview	78%	80%
HOP[169]	Novel Risk Factors and Potential Early Detection Markers for Ovarian Cancer	USA	2003-2009	Interview	71%	68%
MAL[161,162,164]	Malignant Ovarian Cancer Study	Denmark	1994-1999	Interview	81%	68%
NCO[170,171]	North Carolina Ovarian Cancer Study	USA	1999-2008	Interview	67%	60%
NEC[172,173]	New England Case-Control Study of Ovarian Cancer	USA	1992-2003	Interview	71%	64%
NTH[101,102]	Nijmegen Ovarian Cancer Study	Netherlands	2008 (cases); 2002-2003 (controls)	Questionnaire	63%	42%
POL[160]	Polish Ovarian Cancer Case Control Study	Poland	2000-2003	Interview	78%	69%
STA[174]	Genetic Epidemiology of Ovarian Cancer Study	USA	1997-2001	Interview	75%	75%
UCI[175]	University of California Irvine Ovarian Study	USA	1993-2005	Interview or Questionnaire	67%	80%
UKO[159]	United Kingdom Ovarian Cancer Population Study	UK	2006-2010 (cases); 2000-2005 (controls)	Interview	86%	97%
USC[176-178]	Los Angeles County Case-Control Studies of Ovarian Cancer	USA	1992-2002	Interview	60%	72%

UK=United Kingdom; USA=United States of America.

[a] Questionnaires were self-completed by the study participant; Interviews were administered either in person or over the phone.

[b] Response rates were calculated differently across studies; algorithms are available upon request.

[c] Combined for the purpose of the analysis.

Table 3. Counts of cases and controls with hysterectomy more than two years prior to diagnosis (cases) or interview/reference date (controls) according to study and subtype of epithelial ovarian tumors for 15 studies from the Ovarian Cancer Association Consortium

Study	Controls	All LMP	All Invasive	LMP Serous	Invasive Serous	Invasive Endometrioid	Invasive Clear Cell	Mucinous[a]	Other[b]
AUS	281/1,488 (19)	61/306 (20)	255/1,114 (23)	28/143 (20)	163/682 (24)	27/134 (20)	18/86 (21)	38/193 (20)	42/182 (23)
CON	59/549 (11)	15/107 (14)	51/369 (14)	12/68 (18)	28/219 (13)	10/72 (14)	3/35 (9)	9/54 (17)	4/28 (14)
DOV	342/1,849 (18)	75/417 (18)	234/1,159 (20)	46/235 (20)	164/675 (24)	23/187 (12)	5/88 (6)	28/191 (15)	43/200 (22)
GER	130/533 (24)	3/30 (10)	47/228 (21)	2/18 (11)	21/114 (18)	5/26 (19)	1/6 (17)	6/36 (17)	15/58 (26)
HAW	109/1,104 (10)	16/187 (9)	94/709 (13)	11/89 (12)	48/315 (15)	15/117 (13)	8/82 (10)	11/162 (7)	17/131 (13)
HOP	298/1,804 (17)	19/97 (20)	126/674 (19)	14/58 (24)	74/364 (20)	21/97 (22)	4/52 (8)	7/65 (11)	25/135 (19)
MAL	139/1,564 (9)	20/202 (10)	79/553 (14)	12/104 (12)	46/342 (13)	13/75 (17)	7/44 (16)	14/138 (10)	7/52 (13)
NCO	242/1,081 (22)	35/225 (16)	214/867 (25)	24/158 (15)	137/472 (29)	30/140 (21)	11/90 (12)	16/107 (15)	31/125 (25)
NEC	100/1,243 (8)	21/294 (7)	69/825 (8)	11/173 (6)	47/462 (10)	9/167 (5)	8/112 (7)	9/154 (6)	6/51 (12)
NTH	79/594 (13)	N/A[c]	36/255 (14)	N/A[c]	20/116 (17)	5/65 (8)	3/18 (17)	4/34 (12)	4/22 (18)
POL	47/1,101 (4)	0/21 (0)	8/272 (3)	0/17 (0)	4/123 (3)	0/40 (0)	1/11 (9)	1/22 (5)	2/80 (3)
STA	55/566 (10)	11/161 (7)	47/499 (9)	8/105 (8)	32/262 (12)	3/60 (5)	0/51 (0)	6/88 (7)	9/94 (10)
UCI	145/569 (25)	21/197 (11)	101/392 (26)	12/122 (10)	64/213 (30)	16/72 (22)	4/37 (11)	15/102 (15)	11/43 (26)
UKO	198/1,033 (19)	N/A[c]	92/603	N/A[c]	44/310 (14)	22/96 (23)	6/56 (11)	10/60 (17)	10/81 (12)
USC	205/1,809 (11)	46/410 (11)	233/1,326 (18)	31/243 (13)	158/833 (19)	28/183 (15)	10/87 (11)	34/274 (12)	18/116(16)
All	**2,429/16,887**	**343/2,654**	**1,686/9,845**	**211/1,533**	**1,050/5,502**	**227/1,531**	**89/855**	**208/1,680**	**244/1,398**

LMP=low malignant potential; N/A=not applicable.

[a] Includes low malignant potential and invasive epithelial ovarian cancers of mucinous histology.

[b] Includes mixed cell, other specified epithelial ovarian cancer (e.g., Brenner tumor), undifferentiated or poorly differentiated epithelial ovarian cancer and known epithelial ovarian cancer of unknown histology. Both low malignant potential and invasive behaviors are included.

[c] Study only enrolled invasive epithelial ovarian cancer cases; no cases of low malignant potential are included.

Table 4. Association between hysterectomy more than two years prior to diagnosis (cases) or interview/reference date (controls) and subtypes of epithelial ovarian tumors in 15 studies from the Ovarian Cancer Association Consortium

Study	All LMP Combined[a]		All Invasive Combined[a]		LMP Serous[a]		Invasive Serous[a]		Invasive Endometrioid[b]		Invasive Clear Cell[b]		Mucinous[c]	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
AUS	1.85	1.28, 2.67	1.20	0.96, 1.49	1.82	1.09, 3.05	1.14	0.89, 1.47	0.94	0.55, 1.60	0.85	0.44, 1.65	1.72	1.13, 2.60
CON	1.60	0.81, 3.16	1.28	0.82, 2.00	2.22	1.01, 4.87	0.99	0.58, 1.70	1.05	0.46, 2.39	0.63	0.15, 2.58	1.71	0.76, 3.83
DOV	1.40	1.03, 1.90	1.27	1.03, 1.55	1.56	1.06, 2.28	1.41	1.12, 1.77	0.87	0.53, 1.44	0.28	0.11, 0.73	1.00	0.65, 1.54
GER	0.52	0.11, 2.47	0.83	0.54, 1.29	0.59	0.07, 5.35	0.57	0.31, 1.04	0.85	0.27, 2.65	1.33	0.10, 17.11	0.88	0.34, 2.29
HAW	1.42	0.77, 2.62	1.33	0.96, 1.82	2.10	0.98, 4.50	1.32	0.90, 1.95	1.35	0.70, 2.62	1.07	0.46, 2.45	0.95	0.48, 1.85
HOP	2.25	1.26, 4.00	1.21	0.94, 1.54	3.61	1.77, 7.35	1.21	0.89, 1.64	1.66	0.96, 2.86	0.44	0.14, 1.33	0.76	0.34, 1.72
MAL[d]	1.25	0.75, 2.08	1.55	1.14, 2.12	1.35	0.71, 2.58	1.42	0.97, 2.07	2.04	1.04, 4.01	2.07	0.86, 4.95	1.22	0.68, 2.18
NCO	1.13	0.72, 1.77	1.07	0.84, 1.37	0.99	0.58, 1.69	1.25	0.95, 1.65	0.85	0.51, 1.41	0.35	0.16, 0.77	0.92	0.51, 1.64
NEC	1.52	0.89, 2.60	1.05	0.74, 1.48	1.52	0.76, 3.05	1.08	0.73, 1.61	0.74	0.35, 1.58	0.89	0.40, 2.00	1.00	0.48, 2.06
NTH[e]	N/A	N/A	1.27	0.73, 2.22	N/A	N/A	1.42	0.72, 2.81	0.53	0.15, 1.86	4.39	0.58, 32.95	1.30	0.42, 4.07
POL[f]	N/A	N/A	0.54	0.23, 1.27	N/A	N/A	0.59	0.17, 2.01	N/A	N/A	2.16	0.23, 20.01	1.08	0.14, 8.37
STA[g]	1.02	0.45, 2.32	0.72	0.45, 1.17	0.96	0.38, 2.42	0.80	0.47, 1.39	0.63	0.17, 2.33	N/A	N/A	0.95	0.33, 2.70
UCI	0.44	0.24, 0.81	0.96	0.63, 1.45	0.41	0.19, 0.87	1.11	0.68, 1.80	0.88	0.40, 1.93	0.26	0.06, 1.09	0.56	0.30, 1.06
UKO	N/A	N/A	1.21	0.88, 1.67	N/A	N/A	0.88	0.58, 1.34	1.28	0.47, 3.47	0.98	0.30, 3.18	1.47	0.69, 3.14
USC	1.44	0.98, 2.11	1.44	1.15, 1.79	1.60	1.01, 2.53	1.45	1.14, 1.85	1.44	0.89, 2.32	0.74	0.33, 1.67	1.43	0.94, 2.19
Random Effects	1.32	1.02, 1.71	1.19	1.08, 1.32	1.45	1.03, 2.03	1.20	1.07, 1.35	1.09	0.89, 1.35	0.75	0.50, 1.12	1.12	0.91, 1.38
$\hat{\tau}^2$	0.07088		0.001531		0.1349		0.003611		0.01206		0.1683		0.02559	
95% PI	0.69 to 2.52		1.05 to 1.36		0.60 to 3.52		1.01 to 1.43		0.79 to 1.51		0.28 to 2.00		0.75 to 1.68	
OEP	15%		<1%		16%		<1%		21%		24%		23%	

CI=confidence interval; LMP=low malignant potential; N/A=not available; OEP=opposite effects proportion; OR=odds ratio; PI=prediction interval.

[a] Odds ratios and 95% confidence intervals for the association between hysterectomy more than two years prior to diagnosis or interview/reference date and all LMP tumors combined, all invasive EOC combined, LMP serous tumors, and invasive serous EOC were estimated by conditional logistic regression models stratified by age in 5-year groups and race/ethnicity (non-Hispanic white, Hispanic white, black, Asian, other) and adjusted for body mass index in early adulthood (<18.5, 18.5-<25, 25-<30, ≥30), parity (0, 1, 2, 3, 4+ full-term births), duration of oral contraceptive use (0, <24 months, 24-<60 months, 60-<120 months, ≥120 months), tubal ligation (yes/no), and history of breast or ovarian cancer in the mother (yes/no).

[b] Conditional logistic regression models for the association between hysterectomy more than 2 years prior to diagnosis or interview/reference date and invasive endometrioid and invasive clear cell EOC are adjusted for endometriosis (yes/no) in additional to the covariates in the models for all LMP tumors combined, all invasive EOC combined, LMP serous tumors, and invasive serous EOC.

[c] Includes LMP tumors and invasive EOC of mucinous histology. The conditional logistic regression model for the association between hysterectomy more than two years prior to diagnosis or interview/reference date and mucinous epithelial ovarian cancer is stratified by age in 5-year groups and race/ethnicity (non-Hispanic white, Hispanic white, black, Asian, other); model is not adjusted for any additional covariates.

