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14. ABSTRACT
The objective of this application is to study the etiologic heterogeneity of ovarian cancer in multiple cohorts and to build the infrastructure of the Ovarian Cancer Cohort Consortium (OC3), an international consortium of cohort studies, to address scientific aims important for understanding ovarian cancer risk, early detection, and tumor heterogeneity that are only feasible in a consortium setting. Specifically we will examine associations of risk factors with invasive ovarian cancer, including (but not limited to) age, OCs, tubal ligation, parity, postmenopausal hormone use, family history of ovarian cancer, BMI, height, analgesic use, and lifetime ovulatory cycles, differ by histologic subtype, tumor dominance (as a surrogate for cell of origin), and tumor aggressiveness (tumors fatal within three years vs. all others). Then we will determine if risk prediction models for ovarian cancer can be improved by accounting for differential associations by cancer phenotype. In addition, the proposed efforts will create an infrastructure with a core dataset of important variables for ovarian cancer epidemiology that will be available for future efforts to study ovarian cancer risk, including projects that will use prospectively collected biological specimens. Currently, 27 cohorts have agreed to participate in the OC3, 25 of which have sent data that has been harmonized. We have executed data use agreements between the Brigham and Women's Hospital (data coordinating center) with all studies. Data harmonization is complete for the cohorts for which we have received data. Analyses of primary ovarian cancer risk factors (e.g., oral contraceptive use, parity) by histology are complete and a manuscript is published. A draft manuscript examining risk factors by tumor aggressiveness has been sent to co-authors and on-going analyses are nearly completed for the association by tumor dominance and for the development of a baseline risk prediction model. Several projects have been funded to utilize this resource and additional data collection and variable harmonization are underway, with one manuscript in press, one under review, and one drafted and sent to co-authors.

15. SUBJECT TERMS
Ovarian Cancer, tumor heterogeneity, histology, cell of origin, tumor aggressiveness, risk prediction

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INTRODUCTION

The objective of this translational leverage award is to study the etiologic heterogeneity of ovarian cancer in multiple cohorts and to build the infrastructure of the Ovarian Cancer Cohort Consortium (OC3). The OC3 is an international consortium of cohort studies designed to address scientific aims important for understanding ovarian cancer risk, early detection, and tumor heterogeneity. The OC3 is part of the NCI Cohort Consortium, which is an extramural-intramural partnership to address the need for large-scale collaborations and provides the super-structure (but not funding) for managing the OC3. The OC3 currently has 27 participating, on-going cohort studies and we expect there to be over 8,000 invasive ovarian cancer cases among more than 1.5 million women. The goals of the OC3 are to bring together cohorts with ovarian cancer endpoints for pooled projects, build a focused group of ovarian cancer researchers, and develop a comprehensive approach that integrates questionnaire and pathology data with biomarkers, genetics, and tissue. In addition to building the OC3 infrastructure, we propose to evaluate associations of ovarian cancer risk factors by different metrics of tumor heterogeneity. The first specific aim of this application is to examine whether associations of known and putative ovarian cancer risk factors, including (but not limited to) age, oral contraceptive use, tubal ligation, parity, postmenopausal hormone use, family history of ovarian cancer, body mass index, height, analgesic use, and lifetime ovulatory cycles, differ by (a) histologic subtype, (b) tumor dominance (as a surrogate for cell of origin), and (c) tumor aggressiveness (tumors fatal within three years vs. all others). We will use this data to develop ovarian cancer risk prediction models accounting for differential associations by cancer phenotype.

KEYWORDS

Ovarian Cancer, tumor heterogeneity, histology, cell of origin, tumor aggressiveness, risk prediction

OVERALL PROJECT SUMMARY

This grant began on September 30, 2012. Currently, 27 cohorts have agreed to participate in projects addressing the risk factor associations by tumor heterogeneity and to develop an improved risk prediction model for ovarian cancer. The tasks completed during this grant period included: (1) Collecting questionnaire and tumor data from participating cohorts in the OC3; (2) Evaluate associations of ovarian cancer risk factors with tumor subtypes, tumor fatality and tumor dominance; and (3) Develop risk prediction models using results from Aim 1. Below we discuss the accomplishments within each task as well as challenges.

For Task 1, we invited 29 eligible prospective cohorts, 27 of whom agreed to participate in at least 1 on-going or completed analysis (Table 1). The most challenging aspect of this was setting up appropriate Data Use Agreements (DUA) to ensure that all studies were comfortable with sharing their data. To facilitate this process, we developed a template DUA for studies sending their data and a template DUA for investigators wanting to use the data for approved analyses. As such, we also developed standardized protocols for submitting a proposal and authorship guidelines. These are at: https://sites.google.com/a/channing.harvard.edu/oc3/. We also are a part of the National Cancer Institute Cohort Consortium, which facilitating identifying and inviting cohorts. We then set up a data dictionary and associated questionnaire and set up secure file transfer protocols to receive data; 10 studies completed additional abstraction of pathology reports for grade and tumor dominance. All data harmonization was conducted at the Brigham and Women's Hospital (BWH) data coordinating center (DCC).