[d] Models for MAL all LMP tumors combined and LMP serous are not adjusted for history of breast or ovarian cancer in the mother due to missing data.

[e] Models for NTH are not adjusted for body mass index in early adulthood.

[f] Models for POL are not adjusted for endometriosis.
[g] Models for STA are not adjusted for body mass index in early adulthood or endometriosis.

Table 5. Association[a] between age at hysterectomy and invasive serous epithelial ovarian cancer in 15 studies from the Ovarian Cancer Association Consortium

Study	No Hysterectomy (Referent)	Hysterectomy at <40 Years of Age			Hysterectomy at ≥40 Years of Age		
	Ca/Cn	Ca/Cn	OR	95% CI	Ca/Cn	OR	95% CI
AUS	519/1,207	68/101	1.40	0.98, 2.00	95/180	0.99	0.73, 1.35
CON	191/490	10/27	1.01	0.45, 2.29	18/32	0.98	0.50, 1.93
DOV	511/1,507	114/196	1.70	1.30, 2.22	50/146	1.00	0.70, 1.43
GER	93/403	3/35	0.24	0.06, 1.07	18/95	0.70	0.36, 1.33
HAW	267/995	25/48	1.52	0.90, 2.58	23/61	1.16	0.68, 1.96
HOP	290/1,506	37/154	1.13	0.76, 1.70	37/144	1.29	0.86, 1.94
MAL	296/1,425	16/48	1.54	0.85, 2.79	30/91	1.36	0.85, 2.16
NCO	335/839	85/156	1.23	0.89, 1.71	52/86	1.29	0.84, 1.96
NEC	415/1,143	25/49	1.30	0.77, 2.20	22/51	0.90	0.51, 1.56
NTH[b]	96/515	5/19	1.33	0.35, 5.02	15/60	1.44	0.68, 3.08
POL	119/1,054	2/8	1.29	0.14, 12.05	2/39	0.46	0.11, 2.02
STA[b]	230/511	19/31	0.93	0.47, 1.85	13/24	0.66	0.30, 1.46
UCI	149/424	25/54	1.68	0.83, 3.41	39/91	0.85	0.48, 1.53
UKO	266/835	18/74	0.86	0.46, 1.62	26/124	0.89	0.53, 1.50
USC	675/1,604	75/91	1.56	1.11, 2.19	83/114	1.36	0.99, 1.87
Random Effects		1.36 (1.18, 1.56)			1.09 (0.95, 1.24)		
τ^2		0.0018			0		
95% PI		1.15 to 1.61			N/A		
OEP		<1%			N/A		

Ca=case; CI=confidence interval; Cn=control; N/A=not applicable; OEP=opposite effects proportion; OR=odds ratio; PI=prediction interval.

[a] Odds ratios and 95% confidence intervals were estimated by conditional logistic regression models stratified by age in 5-year groups and race/ethnicity (non-Hispanic white, Hispanic white, black, Asian, other) and adjusted for body mass index in early adulthood (<18.5, 18.5-<25, 25-<30, ≥30), parity (0, 1, 2, 3, 4+ full-term births), duration of oral contraceptive use (0, <24 months, 24-<60 months, 60-<120 months, ≥120 months), tubal ligation (yes/no), and history of breast or ovarian cancer in the mother (yes/no).

[b] Models for NTH and STA are not adjusted for body mass index in early adulthood.

Table 6. Association[a] between time since hysterectomy and invasive serous epithelial ovarian cancer in 15 studies from the Ovarian Cancer Association Consortium

Study	No Hysterectomy (Referent)	Hysterectomy 2- <15 years in the past			Hysterectomy 15- <30 years in the past			Hysterectomy ≥30 years in the past		
	Ca/Cn	Ca/Cn	OR	95% CI	Ca/Cn	OR	95% CI	Ca/Cn	OR	95% CI
AUS	519/1,207	40/96	0.86	0.56, 1.32	91/133	1.38	1.00, 1.91	32/52	1.08	0.65, 1.79
CON	191/490	11/21	1.55	0.67, 3.59	12/25	0.81	0.37, 1.77	5/13	0.69	0.21, 2.20
DOV	511/1,507	22/94	0.70	0.43, 1.16	74/179	1.19	0.87, 1.62	68/69	3.36	2.26, 5.01
GER	93/403	6/58	0.38	0.14, 1.02	13/68	0.71	0.34, 1.52	2/4	0.86	0.08, 8.80
HAW	267/995	10/22	1.63	0.74, 3.61	16/56	0.78	0.43, 1.43	22/31	2.28	1.20, 4.33
HOP	290/1,506	10/65	0.89	0.43, 1.85	28/130	1.11	0.72, 1.73	36/103	1.50	0.96, 2.34
MAL	296/1,425	11/54	0.79	0.39, 1.60	25/70	1.66	1.01, 2.74	10/15	2.77	1.14, 6.71
NCO	335/839	22/56	1.01	0.58, 1.76	70/118	1.28	0.89, 1.84	45/68	1.41	0.88, 2.26
NEC	415/1,143	16/32	1.21	0.62, 2.35	11/48	0.50	0.25, 1.00	20/20	2.44	1.23, 4.82
NTH[b]	96/515	8/23	1.56	0.55, 4.49	9/52	1.13	0.45, 2.82	3/4	3.44	0.53, 22.51
POL	119/1,054	3/23	0.89	0.20, 4.11	0/20			1/4	2.38	0.21, 27.46
STA[b]	230/511	10/19	0.87	0.35, 2.13	20/29	0.86	0.43, 1.72	2/7	0.38	0.07, 2.10
UCI	149/424	9/59	0.40	0.16, 0.98	30/62	1.16	0.61, 2.21	25/24	4.54	1.58, 13.03
UKO	266/835	13/44	0.64	0.27, 1.52	20/120	0.77	0.44, 1.34	11/34	1.70	0.76, 3.76
USC	675/1,604	37/54	1.59	1.01, 2.51	82/117	1.24	0.90, 1.71	39/34	1.94	1.17, 3.21
Random Effects		0.95 (0.75, 1.19)			1.13 (0.97, 1.32)			1.85 (1.39, 2.46)		
τ^2		0.4439			0			0.1015		
95% PI		0.57 to 1.58			N/A			0.88 to 3.90		
OEP		39%			N/A			3%		

Ca=case; CI=confidence interval; Cn=control; N/A=not applicable; OEP=opposite effects proportion; OR=odds ratio; PI=prediction interval.

[a] Odds ratios and 95% confidence intervals were estimated by conditional logistic regression models stratified by age in 5-year groups and race/ethnicity (Non-Hispanic white, Hispanic white, black, Asian, other) and adjusted for body mass index in early adulthood (<18.5, 18.5-<25, 25-<30, ≥30), parity (0, 1, 2, 3, 4+ full-term births), duration of oral contraceptive use (0, <24 months, 24-<60 months, 60-<120 months, ≥120 months), tubal ligation (yes/no), and history of breast or ovarian cancer in the mother (yes/no).

[b] Models for NTH and STA are not adjusted for body mass index in early adulthood.

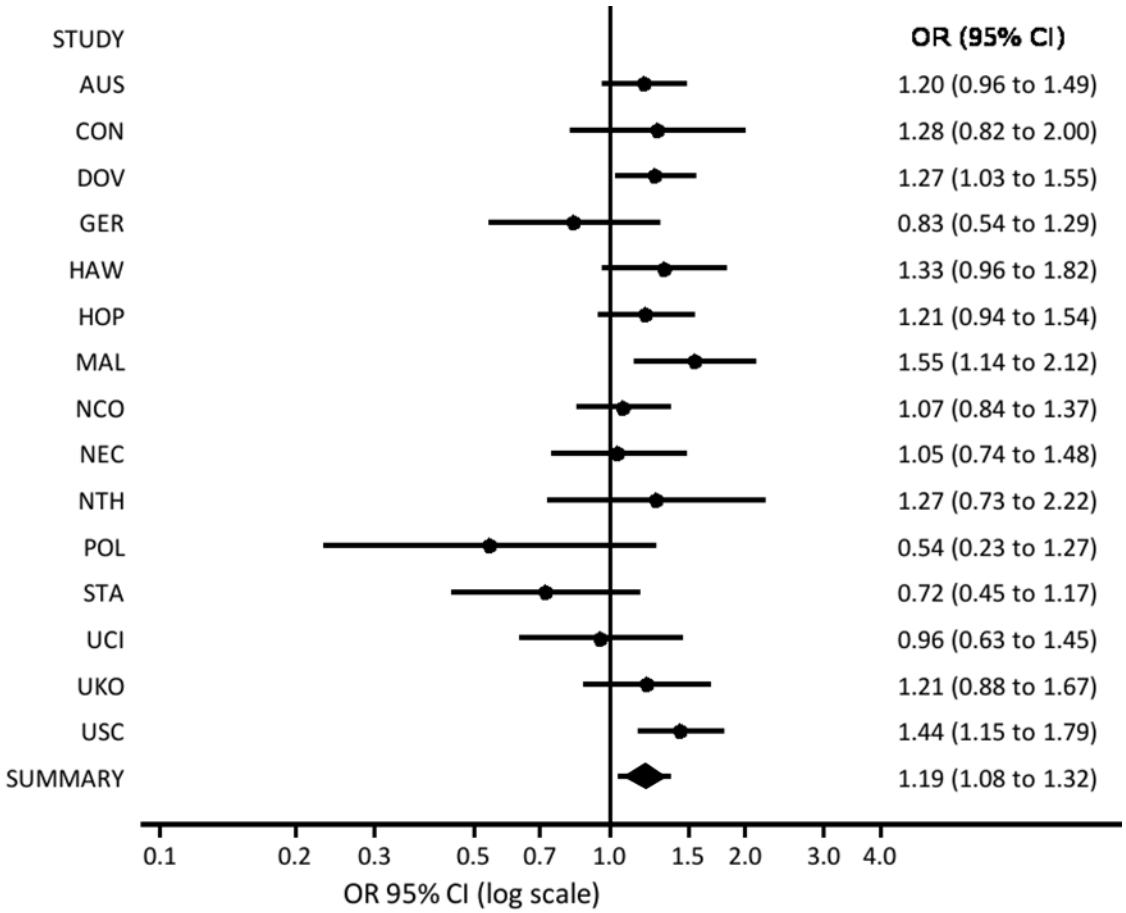


Figure 1. Results from a random-effects analysis of hysterectomy more than two years prior to diagnosis (cases) or interview/reference date (controls) and invasive epithelial ovarian cancer in 15 studies in the Ovarian Cancer Association Consortium. The solid circles are centered on the study-specific odds ratio (OR) estimates; the horizontal line through each circle indicates the 95% confidence interval (CI) for the study-specific estimate. The center of the diamond indicates the estimated average OR of a presumptively normal distribution of true values. The horizontal tips of the diamond indicate the 95% CI for the estimated average OR and the horizontal line through the diamond indicates the 95% prediction interval (PI) for the "next" study. Study-specific conditional logistic regression models were conditioned on age and race/ethnicity and adjusted for body mass index in early adulthood, number of full-term births, duration of oral contraceptive use, tubal ligation, and history of breast or ovarian cancer in the mother. See Table 2 for published references for each study. (AUS, Australian Ovarian Cancer Study and Australian Cancer Study (Ovarian Cancer); CON, Connecticut Ovarian Cancer Study; DOV, Diseases of the Ovary and their Evaluation; GER, German Ovarian Cancer Study; HAW, Hawaii Ovarian Cancer Case-Control Study; HOP, Novel Risk Factors and Potential Early Detection Markers for Ovarian Cancer; MAL, Malignant Ovarian Cancer Study; NCO, North Carolina Ovarian Cancer Study; NEC, New England Case-Control Study of Ovarian Cancer; NTH, Nijmegen Ovarian Cancer Study; POL, Polish Ovarian Cancer Case Control Study; STA, Genetic Epidemiology of Ovarian Cancer Study; UCI, University of California Irvine Ovarian Study; UKO, United Kingdom Ovarian Cancer Population Study; USC, Los Angeles County Case-Control Studies of Ovarian Cancer).