Table 1. Details on the OC3 cohorts

Cohort (Acronym)	N[1]	Invasive Cases[2]	Median age	Data available[3]
Adventist Health Study II (AHS2)	46,226	86	54	B
Black Women's Health Study (BWHS)[5]	59,000	122	50	B, F, D
Breast Cancer Detect. Demonstration Proj. (BCDDP)	36,055	145	61	B, FU, D
Breakthrough Generations Study (BGS)	101,881	330	48	B
California Teacher's Study (CTS)	43,782	185	50	B, FU, D
Canadian Study of Diet, Lifestyle, & Health (CSDLH)[4]	39,618	90	58	B, D
Cancer Prevention Study II (CPS2)	65,975	549	62	B, FU, D
Campaign Against Cancer & Heart Disease (CLUEII)	12,393	82	46	B, FU
European Pros. Invest. into Cancer & Nutrition (EPIC)	264,217	704	51	B, D

Iowa Women's Health Study (IWHS)	30,595	268	61	B, FU, D
Melbourne Collab. Cohort Study (MCCS)	23,249	136	55	B, D
Multi-ethnic Cohort Study (MEC)	6,474	75	57	B, FU, D
Netherlands Cohort Study (NCS)[4]	62,573	448	62	B, D
NIH-AARP Diet and Health Study (AARP)	153,084	703	62	B, FU, D
Nurses' Health Study (NHS)	103,298	770	46	B, FU, D
Nurses' Health Study II (NHS2)	111,801	215	35	B, FU, D
NYU Women's Health Study (NYUWHS)	12,431	129	49	B, D
Northern Sweden Health & Disease Study (NSHDS)[5]	43,000	155	55	B, D
Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO)	60,219	363	62	B, FU, D
Shanghai Women's Health Study (SWHS)[5]	74,914	202	60	B, F, T
Singapore Chinese Health Study (SCHS)	31,945	96	56	B, FU, D
Sister Study (SS)	39,196	39	55	B, FU, D
Swedish Mammography Cohort (SMC)	33,418	39	60	B, FU, D
Vitamins and Lifestyle Study (VITAL)	28,331	130	60	B, D
Women's Health Initiative (WHI)	93,676	1032	63	B, F, I, T
Women's Health Study (WHS)	33,548	204	53	B, FU, D
Women's Lifestyle & Health Study (WLHS)	49,087	201	40	B, FU
Total	**1,659,986**	**7,498**		

[1]Eligible for inclusion in our analyses, including having a least one ovary and no baseline cancer; [2]There are 491 borderline cases in addition to invasive disease; [3]B=baseline data; FU=Follow-up questionnaires; D=Diet/food frequency questionnaire; [4]Case-cohort design, numbers show full cohort size; [5]Baseline data not yet sent to DCC.

Data harmonization for the key variables is complete for 24 cohorts from which we have received data. Specifically we have cleaned and harmonized the following variables: ovarian cancer diagnosis characteristics (date/age of diagnosis, date of death, type of tumor, morphology, histology, grade), study enrolment and follow-up data (date/age of enrolment, date/age of death, date/age of last follow-up), race, prior cancer diagnoses, family history of ovarian or breast cancers, menopausal status, postmenopausal hormone use (ever/never, duration, and type), use of oral contraceptives (ever/never, duration), tubal ligation, parity, hysterectomy status, oophorectomy status, age at menarche, age at menopause, smoking, height, body mass index (BMI), BMI at age 18, alcohol intake, endometriosis, other cancer diagnoses, diagnosis of cardiovascular disease, diagnosis of auto-immune disease, diagnosis of diabetes, and NSAIDs. We also have cleaned grade as abstracted from tumor registries or pathology reports; in our initial submission of the histology paper, we were criticized for not examining high and low grade serous tumors separately. To increase power, we abstracted grade from the NHS and NHSII pathology reports (which had not been previously done); in total 17 studies provided grade information. In these studies, among serous tumors, 135 are Grade I, 522 are Grade II, and 1683 are Grade III; 793 have unknown grade. We have developed SAS macros for conducting analyses in a standardized manner, including a macro to meta-analyze results for a particular exposure across studies, one to conduct a pooled analysis, and macros to assess risk factor association heterogeneity by tumor subtype.