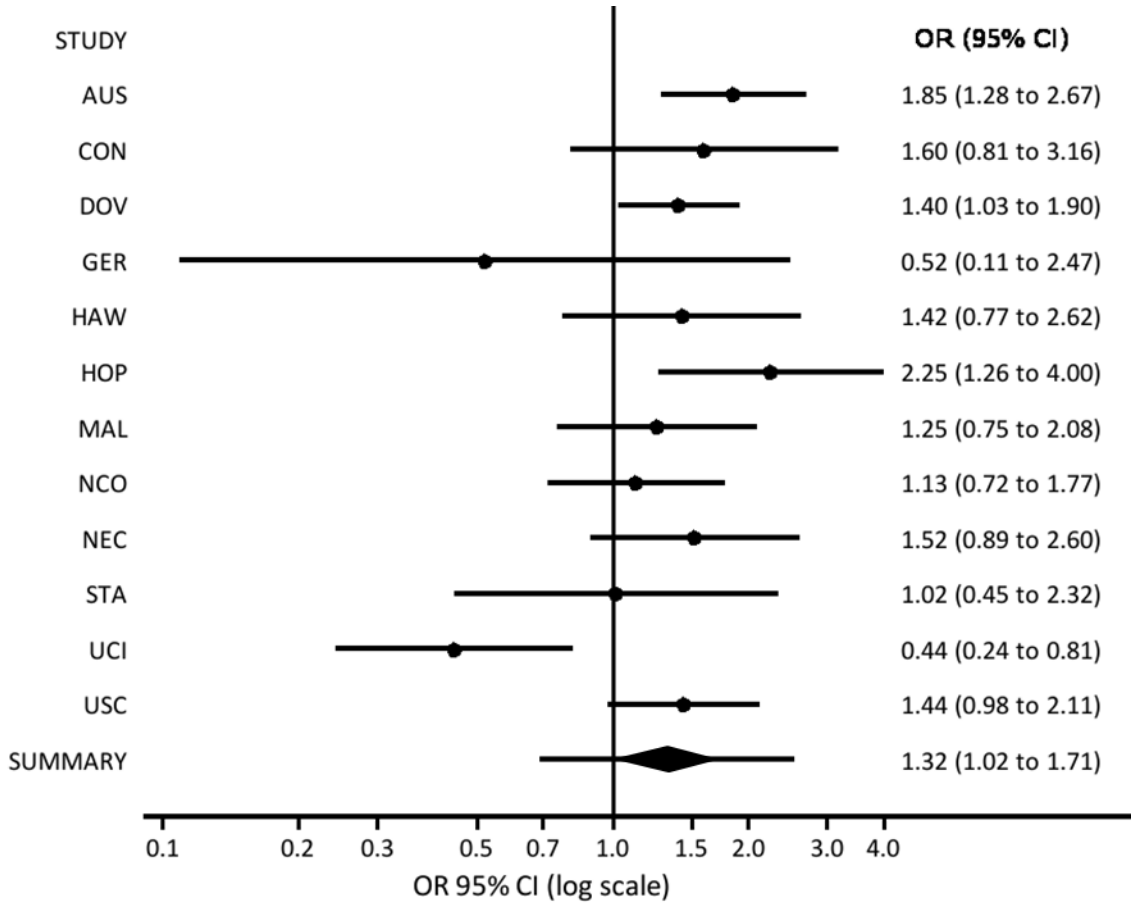


Figure 2. Results from a random-effects analysis of hysterectomy more than two years prior to diagnosis (cases) or interview/reference date (controls) and low malignant potential epithelial ovarian tumors in 12 studies in the Ovarian Cancer Association Consortium. The solid circles are centered on the study-specific odds ratio (OR) estimates; the horizontal line through each circle indicates the 95% confidence interval (CI) for the study-specific estimate. The center of the diamond indicates the estimated average OR of a presumptively normal distribution of true values. The horizontal tips of the diamond indicate the 95% CI for the estimated average OR and the horizontal line through the diamond indicates the 95% prediction interval (PI) for the "next" study. Study-specific conditional logistic regression models were conditioned on age and race/ethnicity and adjusted for body mass index in early adulthood, number of full-term births, duration of oral contraceptive use, tubal ligation, and history of breast or ovarian cancer in the mother. See Table 2 for published references for each study. (AUS, Australian Ovarian Cancer Study and Australian Cancer Study (Ovarian Cancer); CON, Connecticut Ovarian Cancer Study; DOV, Diseases of the Ovary and their Evaluation; GER, German Ovarian Cancer Study; HAW, Hawaii Ovarian Cancer Case-Control Study; HOP, Novel Risk Factors and Potential Early Detection Markers for Ovarian Cancer; MAL, Malignant Ovarian Cancer Study; NCO, North Carolina Ovarian Cancer Study; NEC, New England Case-Control Study of Ovarian Cancer; STA, Genetic Epidemiology of Ovarian Cancer Study; UCI, University of California Irvine Ovarian Study; USC, Los Angeles County Case-Control Studies of Ovarian Cancer).

CHAPTER 5. RISK PREDICTION FOR PREMENOPAUSAL HYSTERECTOMY WITH THE INTENTION OF OVARIAN CONSERVATION

5.1 Abstract

We sought to develop and internally validate a predictive model for hysterectomy with ovarian conservation among premenopausal women. We also evaluated the contribution of family history of benign gynecologic conditions to the model because it may be a proxy for more severe conditions that require invasive treatment such as hysterectomy. The study included 457 premenopausal women who had undergone hysterectomy for benign conditions (cases) and 499 premenopausal women attending general medicine and gynecology practices in the catchment area of Durham, North Carolina who had not undergone hysterectomy (controls). We fit unconditional logistic regression models including the following regressors to predict hysterectomy: age, race, marital status, body size, and history of education, smoking, oral contraceptive use, full-term pregnancies, tubal ligation, fibroids, endometriosis, ovarian cysts, myomectomy, and benign gynecologic conditions in the immediate family. Model performance was evaluated by discrimination (area under the receiver operating characteristic curves (AUC)) and calibration (goodness-of-fit test and plots of observed versus predicted probabilities) metrics. Contribution of family history to the model was assessed by comparing the AUCs of the models without and with the variable and calculating net reclassification improvement (NRI). We internally validated the model by repeating the model fit in 200 bootstrap samples to produce bias-corrected AUC values. Both models (without and with family history) exhibited very good discriminatory power (bias-corrected AUC=0.84), and were not poorly calibrated (goodness-of-fit p=0.2). A test of the hypothesis of no difference in AUC yielded p=0.1. In the NRI analysis, the net proportions reclassified correctly were <1% for cases and 1% for controls. Family history resulted in slightly worsened classification. This initial prediction

model for premenopausal, ovarian-conserving hysterectomy warrants additional development, especially among women followed longitudinally for the occurrence of hysterectomy. Such models have the potential of identifying women for earlier and less invasive treatment than hysterectomy for benign conditions. Prediction models may also enhance the selection of participants for studies of hysterectomy and the imputation of missing data in studies in which hysterectomy is an outcome, exposure, or covariate.

5.2 Introduction

Hysterectomy is the most common non-obstetric, surgical procedure among women in the United States (US).[1] Although numbers of hysterectomy in the US have declined from their peak of 725,000 in 1975, approximately 600,000 hysterectomies are currently performed annually in the US. More than one-third of women will have a hysterectomy by age 60.[2-6] Younger women who undergo hysterectomy are more likely to do so for uterine leiomyomas, while for older women the indication is more likely to be prolapse or cancer.[5] The American College of Obstetricians and Gynecologists recommended in 1999[12] and 2008[13] that bilateral salpingo-oophorectomy (BSO) be avoided in premenopausal women because negative outcomes associated with earlier loss of ovarian function outweigh the potential benefits of avoiding future ovarian pathology including ovarian cancer.[14] Thus, between 2000 and 2004, only 37% of hysterectomies among women aged 15-44 years included concomitant BSO versus 78% of hysterectomies among women aged 50-54 years.[3,15,16]

In a study of the 2003 Nationwide Inpatient Sample of discharge data in a 20% stratified sample of US hospitals, the most frequent indications for hysterectomy were fibroids (33%), menstrual disorders (21%), uterine prolapse (16%), and endometriosis (14%) among white women aged 16 or older; among African American women aged 16 or older, hysterectomies were predominantly performed to treat fibroids (70%) rather than menstrual disorders (12%), uterine prolapse (4%), or endometriosis (6%).[11]

The most common indications for hysterectomy appear to have a familial component. Women with fibroids and with endometriosis are more likely to report a family history of these

conditions in first degree relatives. Women with fibroids who have a family history of fibroids may be more likely than women without a family history to present with more severe symptoms, and thus be more likely to undergo hysterectomy[43,44] and at an earlier age.[45] In a recent study, women with pathologically confirmed endometriosis were more likely to report a family history of endometriosis than women without endometriosis; however, the investigators did not find differences in the characteristics or symptoms of the women with endometriosis by family history of endometriosis.[40] Earlier studies have also reported associations between family history of endometriosis and clinically-confirmed endometriosis[49,199-201] and suggested that the severity of endometriosis may be higher among women with a family history of endometriosis.[49,202]

Although it is generally assumed that women with fibroids, endometriosis, uterine prolapse, and other menstrual-related conditions choose to undergo hysterectomy for treatment of their benign gynecologic condition(s), there is a need to precisely estimate the multivariable predictive capability of these potential predictors of hysterectomy and to further evaluate any other additional traits, including family history of common, benign gynecologic conditions. These predictive models may help identify at-risk women for clinical monitoring and potentially earlier (and less invasive) treatment. To the best of our knowledge, there have been no prediction models developed for hysterectomy among premenopausal women who intend to conserve one or both ovaries. To that end, we used data from a prospective cohort study of premenopausal women undergoing hysterectomy and control women with intact uteri and ovaries to develop a predictive model for hysterectomy with the intention of ovarian conservation. Because family history of gynecologic conditions may be a proxy for more severe indications for hysterectomy, we also sought to evaluate the contribution of family history of common, benign gynecologic conditions to the model's performance.