With respect to the OC3 structure, we continue to have monthly conference calls run by the PI with the Steering Committee. The calls focus on discussing on-going and future collaborations or projects, and vetting preliminary results. Further, given the number of on-going projects, we have a bi-weekly analysis conference call to discuss data cleaning, next steps, and results. This meeting includes Dr. Elizabeth Poole (a junior faculty member working on the project) and the OC3 programmer. The OC3 has had five in-person meetings since the grant started, including at the 2016 Annual NCI Cohort Consortium Meeting. Our next in-person meeting is in November 2017 at the Cohort Consortium annual meeting. We chose these meeting times because many investigators attend these associated meetings so we have very good attendance.
For task 2, we have completed the analysis for examination of ovarian cancer risk factors by histology, which was published in the Journal of Clinical Oncology. The published manuscript is in Appendix 1 and the details of the analytic approach are outlined there. Briefly, among 1.3 million women from 21 studies, 5,584 invasive epithelial ovarian cancers were identified (3,378 serous, 606 endometrioid, 331 mucinous, 269 clear cell, 1,000

other). By using competing risks Cox proportional hazards regression stratified by study and birth year and adjusted for age, parity, and oral contraceptive use, we assessed associations for all invasive cancers by histology. Heterogeneity was evaluated by likelihood ratio test. Most risk factors exhibited significant heterogeneity by histology. Higher parity was most strongly associated with endometrioid (relative risk [RR] per birth, 0.78; 95% CI, 0.74 to 0.83) and clear cell (RR, 0.68; 95% CI, 0.61 to 0.76) carcinomas (P value for heterogeneity [P-het] , .001). Similarly, age at menopause, endometriosis, and tubal ligation were only associated with endometrioid and clear cell tumors (P-het=0.01). Family history of breast cancer (P-het = .008) had modest heterogeneity. Smoking was associated with an increased risk of mucinous (RR per 20 pack-years, 1.26; 95% CI, 1.08 to 1.46) but a decreased risk of clear cell (RR, 0.72; 95% CI, 0.55 to 0.94) tumors (P-het = .004). Unsupervised clustering by risk factors separated endometrioid, clear cell, and low-grade serous carcinoma as from high-grade serous and mucinous carcinomas. The heterogeneous associations of risk factors with ovarian cancer subtypes emphasize the importance of conducting etiologic studies by ovarian cancer subtypes. Most established risk factors were more strongly associated with nonserous carcinomas, which demonstrate challenges for risk prediction of serous cancers, the most fatal subtype.

With respect to the rapidly fatal study, all analyses are complete and a draft manuscript will be circulated to co-authors shortly (Appendix 2). Among 1.3 million women from 21 studies with sufficient cases with at least 5 years of follow-up, 5,577 invasive epithelial ovarian cancers were identified and classified as very aggressive (death in <1 year, n=816), aggressive (death in 1-<3 years, n=1347), moderately aggressive (death in 3-<5 years, n=618), and less aggressive (lived 5+ years, n=1645). Using competing risks Cox proportional hazards regression stratified by study and birth year and adjusted for age, parity, and oral contraceptive use, we assessed associations by tumor aggressiveness for all invasive cancers and separately among serous and endometrioid/clear cell tumors. Heterogeneity was evaluated by likelihood ratio test with a trend test across the ordinal aggressiveness subtype beta coefficients using meta-regression. Most risk factors did not significant heterogeneity by aggressiveness, overall or for serous or endometrioid/clear cell tumors. Parity had a significantly different association by aggressiveness (p-heterogeneity=0.01), with a stronger inverse association for the first pregnancy (HR=0.72; 95%CI: 0.58,0.88) and no association for subsequent pregnancies (HR=0.97; 95%CI: 0.92,1.02) for very aggressive disease but a similar association for the first and subsequent pregnancies for less aggressive disease (HR=0.87 for both). Both long duration of hormone therapy (p-trend=0.03) and family history of ovarian cancer (p-trend=0.01) had stronger positive associations for less aggressive disease phenotypes. Only very aggressive disease was associated with current versus never smoking (HR=1.14, p-trend=0.005) and body mass index at study entry <20 and ≥35 kg/m^2 compared to 20-<25 (HR=1.36, p-trend=0.06 and 1.93, p-trend=0.0002, respectively). Results were largely similar by histologic subtype. In clustering analysis of risk factor associations, very aggressive and aggressive tumor phenotypes clustered regardless of histology, but otherwise clustered by histology. Our results suggest that risk factor profiles may drive tumor aggressiveness. Additional work to assess biological pathways for these relationships is warranted. The potentially stronger association of a family history of ovarian cancer with less aggressive disease is supported by reports of better survival in BRCA mutation carriers. The BMI association with rapidly fatal disease suggests that metabolic dysfunction may play a role in tumor aggressiveness.