5.3 Materials and methods

5.3.1 Study population and data sources

Data for the analyses came from the *Prospective Research on Ovarian Function (PROOF)* Study, a prospective cohort of premenopausal women that enrolled women in Durham, North

Carolina from April 2004 through December 2007.[34,121-123] Women between the ages of 30 and 47 years undergoing hysterectomy at Duke University Medical Center or Durham Regional Hospital, both part of the Duke University Health System and located in Durham, were enrolled. Women with intact uteri were recruited using brochures and advertisements in publications that were placed in clinics and offices of gynecology and family medicine practices and were frequency matched to the women undergoing hysterectomy on age and race. The aims of the original study were to prospectively determine whether women undergoing hysterectomy were more likely to experience ovarian failure during the four years after hysterectomy than the women with intact uteri and to identify medical, reproductive, and lifestyle characteristics that may be associated with earlier ovarian failure.[121] The present analysis includes only baseline data for the women who underwent hysterectomy, hereafter identified as cases, and the women with intact uteri, hereafter identified as controls.

Cases were women, aged 30 to 47 years at baseline, who were scheduled to undergo hysterectomy for benign gynecologic conditions. In addition, they could not be pregnant, were premenopausal as evidenced by at least one menstrual period in the previous 3 months, had to have at least one intact ovary prior to and following the hysterectomy, had no personal history of cancer (except nonmelanoma skin cancer), and had to be able to complete the interview in English. Premenopausal status was confirmed by a pre-operative blood level of follicle-stimulating hormone less than 40 international units per liter. Controls had the same eligibility criteria as the cases.

Participants completed a baseline interview visit during which they completed an extensive interviewer-administered questionnaire, provided a blood specimen, and had anthropometric measurements taken. The baseline interview for cases occurred prior to their hysterectomies, most at the time of their pre-operative visits. The questionnaire ascertained information on the following: demographics, menstrual cycle history, reproductive history, medical and gynecologic condition history, family history (mothers, sisters) of benign gynecologic conditions, and lifestyle characteristics related to alcohol, smoking, diet, and physical activity. The study protocol was

approved by the Duke University Medical Center's Institutional Review Board and all participants provided written, informed consent.

5.3.2 Predictors

Based on a review of the scientific literature, we selected the following predictors and parameterizations, *a priori*, for inclusion in the prediction model: age (continuous, linear); self-identified race (white, black, other); marital status (single/never married, married/living with significant other, divorced/separated/widowed); education level (high school graduate or lower, some college or post-high school training, college graduate or higher); tubal ligation (yes, no); fibroids (yes, no); endometriosis (yes, no); ovarian cysts (yes, no), previous myomectomy (yes, no); and smoking status (never, former, current). Because it has been reported that dichotomization of predictors results in a loss of discriminative ability[124,125], we included the following variables as restricted cubic splines:[126] duration of oral contraceptive (OC) use (knots at 1, 3, 5, and 10 years); number of full-term pregnancies (knots at 1, 2, and 3 pregnancies); and BMI (knots at 18.5, 25, and 30). To evaluate the contribution of family history to the prediction model, we included a dichotomous variable that indicated a history of common, benign gynecologic conditions (i.e., fibroids, endometriosis, or ovarian cysts) in the woman's mother or sisters.

5.3.3 Model specification, estimation, and performance

We used unconditional logistic regression; coefficients were estimated with maximum likelihood methods. The baseline model (model A) included the predictors outlined above except family history; family history was added to form the full model (model B).

We evaluated model performance by examining both discrimination and calibration.[133,134] Discrimination, the ability of the model to classify women with and without hysterectomy correctly, is most commonly assessed using the concordance statistic (*c* statistic), a rank-order statistic for predictions against actual outcomes. For a binary outcome, the *c* statistic is equivalent to the area under the receiver operating characteristic curve (AUC). It is interpreted as the probability that a randomly drawn case will have a higher predicted probability of being a case from the fitted model

than a randomly drawn control. The discrimination slope, which can be visualized with a box plot or histogram, is calculated as the difference in average predicted probabilities for women with and without hysterectomy.[135]

Calibration, the agreement between the observed and predicted outcomes, was assessed with the Hosmer-Lemeshow Goodness of Fit (GOF) test, a chi-squared test based on the grouping of similar participants into *n* strata (often 10, as here) on the basis of their predicted probabilities, and the graphical representation of observed versus predicted agreement.[203] Additionally, we estimated the intercept and slope of the calibration line in a logistic regression model by regressing the observed outcome on the linear predictor (i.e., the predicted probabilities). Ideally, if the observed distributions of outcomes and predicted probabilities agree perfectly, the intercept of that line is 0 and the slope is 1.[136]

5.3.4 Model validation

Internal validation, the validity of the model in the underlying population that the data came from, may address issues of stability in predictor selection and quality of the predictions. The apparent performance of a prediction model is always better in the training/development data set than it is in a validation set, even when both come from the same population. The magnitude of this "optimism" can be used to correct performance metrics such as the AUC.

As an alternative to a split-sample approach, in which part of the study population is used to develop the prediction model and the remainder is used to validate it, we employed cross-validation with bootstrapping methods, which result in stable and nearly unbiased estimates of performance, as well as the lowest root mean squared error across methods.[137] We generated 200 bootstrap samples (i.e., with replacement) from the set of study participants with non-missing predictor data,[137,138] fitted the logistic regressions for models A and B in each sample, and generated a set of 200 *c* statistics (i.e., AUCs). The average of the 200 differences between the original AUC and the bootstrap samples' AUCs reflects the optimism of the prediction model. The optimism-corrected AUC was then calculated by subtracting the optimism from the AUC estimate from the original model.[134]

5.3.5 Evaluation of family history's contribution to the model

Whether the addition of the family history information substantially improved prediction was examined by calculating the difference between model A's AUC and model B's AUC.[140] Additionally, we present a reclassification table,[142] overall and stratified by outcome,[143] which shows how many cases and controls were reclassified, overall and correctly, by adding family history to the prediction model [144] as well as the Net Reclassification Improvement (NRI).[143,145] The NRI, which depends mainly on the estimated regression coefficient for the added predictor,[141] is similar to the percent of patients reclassified, but it takes into account movements in the correct direction. Negative percentages of the components of the NRI (i.e., event NRI and non-event NRI), as well as a negative overall NRI, are interpreted as a net worsening in classification.[204]

5.4 Results

There were 457 cases and 499 controls; the median age of both groups was 41 years. Women who underwent hysterectomy were more likely to report tubal ligation, previous myomectomy, endometriosis, fibroids, and ovarian cysts; they were also more likely to be black, married, less educated, current smokers, parous, and have heavier BMI. OC use was similar among the cases and controls (Table 7).

Sixty-one cases (13%) and 78 controls (17%) were missing data for one or more variables in the risk prediction models (Appendix Table 6). Data on BMI, duration of OC use, endometriosis, ovarian cysts, and previous myomectomy were missing less than 3% of the time while 11% of cases and 14% of controls were missing data on family history of common, benign gynecologic conditions. Participants missing family history data were more likely to be former or current smokers, have less than a college degree, and were less likely to report endometriosis or previous myomectomy (Appendix Table 7). We performed a sensitivity analysis of the impact of missing family history data by coding missing family history as "no" for one full model and as "yes" for another full model; results were similar to those from the complete-participant analysis that included a total of 817 women with non-missing data (Appendix Table 8).

The results for both models A & B were similar and indicated excellent performance, with AUC values of 0.85 (Table 8). The discrimination slopes were similar (model A: 0.376; model B: 0.384) (Figure 3). Both models were also well calibrated; the p-values for the Hosmer-Lemeshow GOF tests were 0.2 for both models (Figure 4). The p-values for the GOF test were similar when participants were grouped into 20 (p=0.2) and 40 (p=0.3) strata. The slopes of both models' calibration lines were 1.00 and the intercepts were approximately zero (model A: -4.25×10^{-8}; model B: -1.61×10^{-6}).

The mean differences between the apparent *c* statistics from the original models and the 200 bootstrapped models were 0.0085 for model A and 0.0091 for model B. The bias-corrected AUCs were therefore 0.8410 for model A and 0.8445 for model B.

The nonparametric test of the difference between the estimated AUCs in models A and B yielded p=0.1 (Figure 5). Overall, 16% of participants were reclassified when family history was added to the model; only 33% of reclassified cases and 48% of controls were reclassified correctly (Table 9). According to the NRI, the net percentage of cases reclassified correctly was <1% and the net percentage of controls reclassified correctly was 1%. Hence, the event and non-event NRIs were negative (-0.003 and -0.010, respectively), as was the overall NRI (-0.012, 95% CI: -0.041 to -0.015), indicating a net worsening in classification.

5.5 Discussion

We developed, *a priori*, a well-calibrated predictive model for hysterectomy with intention of ovarian conservation among premenopausal women that had very good discriminatory power. Internal validation resulted in minimal correction of the model's performance. Adding family history of common, benign gynecologic conditions to the model resulted in little to no impact on the model's performance and in fact, a small net worsening of classification.

There were minimal missing data and we were able to include an extensive list of predictors that have been reported to be associated with hysterectomy or its indications; with 22 degrees of freedom in model B, the events-per-variable (EPV) was 18. In simulation studies, no major problems

occurred when the EPV was 10 or greater;[127,129] evidence suggests that the inclusion of nuisance predictors (i.e., predictors that don't substantially contribute to the model) only marginally affects discriminatory accuracy.[124] This is demonstrated by the minimal change in model performance with the addition of the family history variable.

Although stepwise selection methods for inclusion (or exclusion) of predictors in such models are common, several studies have concluded that such methods are deficient, often resulting in overestimation of regression coefficients and loss of predictive power.[124,132,133] Steyerberg et al.[124] cautioned against basing the structure of prediction models solely on the data under study, especially when the dataset is small, and suggested that previously published or clinically practical parameterizations of variables are preferred over classifications that best fit the data. As we selected predictors *a priori*, and examined only the sensitivity of a single model to the inclusion or exclusion of a single predictor (family history of benign gynecologic conditions), our analyses did not suffer from the typical overfitting that comes from stepwise selection, as evidenced by the negligible decrease in the AUC from 0.85 to 0.84 when we adjusted for overfitting optimism.

However, given the near-complete absence of model development, nuisance predictors may have been included at the cost of degrees of freedom. The study was moderately sized and may represent a geographically limited study population. Both cases and controls were from Durham and surrounding counties, thus they represent a small area of central North Carolina and may not be generalizable to other locations or clinical settings. Additionally, though the control women were volunteers, they were recruited from, and likely represent, the source population of women under gynecologic care who could potentially have hysterectomies in Durham County.

With common and easy-to-define demographic, behavioral, and reproductive factors, we showed that we have an ability to predict, with very good discriminatory accuracy, hysterectomy among premenopausal women who aim to conserve one or both ovaries. Application of such a model throughout a woman's gynecologic care may lead to earlier and less invasive treatment for common, benign gynecologic conditions. Family history of common, benign gynecologic conditions in first-

degree relatives did not contribute positively to the prediction model. This may be due to the model's already strong performance and the need for a very strong predictor to substantially improve upon the AUC. [141] In fact, the addition of family history to the model resulted in a slight net worsening of the classification, suggesting that it might be counter-productive to use this variable for predictive purposes. Additional to its clinical application, information from our ability to predict premenopausal hysterectomy may also inform future analyses with respect to missing hysterectomy status or for choosing appropriate studies of hysterectomy.

The apparent success of this initial prediction model warrants further exploration and development in additional datasets representing different populations of premenopausal women. Future models could consider additional potential predictors such as symptom index(es) or additional medical treatments; prediction of different surgical modalities for hysterectomy could also be explored. There is room for improvement upon our preliminary model, including the possibility of making it more parsimonious while retaining its strong discriminatory power. As with all prediction models, external validation of this model is required before it can be adopted in clinical or other settings.

Table 7. Baseline characteristics of premenopausal women undergoing hysterectomy with ovarian conservation and control women in the *Prospective Research on Ovarian Function (PROOF) Study*, 2004-2007[a]

	Cases (n=457)		Controls (n=499)	
	n	(%)	n	(%)
Age				
Mean (SD)	40.53	(4.20)	40.12	(4.48)
Median	41		41	
Range (IQR)	30-47	(38-44)	30-47	(37-44)
Race				
White	210	(46)	258	(52)
Black	236	(52)	214	(43)
Other	11	(2)	27	(3)
Marital Status				
Single/Never married	82	(18)	128	(26)
Married/Living with significant other	283	(62)	260	(52)
Divorced/Separated/Widowed	92	(20)	111	(22)
Education				
High school graduate or below	112	(25)	86	(17)
Some college/trade/tech school	177	(39)	143	(29)
College graduate or higher	168	(37)	270	(54)
Smoking				
Never	271	(59)	328	(66)
Former	88	(19)	88	(18)
Current	98	(21)	83	(17)
BMI at Baseline (kg/m^2)				
Mean (SD)	31.07	(7.31)	29.31	(7.54)
Median	30.27		27.79	
Range (IQR)	17.85-55.52	(25.34-35.75)	16.26-66.61	(23.42-34.16)
Underweight (<18.5)	3	(1)	7	(1)
Normal (18.5- <25)	99	(22)	166	(33)
Overweight (25- <30)	117	(26)	126	(25)
Obese (≥30)	237	(52)	199	(40)
Oral Contraceptive Use				
Never	39	(9)	46	(9)
Ever	418	(91)	453	(91)
Years of Oral Contraceptive Use[b]				
Mean (SD)	8.01	(6.93)	7.45	(6.25)
Median	6.00		6.00	
Range (IQR)	0.08-30.00	(2.00-12.00)	0.08-28.00	(2.50-11.92)
< 1 year	48	(12)	54	(12)
1- <3 years	67	(16)	61	(14)
3- <5 years	47	(12)	64	(14)
5- <10 years	98	(24)	120	(27)
≥10 years	147	(36)	145	(33)
Gravidity				
Never pregnant	50	(11)	105	(21)
Ever pregnant	407	(89)	394	(79)
Number of Full-term Pregnancies[c]				
Mean (SD)	2.04	(1.10)	1.87	(1.31)
Median	2		2	
Range (IQR)	0-8	(2-3)	0-8	(1-2)

0	23	(6)	52	(13)
1	100	(25)	103	(26)
2	168	(41)	141	(36)
3	82	(20)	66	(17)
≥4	34	(8)	32	(8)
Reproductive Health (History of)				
Tubal ligation	210	(46)	135	(27)
Fibroids	341	(75)	106	(21)
Endometriosis	69	(15)	40	(8)
Ovarian cysts	151	(33)	88	(18)
Previous myomectomy	43	(9)	17	(3)
Family History of Benign Gynecologic Conditions[d]	257	(63)	189	(44)

BMI=Body mass index; IQR=Interquartile range; SD=Standard deviation.

[a] All baseline characteristics were included in the risk prediction models for hysterectomy with the exception of gravidity (nulligravid/gravid) and oral contraceptive use (never/ever). Women reporting never use of oral contraceptives or nulligravidity were included in the models as 0 years of oral contraceptive use and 0 full-term pregnancies, respectively. Numbers (%) are reported for cases and controls except where noted.

[b] Among women who reported ever use of oral contraceptives.

[c] Among gravid women.

[d] History of endometriosis, fibroids, or ovarian cysts in mother or sister(s).

Table 8. Summary of model performance for the prediction models for hysterectomy with ovarian conservation among premenopausal women, without and with family history of common, benign gynecologic conditions

Model	AIC	Neg 2 Log L	AUC	AUC SE
A	832.596	788.596	0.8495	0.0136
B	824.560	778.560	0.8536	0.0133

AIC=Akaike's information criteria; AUC= Area under the curve; AUC SE: Standard error of the area under the curve; Neg 2 Log L=Negative 2 Log-Likelihood.

Table 9. Reclassification among all baseline *Prospective Research on Ovarian Function (PROOF)* participants, cases only, and controls only after including family history of common, benign gynecologic conditions in the prediction model for hysterectomy with ovarian conservation among premenopausal women

Among All Participants		**(Model B)**			
		Controls	**Cases**	**Total**	**% Reclassified**
(Model A)	**Controls**	405	19	424	4
	Cases	16	377	393	4
Among Cases		**(Model B)**			
		Controls	**Cases**	**Total**	**% Reclassified**
(Model A)	**Controls**	76	11	87	13
	Cases	12	297	309	4
Among Controls		**(Model B)**			
		Controls	**Cases**	**Total**	**% Reclassified**
(Model A)	**Controls**	329	8	337	2
	Cases	4	80	84	5

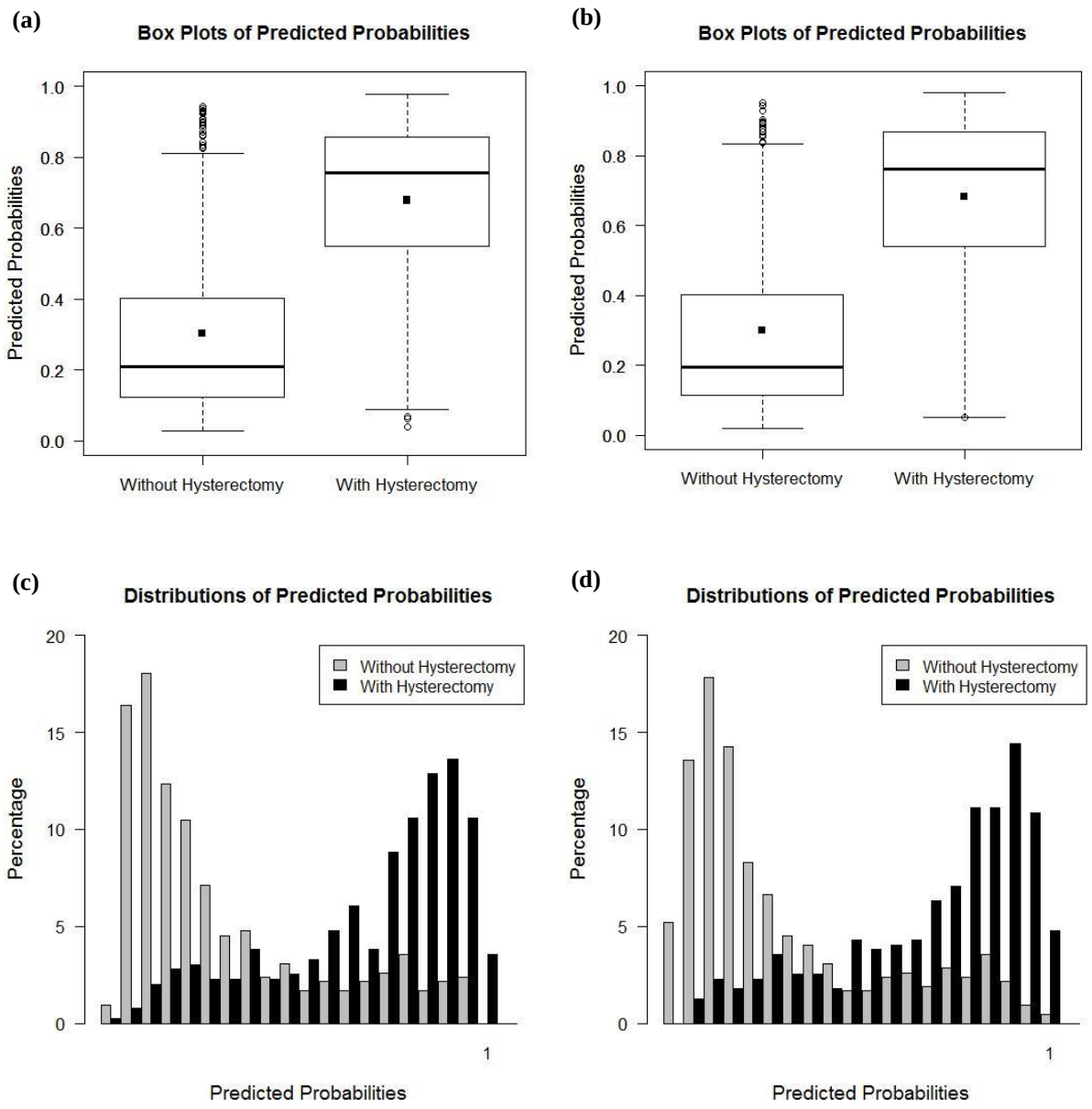
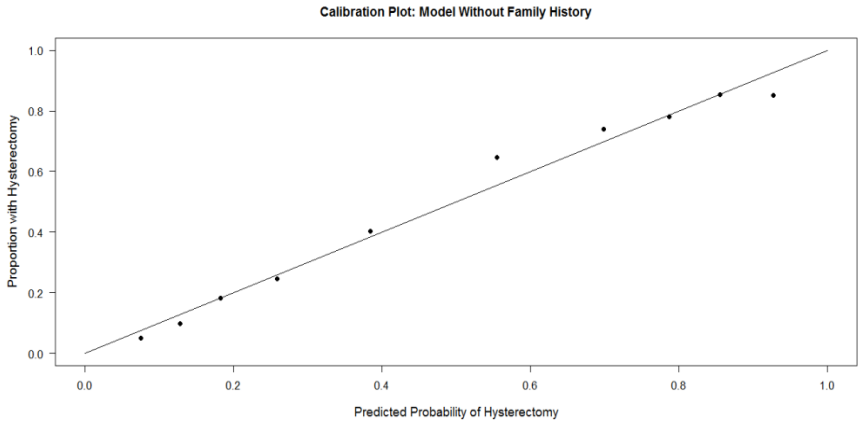


Figure 3. Discrimination of the prediction models for hysterectomy with ovarian conservation among premenopausal women, without and with family history of common, benign gynecologic conditions. Panels (a) and (b) display boxplots for the distributions of predicted probabilities among controls and cases for Model A (a) and Model B (b). Panels (c) and (d) display the histograms of risks separated for controls (light grey columns) and cases (black columns), separately, for Model A (c) and Model B (d).

(a)



(b)

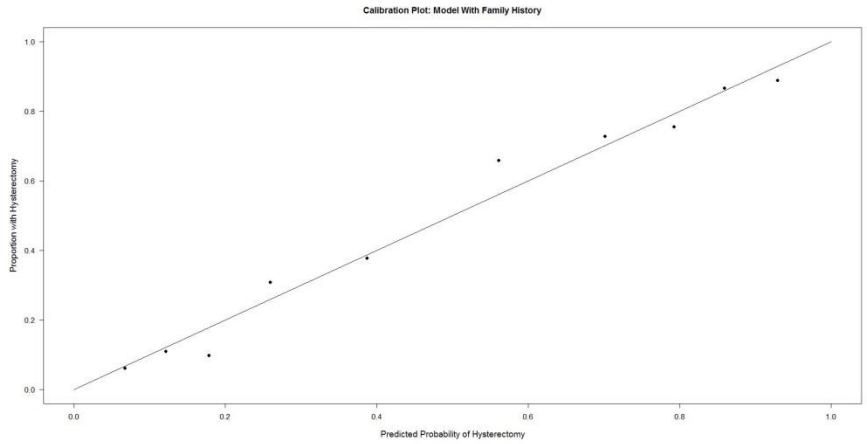


Figure 4. Calibration of the prediction models for hysterectomy with ovarian conservation among premenopausal women, without and with family history of common, benign gynecologic conditions. The calibration plot compares observed and predicted risks for deciles of participants, as grouped by the Hosmer-Lemeshow Goodness of Fit test, for Models A (panel a) and B (panel b). The ten solid dots along the 45-degree line represent the average estimated probability for each decile of participants; perfect calibration would be represented by dots that fall directly on the 45-degree line.

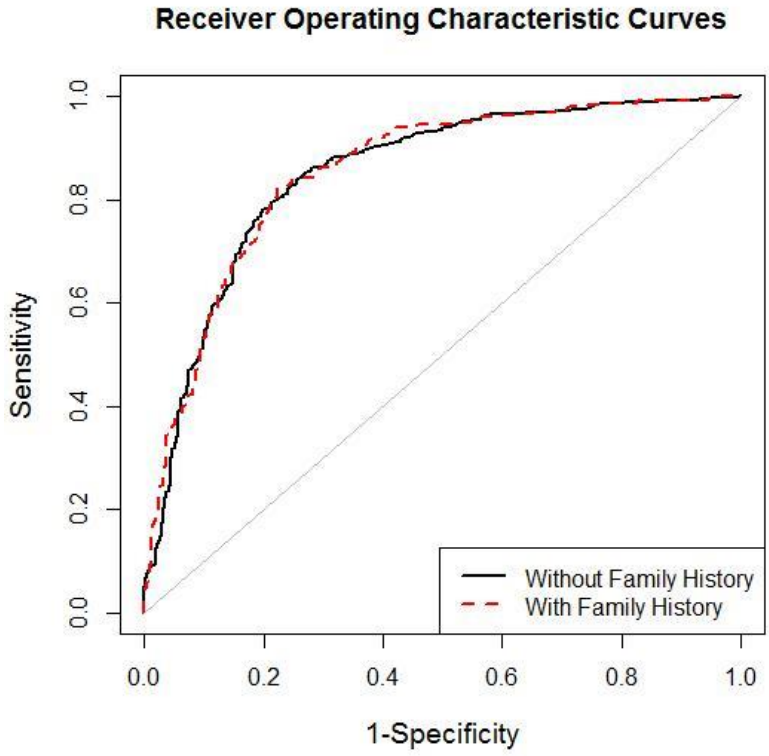


Figure 5. Receiver operating characteristic curves for the prediction models for hysterectomy with ovarian conservation among premenopausal women, without and with family history of common, benign gynecologic conditions. The receiver operating characteristic curve plots the true positive rate (i.e., sensitivity) on the y-axis against the false positive rate (i.e., 1-specificity) on the x-axis for Model A (solid black line) and Model B (dashed red line); the solid diagonal line indicates no discrimination.

CHAPTER 6. DISCUSSION

6.1 Recapitulation of aims and results

The goal of this dissertation was to explore the relationship between hysterectomy and subtypes of epithelial ovarian cancer and to build a multivariate prediction model for premenopausal, ovarian-conserving hysterectomy. We accomplished each of the proposed aims through the use of 15 case-control studies in the Ovarian Cancer Association Consortium and the recent *Prospective Research on Ovarian Function* Study. These aims were: to determine the associations between epithelial ovarian cancer and (1) hysterectomy more than two years prior to diagnosis (cases) or interview (controls), (2) age at hysterectomy, and (3) time since hysterectomy as well as to (1) develop and internally validate a predictive model for premenopausal, ovarian-conserving hysterectomy and (2) evaluate the contribution of family history of benign, gynecologic conditions among mothers or sisters to the predictive model.

Our findings do not support the long-held belief that hysterectomy is protective against ovarian cancer. In fact, we found average relative increases in the odds of most subtypes of epithelial ovarian cancer with hysterectomy more than two years prior to diagnosis (cases) or interview (controls), including hysterectomies prior to age 40 or hysterectomies 30 years or more in the past. In support of the published literature, we found an average relative decrease in the odds of invasive clear cell epithelial ovarian cancer.

We were able to predict ovarian-conserving hysterectomy among premenopausal women with very good discriminatory accuracy. The multivariate prediction model was well-calibrated; there was good agreement between the predicted probabilities of hysterectomy and the observed outcomes. Family history of common, benign gynecologic conditions in the mother or sisters of premenopausal

women did not contribute to the performance of the prediction model in an important way, and in fact, resulted in a slight net worsening of hysterectomy classification.

6.2 Strengths

One key strength of this research was the opportunity to evaluate the associations between aspects of hysterectomy and epithelial ovarian cancer using standardized variable parameterizations and the same analytic and statistical approach among women from 15 independent case-control studies of ovarian cancer from across the globe. Only one previous study pooled results from multiple studies, but it was from an older time period (studies were conducted from 1956 to 1986) when we knew far less about the heterogeneous nature of epithelial ovarian cancer; the median years of ovarian cancer diagnosis among our studies ranged from 1994 to 2005 and more recent hysterectomies were represented. Though rates of hysterectomy have not dramatically changed over the previous several decades, surgical approaches have evolved, the rates of concomitant bilateral salpingo-oophorectomy are more age-dependent than in previous decades, and women who undergo hysterectomy now may have undergone different treatments for their gynecologic condition(s) prior to their hysterectomy that did not exist before. Additionally, our study is the largest examination of hysterectomy and epithelial ovarian cancer to date; the largest analysis prior to this study included less than half the number of women we were fortunate to include. The inclusion of almost 30,000 women allowed us to determine the associations between aspects of hysterectomy and subtypes of epithelial ovarian cancer, which were not previously studied due to (a) lack of knowledge that risk factors for ovarian cancer may differ by subtype and (b) lack of adequate sample size due to ovarian cancer being rare, in general, and including only small percentages of each of the non-serous subtypes.

Though the number of participants included from the PROOF study was not appreciably large, there were minimal missing data and we were able to include an extensive list of 14 variables related to demographics, anthropomorphic characteristics, behavioral risk factors, and reproductive and medical history that have been reported to be associated with hysterectomy or its indications among premenopausal women. Because we selected the prediction model's regressors *a priori*, there

was minimal overfitting that is typically a result of data-driven model development being performed in the same population as evaluation of the model. Again, though our sample size for this study was not on the same scale as our analysis of 30,000 women from 15 studies, we were able to use bootstrap methods to internally validate our prediction model. The apparent success of our initial prediction model for premenopausal hysterectomy with ovarian conservation represents a solid first step toward informing future analyses where hysterectomy status may be missing or where there needs to be appropriate selection of women for studies of hysterectomy. Additionally, there may be clinical utility in such a prediction model, whereby women may be identified for earlier and hopefully, less invasive treatments for their common, benign gynecologic condition(s).

6.3 Limitations

With respect to the prediction model for premenopausal women, one limitation was our population of women included in the analysis. Though we are not concerned about the likelihood that they represent the population of premenopausal women who may eventually undergo hysterectomy for common, benign gynecologic conditions, even though the controls were volunteers who were on average, more educated than the source population, they still represent a geographically limited population; our findings may not be generalizable to other locations or clinical settings.

In our analysis of the associations between aspects of hysterectomy and epithelial ovarian cancer, there may have been misclassification of self-reported oophorectomy status. Over time, the likelihood of concomitant bilateral salpingo-oophorectomy with hysterectomy has changed such that ovarian conservation prior to and during the earlier years of menopause is encouraged. Because of that, women who undergo hysterectomy prior to menopause are more likely to retain one or both of their ovaries, leaving them at risk for epithelial ovarian cancer. Though misreporting bilateral-oophorectomy seems unlikely among these women due to the noticeable sequelae of removing both ovaries, there may be some misclassification among women whose hysterectomies were performed during the perimenopause period or when the indication for hysterectomy involved symptoms that mimicked those of menopause (e.g., abnormal uterine bleeding). Women who undergo hysterectomy

after menopause are less likely to retain their ovaries, resulting in a small group of women who would still be at risk for ovarian cancer. Not only could there be more misclassification of oophorectomy status among these women, because the loss of ovarian function has already been experienced, these women also likely represent a very particular group of post-menopausal women who retain one or both ovaries. The reasons for ovarian conservation among these women may, or may not be related to their future ovarian cancer risk. Unfortunately, even with a large sample size of 30,000 women, an analysis among these women would be considerably underpowered, even if the appropriate data had been collected in the original studies.

6.4 Public health significance

The public health impact of a single study of modest size such as the PROOF study of approximately 1,000 women is difficult to evaluate, but our analysis does provide insight into premenopausal hysterectomy with ovarian conservation. We were able to predict, with very good discriminatory accuracy, premenopausal hysterectomy using easy-to-define variables that could be easily collected in a clinical setting. The identification of women "at risk" for future hysterectomy could theoretically result in earlier and less-invasive treatments for their gynecologic conditions. Though adoption of a prediction model for premenopausal hysterectomy with ovarian conservation in a clinical setting cannot and should not occur until additional model development can be done prospectively among larger and more diverse groups of women (and subsequently externally validated), there is the potential for decreasing the incidence and prevalence of one of the most common, and often invasive, surgeries among women in the United States. Our study provides a framework around which future prediction models could be developed and validated.

Hysterectomy is the most common non-obstetric (e.g., Cesarean delivery) surgery performed among women in the United States and impacts approximately 600,000 women each year. More than one-third of women will have a hysterectomy by age 60. Like any surgery, there are numerous risks and costs associated with having a hysterectomy and downstream consequences of having a hysterectomy prior to menopause (e.g., exogenous hormone use) may impact future risk of medical

conditions such as ovarian cancer, breast cancer, osteoporosis, and stroke. Ovarian cancer may be much rarer than conditions such as breast cancer and osteoporosis, but it is a highly fatal disease for which no screening or early detection measures exist. In fact, due to the rarity of ovarian cancer, it is unlikely that screening for the disease in the general population, even with a perfectly sensitive and specific test, would be beneficial. Most cases are diagnosed at a late stage when five-year survival is an abysmal 27%, a fact that has led to ovarian cancer being dubbed the "whispering disease." Though questions remain after our analysis of almost 30,000 women from 15 studies across the globe, our study provides a reason for critically challenging the status quo in ovarian cancer research and for thinking about factors related to hysterectomy that may not have been evaluated at a level of detail necessary for identifying direct and indirect effects of exposures and their sequelae.

6.5 Future research

Future research regarding ovarian-conserving hysterectomy among premenopausal women should focus, at a much more detailed level, on the factors that predict future hysterectomy so that unnecessary hysterectomies might ultimately be avoided; this level of detail may only be available in medical records since self-report of complicated medical information is often unknown or misclassified. In addition to better understanding the common indications for hysterectomy (fibroids, endometriosis, non-specific menstrual disorders), we need to also understand the reasons for and outcomes associated with other treatments that may be utilized in place of or prior to the use of hysterectomy to treat a condition. Likewise, the downstream consequences of hysterectomy, especially prior to menopause, must be studied with far greater detail. It has been shown that women who undergo hysterectomy prior to menopause experience menopause earlier than they would have without the hysterectomy[121]. This may result in the earlier use of menopausal hormone replacement use; the duration of use may also be longer if symptoms of menopause start at an earlier age and continue through multiple decades. The risks associated with the types of hormones used, the duration of use, and the proximity of use to other risk factors that may impact the incidence or prevalence of other diseases are imperative to study. This research will require very detailed information that may

only be accurately collected prospectively and/or from medical charts or claims records. The methods of analyzing data that relate to different time points along a woman's reproductive health history are complicated but more careful consideration of the decisions made by women and their health providers could lead to a better understanding and possible prevention of disease such as ovarian cancer.

APPENDIX. SUPPLEMENTARY TABLES AND FIGURES FOR CHAPTER 4 and 5

Appendix Table 1. Association between hysterectomy more than two years prior to diagnosis (cases) or interview/reference date (controls) and subtypes of invasive serous and mucinous epithelial ovarian tumors

Study	Low-grade Invasive Serous[a]		High-grade Invasive Serous[a]		LMP Mucinous[b]		Invasive Mucinous[b]	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
AUS	0.75	(0.29, 1.93)	1.14	0.88, 1.48	2.00	1.27, 3.16	0.95	0.37, 2.41
CON	N/A	N/A	0.72	0.39, 1.33	0.81	0.23, 2.85	3.91	1.33, 11.50
DOV	0.69	(0.15, 3.18)	1.28	1.00, 1.64	1.12	0.70, 1.78	0.51	0.15, 1.72
GER	N/A	N/A	0.67	0.35, 1.28	0.63	0.07, 5.53	0.96	0.33, 2.82
HAW	3.58	(0.63, 20.42)	1.28	0.85, 1.92	0.82	0.32,2.15	1.12	0.46, 2.75
HOP	0.33	(0.04, 2.63)	1.25	0.91, 1.71	0.85	0.25, 2.93	0.70	0.24, 2.04
MAL	1.85	(0.96, 3.57)	1.22	0.77, 1.92	1.11	0.52, 2.35	1.40	0.58, 3.36
NCO	1.27	(0.57, 2.85)	1.25	0.94, 1.67	1.07	0.51, 2.22	0.70	0.28, 1.74
NEC	2.11	(0.66, 6.81)	1.02	0.68, 1.54	1.41	0.62, 3.23	0.51	0.12, 2.15
NTH[c]	1.11	(0.11, 10.99)	1.30	0.58, 2.93	N/A	N/A	1.30	0.42, 4.07
POL	N/A	N/A	N/A	N/A	N/A	N/A	1.21	0.15, 9.49
STA[d]	1.26	(0.30, 5.42)	0.81	0.46, 1.42	1.43	0.29, 7.05	0.71	0.19, 2.70
UCI	2.05	(0.51, 8.25)	1.05	0.63, 1.75	0.47	0.22, 1.01	0.86	0.31, 2.34
UKO	N/A	N/A	0.99	0.64, 1.54	N/A	N/A	1.47	0.69, 3.14
USC	1.18	(0.48, 2.93)	1.47	1.14, 1.91	1.18	0.64, 2.19	1.64	0.95, 2.84
Random Effects	1.35 (0.92, 1.97)		1.18 (1.06, 1.32)		1.11 (0.82, 1.51)		1.15 (0.88, 1.52)	
τ^2	0		0		0.05974		0.005291	
95% PI	N/A		N/A		0.59 to 2.08		0.84 to 1.59	
OEP	N/A		N/A		33%		3%	

CI=confidence interval; LMP=low malignant potential; N/A=not available/applicable; OEP=opposite effects proportion; OR=odds ratio; PI=prediction interval.

[a] Odds ratios and 95% confidence intervals for the association were estimated by conditional logistic regression models stratified by age in 5-year groups and race/ethnicity (non-Hispanic white, Hispanic white, black, Asian, other) and adjusted for body mass index in early adulthood (<18.5, 18.5-<25, 25-<30, ≥30), parity (0, 1, 2, 3, 4+ full-term births), duration of oral contraceptive use (0, <24 months, 24-<60 months, 60-<120 months, ≥120 months), tubal ligation (yes/no), and history of breast or ovarian cancer in the mother (yes/no).

[b] Conditional logistic regression models were stratified by age in 5-year groups and race/ethnicity (non-Hispanic white, Hispanic white, black, Asian, other); models are not adjusted for any additional covariates.

[c] Models for NTH low-grade and high-grade invasive serous EOC are not adjusted for body mass index in early adulthood.

[d] Models for STA low-grade and high-grade invasive serous EOC are not adjusted for body mass index in early adulthood. Model for low-grade invasive serous EOC is not adjusted for endometriosis.

Appendix Table 2. Characteristics related to study validity and time/place evaluated as potential sources for heterogeneity of results

Study	Cases			Controls				
	Source	**Median Year of Diagnosis**	**Mean days between diagnosis and interview**	**Timing of Sampling with Cases**	**Eligibility of Women Unsure of Ovary Status[a]**	**Median Year of Hysterectomy**	**Prevalence of Hysterectomy**	**Age Standardized Prevalence of Hysterectomy[b]**
AUS	Population	2003	161	Concurrent	Included	1984	19%	19%
CON	Population	2001	281	Concurrent	Excluded	1982	11%	13%
DOV	Population	2005	278	Concurrent	Excluded	1982	18%	18%
GER	Population	1994	218	Concurrent	Included	1980	24%	24%
HAW	Population	1996	328	Concurrent	Excluded	1975	10%	10%
HOP	Population	2005	129	Concurrent	Included	1982	17%	15%
MAL	Population	1996	109	Concurrent	Included	1979	9%	8%
NCO	Population	2002	185	Concurrent	Included	1979	22%	22%
NEC	Population	1997	296	Concurrent	Excluded	1977	8%	9%
NTH	Population	2002	2,513	Subsequent	Included	1982	13%	12%
POL	Population	2002	95	Concurrent	Included	1987	4%	4%
STA	Population	1998	287	Concurrent	Excluded	1982	10%	15%
UCI	Population	2000	945	Concurrent	Included	1983	25%	26%
UKO	Hospital/Clinic	2005	743	Subsequent	Included	1983	19%	15%
USC	Population	1996	275	Concurrent	Excluded	1975	11%	12%

[a] Some studies explicitly excluded potential controls if they were not sure they had at least one intact ovary. Studies that included participants who were unsure of their ovary status or who did not give participants the option of a "don't know" answer are grouped together.

[b] The standard population used was the arithmetic mean of the un-weighted distribution of ages in each study's control group (age groups: 18-44, 45-64, 65+).

Appendix Table 3. Counts of hysterectomies that occurred at or prior to diagnosis (cases) or interview/reference date (controls) according to study and outcome

Study	Hysterectomy At or Prior to Diagnosis (Cases) or Interview/Reference Date (Controls)[a]		Hysterectomy in the First Year Prior to Diagnosis (Cases) or Interview/Reference Date (Controls)[b]		Hysterectomy in the Second Year Prior to Diagnosis (Cases) or Interview/Reference Date (Controls)[c]	
	Cases	**Controls**	**Cases**	**Controls**	**Cases**	**Controls**
AUS	327/1420 (23)	291/1488 (20)	0/327 (0)	1/291 (<1)	11/327 (3)	9/291 (3)
CON	116/476 (24)	61/549 (11)	23/116 (20)	1/61 (2)	27/116 (23)	1/61 (2)
DOV	319/1576 (20)	358/1849 (19)	5/319 (2)	7/358 (2)	5/319 (2)	9/357 (3)
GER	53/258 (21)	137/533 (26)	3/53 (6)	4/137 (3)	0/53 (0)	3/137 (2)
HAW	113/896 (13)	114/1104 (10)	1/113 (1)	4/114 (4)	2/113 (2)	1/114 (1)
HOP	151/771 (20)	309/1804 (17)	4/151 (3)	0/309 (0)	2/151 (1)	11/309 (4)
MAL	99/755 (13)	146/1564 (9)	0/99 (0)	4/146 (3)	0/99 (0)	3/146 (2)
NCO	271/1092 (25)	248/1081 (23)	17/271 (6)	2/248 (1)	5/271 (2)	4/248 (2)
NEC	92/1119 (8)	102/1243 (8)	0/92 (0)	1/102 (1)	2/92 (2)	1/102 (1)
NTH	177/255 (69)	82/594 (14)	139/177 (79)	2/82 (2)	2/177 (1)	1/82 (1)
POL	216/293 (74)	51/1101 (5)	207/216 (96)	1/51 (2)	1/216 (<1)	3/51 (6)
STA	78/660 (12)	61/566 (11)	18/78 (23)	3/61 (5)	2/78 (3)	3/61 (5)
UCI	125/589 (21)	159/569 (28)	0/15 (0)	10/159 (6)	3/15 (20)	4/159 (3)
UKO	342/603 (57)	201/1033 (19)	232/342 (68)	0/201 (0)	18/342 (5)	3/201 (1)
USC	287/1736 (17)	211/1809 (12)	3/287 (1)	4/211 (2)	5/287 (2)	2/211 (1)
All	**2766/12499 (22)**	**2531/16887 (15)**	**652/2766 (24)**	**44/2531 (2)**	**85/2766 (3)**	**58/2531 (2)**

[a] The counts and percentages shown represent the proportion of women who reported hysterectomy at the same age, or an earlier age, as their age at diagnosis (cases) or age at interview/reference date (controls).

[b] The counts and percentages shown represent the proportion of hysterectomies where the difference between age at diagnosis/interview and age at hysterectomy is less than or equal to 1.

[c] The counts and percentages shown represent the proportion of hysterectomies where the difference between age at diagnosis/interview and age at hysterectomy is greater than 1 and less than or equal to 2.

Appendix Table 4. Sensitivity analysis of the association[a] between hysterectomy[b] and low malignant potential epithelial ovarian tumors

Study	At or Prior		More Than One Year Prior		More Than Two Years Prior	
	OR	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**
AUS	1.76	1.23, 2.53	1.78	1.24, 2.55	1.85	1.28, 2.67
CON	4.41	2.54, 7.64	3.88	2.20, 6.86	1.60	0.81, 3.16
DOV	1.35	1.00, 1.83	1.36	1.00, 1.85	1.40	1.03, 1.90
GER	0.81	0.21, 3.09	0.50	0.11, 2.37	0.52	0.11, 2.47
HAW	1.31	0.72, 2.39	1.42	0.77, 2.61	1.42	0.77, 2.62
HOP	2.13	1.20, 3.78	2.13	1.20, 3.78	2.25	1.26, 4.00
MAL[c]	1.18	0.71, 1.96	1.22	0.73, 2.02	1.25	0.75, 2.08
NCO	1.23	0.80, 1.91	1.11	0.71, 1.74	1.13	0.72, 1.77
NEC	1.44	0.85, 2.46	1.47	0.86, 2.50	1.52	0.89, 2.60
STA[d]	1.32	0.64, 2.71	1.07	0.49, 2.35	1.02	0.45, 2.32
UCI	0.39	0.22, 0.69	0.46	0.25, 0.83	0.44	0.24, 0.81
USC	1.50	1.03, 2.18	1.46	1.00, 2.14	1.44	0.98, 2.11
Random Effects	1.40	0.98, 1.99	1.37	0.89, 1.91	1.32	1.02, 1.71
$\hat{\tau}^2$	0.2195		0.1656		0.07088	
95% PI	0.46 to 4.21		0.52 to 3.61		0.69 to 2.52	
OEP	21%		22%		15%	

CI=confidence interval; N/A=not available; OEP=opposite effects proportion; OR=odds ratio; PI=prediction interval.

[a] Odds ratios and 95% confidence intervals for the association between hysterectomy and all LMP tumors combined were estimated by conditional logistic regression models stratified by age in 5-year groups and race/ethnicity (non-Hispanic white, Hispanic white, black, Asian, other) and adjusted for body mass index in early adulthood (<18.5, 18.5-<25, 25-<30, ≥30), parity (0, 1, 2, 3, 4+ full-term births), duration of oral contraceptive use (0, <24 months, 24-<60 months, 60-<120 months, ≥120 months), tubal ligation (yes/no), and history of breast or ovarian cancer in the mother (yes/no).

[b] The exposed group is defined in three different ways for the sensitivity analysis: (1) hysterectomy at or prior to diagnosis (cases) or interview/reference date (controls), (2) hysterectomy more than one year prior to diagnosis (cases) or interview/reference date (controls), and (3) hysterectomy more than two years prior to diagnosis (cases) or interview/reference date (controls).

[c] Models for MAL are not adjusted for history of breast or ovarian cancer in the mother due to missing data.

[d] Models for STA are not adjusted for body mass index in early adulthood or endometriosis.

Appendix Table 5. Sensitivity analysis of the association[a] between hysterectomy[b] and invasive epithelial ovarian cancer

Study	At or Prior		More Than One Year Prior		More Than Two Years Prior	
	OR	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**
AUS	1.23	1.00, 1.53	1.24	1.00, 1.54	1.20	0.96, 1.49
CON	2.31	1.54, 3.47	1.61	1.05, 2.47	1.28	0.82, 2.00
DOV	1.24	1.01, 1.51	1.26	1.03, 1.54	1.27	1.03, 1.55
GER	0.82	0.53, 1.26	0.79	0.51, 1.22	0.83	0.54, 1.29
HAW	1.30	0.95, 1.77	1.35	0.98, 1.85	1.33	0.96, 1.82
HOP	1.23	0.96, 1.56	1.19	0.93, 1.51	1.21	0.94, 1.54
MAL[c]	1.47	1.08, 2.00	1.51	1.11, 2.06	1.55	1.14, 2.12
NCO	1.20	0.95, 1.52	1.08	0.85, 1.38	1.07	0.84, 1.37
NEC	1.05	0.74, 1.47	1.06	0.75, 1.49	1.05	0.74, 1.48
NTH[d]	14.33	8.88, 23.15	1.24	0.71, 2.16	1.27	0.73, 2.22
POL[e]	65.75	40.25, 107.40	0.59	0.26, 1.32	0.54	0.23, 1.27
STA[f]	1.01	0.65, 1.55	0.71	0.44, 1.13	0.72	0.45, 1.17
UCI	0.78	0.52, 1.17	0.93	0.62, 1.41	0.96	0.63, 1.45
UKO	5.86	4.39, 7.81	1.38	1.01, 1.89	1.21	0.88, 1.67
USC	1.44	1.16, 1.79	1.46	1.18, 1.82	1.44	1.15, 1.79
Random Effects	2.05	1.04, 4.03	1.21	1.07, 1.36	1.19	1.08, 1.32
$\hat{\tau}^2$	1.436		0.006593		0.001531	
95% PI	0.14 to 29.79		0.98 to 1.49		1.05 to 1.36	
OEP	27%		1%		<1%	
	Excluding NTH, POL, and UKO[g]					
Random Effects	1.23	1.06, 1.43	1.20	1.06, 1.37	1.20	1.07, 1.34
$\hat{\tau}^2$	0.01922		0.008824		0.003232	
95% PI	0.87 to 1.74		0.94 to 1.54		1.01 to 1.42	
OEP	7%		2%		<1%	

CI=confidence interval; N/A=not available; OEP=opposite effects proportion; OR=odds ratio; PI=prediction interval.

[a] Odds ratios and 95% confidence intervals for the association between hysterectomy and all invasive epithelial ovarian cancers combined were estimated by conditional logistic regression models stratified by age in 5-year groups and race/ethnicity (non-Hispanic white, Hispanic white, black, Asian, other) and adjusted for body mass index in early adulthood (<18.5, 18.5-<25, 25-<30, ≥30), parity (0, 1, 2, 3, 4+ full-term births), duration of oral contraceptive use (0, <24 months, 24-<60 months, 60-<120 months, ≥120 months), tubal ligation (yes/no), and history of breast or ovarian cancer in the mother (yes/no).

[b] The exposed group is defined in three different ways for the sensitivity analysis: (1) hysterectomy at or prior to diagnosis (cases) or interview/reference date (controls), (2) hysterectomy more than one year prior to diagnosis (cases) or interview/reference date (controls), and (3) hysterectomy more than two years prior to diagnosis (cases) or interview/reference date (controls).

[c] Models for MAL all LMP tumors combined are not adjusted for history of breast or ovarian cancer in the mother due to missing data.

[d] Models for NTH are not adjusted for body mass index in early adulthood.

[e] Models for POL are not adjusted for endometriosis.

[f] Models for STA are not adjusted for body mass index in early adulthood or endometriosis.

[g] 69%, 74%, and 57% of the cases from the NTH, POL, and UKO studies, respectively, reported ever having a hysterectomy (see Appendix Table 3). As these percentages are much higher than the percentage of cases from other studies reporting hysterectomy, and from the

general populations that these studies represent, we decided to perform sensitivity analyses that (a) defined our exposure using different time periods and (b) excluded these studies from the pooled results.

Appendix Table 6. Accounting of missing data among women undergoing hysterectomy and control women in the PROOF Study, 2004-2007[a]

	Cases (n=457)		Controls (n=499)	
	N	**(%)**	**N**	**(%)**
Age	0	(0)	0	(0)
Race	0	(0)	0	(0)
Marital Status	0	(0)	0	(0)
Education	0	(0)	0	(0)
Smoking	0	(0)	0	(0)
BMI at Baseline	1	(<1)	1	(<1)
Duration Oral Contraceptive Use	11	(3)	9	(2)
Full-term Pregnancies	0	(0)	0	(0)
Tubal ligation	0	(0)	0	(0)
Fibroids	0	(0)	3	(1)
Endometriosis	1	(<1)	3	(1)
Ovarian cysts	0	(0)	2	(<1)
Previous myomectomy	1	(<1)	0	(0)
Family History of Benign Gynecologic Conditions[b]	50	(11%)	68	(14%)

BMI=Body mass index.

[a] Sixty-one cases (13%), representing 44% of the participants with missing data, and 78 controls (17%), representing 56% of the participants with missing data, are missing data for one or more variables in the risk prediction model. Fifty-eight cases and 72 controls are missing data for one variable, three cases and five controls are missing data for two variables, and one control is missing data for four variables.

[b] History of endometriosis, fibroids, or ovarian cysts in mother or sister(s).

Appendix Table 7. Baseline characteristics, by missingness of family history of common, benign gynecologic conditions, of participants in the PROOF Study, 2004-2007[a]

	Missing FamHx (n=118)		Not Missing FamHx (n=838)	
	N	(%)	N	(%)
Age				
Mean (SD)	40.25	(4.68)	40.32	(4.31)
Median	41		41	
Range (IQR)	30-47	(37-44)	30-47	(37-44)
Race				
White	55	(47)	413	(49)
Black	58	(49)	392	(47)
Other	5	(4)	33	(4)
Marital Status				
Single/Never married	28	(24)	182	(22)
Married/Living with significant other	68	(58)	475	(57)
Divorced/Separated/Widowed	22	(19)	181	(22)
Education				
High school graduate or below	33	(28)	165	(20)
Some college/trade/tech school	33	(28)	287	(34)
College graduate or higher	52	(44)	386	(46)
Smoking				
Never	62	(53)	537	(64)
Former	27	(23)	149	(18)
Current	29	(25)	152	(18)
BMI at Baseline (kg/m^2)				
Mean (SD)	29.73	(8.50)	30.21	(7.33)
Median	27.93		29.06	
Range (IQR)	16.41-55.52	(23.23-34.53)	16.26-66.61	(24.53-34.78)
Underweight (<18.5)	3	(3)	7	(1)
Normal (18.5- <25)	36	(31)	229	(27)
Overweight (25- <30)	32	(27)	211	(25)
Obese (≥30)	47	(40)	389	(47)
Oral Contraceptive Use				
Never	11	(9)	74	(8)
Ever	107	(91)	764	(92)
Years of Oral Contraceptive Use[b]				
Mean (SD)	7.03	(7.16)	7.02	(6.59)
Median	5.00		5.00	
Range (IQR)	0.00-28.00	(0.50-11.08)	0.00-30.00	(1.00-11.00)
< 1 year	19	(19)	83	(11)
1- <3 years	12	(12)	116	(15)
3- <5 years	12	(12)	99	(13)
5- <10 years	27	(26)	191	(26)
≥10 years	32	(31)	260	(31)
Gravidity				
Nulligravid	19	(16)	136	(16)
Gravid	99	(84)	702	(84)
Number of Full-term Pregnancies[c]				
Mean (SD)	1.91	(1.24)	1.97	(1.21)
Median	2		2	
Range (IQR)	0-6	(1-3)	0-8	(1-3)

0	19	(16)	136	(16)
1	23	(19)	125	(15)
2	26	(22)	195	(23)
3	25	(21)	161	(19)
≥4	25	(21)	221	(26)
Reproductive Health (History of)				
Tubal ligation	43	(36)	302	(36)
Fibroids	52	(45)	395	(47)
Endometriosis	8	(7)	101	(12)
Ovarian cysts	25	(21)	214	(26)
Previous myomectomy	5	(4)	55	(7)

BMI=Body mass index; IQR=Interquartile range; SD=Standard deviation.

[a] All baseline characteristics are included in the risk prediction model for hysterectomy with the exception of gravidity (nulligravid/gravid) and oral contraceptive use (never/ever). Women reporting never use of oral contraceptives or nulligravidity will be included in the model as 0 years of oral contraceptive use and 0 full-term pregnancies, respectively. Numbers (%) are reported for cases and controls except where noted.

[b] Among women who reported ever use of oral contraceptives.

[c] Among gravid women.

Appendix Table 8. Sensitivity analysis of model performance for the prediction models for hysterectomy with ovarian conservation among premenopausal women where the n=118 observations missing family history of common, benign gynecologic conditions are recoded as no or yes (presence of family history of common, benign gynecologic conditions)

Model	AIC	Neg 2 Log L	AUC	AUC SE
Recoded "No"	946.377	900.377	0.8465	0.0128
Recoded "Yes"	950.292	904.292	0.8452	0.0129

AIC=Akaike's information criteria; AUC= Area under the curve; AUC SE: Standard error of the area under the curve; Neg 2 Log L=Negative 2 Log-Likelihood.

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