For the manuscript examining associations by tumor dominance, analyses are complete and the manuscript has been drafted (Appendix 3). Laterality of epithelial ovarian tumors may reflect the underlying carcinogenic pathways and origins of tumor cells. Predominantly unilateral ovarian cancers (i.e., dominant) are more likely to originate from the ovarian surface epithelium, whereas bilateral ovarian cancers (i.e., non-dominant) are more like to have a fallopian tube origin. Elucidating the associations with ovarian cancer risk factors by tumor dominance may help understand the mechanisms through which these factors influence ovarian cancer risk. We pooled data from 11 prospective studies participating in the OC3 that had information on measures of tumor size or tumor dominance extracted from surgical pathology reports or obtained through cancer registry. We defined dominant tumors as those restricted to one ovary or having dimension on one ovary at least twice as large as the other, and non-dominant tumors as those with similar dimensions across the two ovaries. Competing risks Cox model was used to examine whether associations with reproductive and hormonal risk factors differed by ovarian tumor dominance. Both parity (p-heterogeneity=0.05) and number of pregnancies (p-heterogeneity=0.002) were more strongly inversely associated with dominant than non-dominant ovarian cancer. The inverse associations were similar for left- and right-dominant tumors. Older age at last birth was

associated with lower risk of dominant tumors but higher risk of non-dominant tumors (p-heterogeneity=0.05). Similarly, longer years since last birth had a positive association with dominant tumors but an inverse association with non-dominant tumors (p-heterogeneity=0.04). Current BMI was associated with significantly increased risk of right-dominant ovarian cancer; no associations were observed for left-dominant or non-dominant tumors (p-heterogeneity=0.04). Although the heterogeneity was not statistically significant, an increased risk associated with endometriosis was observed for dominant tumors but not for non-dominant tumors. These data suggest that reproductive risk factors appear to have a stronger impact on dominant tumors, which may have an ovarian origin.

In addition, progress is being made on task 3, the risk prediction model in the OC3 in collaboration with Dr. Ed Iversen at Duke University. A recent ovarian cancer relative risk prediction model based on data drawn from 11 Ovarian Cancer Association Consortium (OCAC) case-control studies had an area under the curve (AUC) of 0.65 for the core set of ovarian cancer risk factors. We set out to extend this model to predict absolute risk of ovarian cancer and to train and evaluate the resulting model using prospective data from the OC3. To this end, we created a Bayesian hierarchical model for absolute risk of ovarian cancer that allowed for the competing hazards of mortality and diagnosis of other (non-ovarian) cancers. We drew age- and birth-cohort-specific baseline hazards rates and associated error estimates from SEER sources and estimated age-specific incidence of bilateral oophorectomy (BSO) post-baseline using NHANES data. The model is stratified by age (<50, ≥50) and includes as risk factors oral contraceptive duration, family history of breast and ovarian cancer, smoking, endometriosis, age at menarche, tubal ligation, menopausal status, hormone use, body mass index, parity, hysterectomy, breastfeeding, and aspirin. Eight US-based cohorts participating in OC3 provided data on baseline risk factors and diagnosis date of ovarian and other cancers post-baseline. Two studies were reserved for validation; the rest were split 80/20 for development and initial validation. Eligible women had no prior cancer or BSO at baseline; 571,194 women and 3,004 ovarian cancer cases were eligible. Data from the OCAC study were used to construct prior distributions for the ovarian cancer conditional hazards parameters and for risk factor imputation of missing data. We compared risk factor distributions among and between the retrospective (OCAC) and prospective (OC3) data sources and observed relatively little heterogeneity within and across study designs, enhancing confidence in our ability to leverage the retrospective data to improve accuracy of the prospective model's ovarian cancer risk parameter estimates. Indeed, preliminary estimates of ovarian cancer associations were similar between OCAC and OC3, with exception of hysterectomy and hormone use. Since most women were missing data on at least one risk factor and because there were non-random patterns of missingness, we incorporated an ancillary multivariate model for coherent multiple imputation of missing data as part of the risk model. The resulting hierarchical model specifies a joint distribution on outcomes and risk factors. A preliminary run utilizing only 16% of the OC3 data for modeling construction has an all events AUC of 0.57, an AUC for mortality of 0.69, an AUC for other cancer of 0.52 and an AUC for ovarian cancer of 0.52. This is a proof-of-concept model that utilizes a relatively small number of prospective cancer events in its training data set, explaining, at least in part, the modest prospective cancer AUC estimates it provides. We are currently fitting the model using the full 80% training set, which represents five times as many outcomes. This project has presented particular challenges because no one study had asked all of the variables in the model. This precluded a complete data approach to the analysis and required imputation. However, because we had to use other studies to impute data in studies that were missing by design (i.e., did not ask a specific question), the modeling was very complex and computationally intensive. Unfortunately the computer resources at the DCC were insufficient to run the programs and thus we had to update multiple DUAs to allow cohort data to be transferred to the Duke supercomputing clusters. After validation of all invasive disease, we will assess serous and endometrioid/clear cell tumors separately.

One of the key goals of the OC3 is to foster collaborations and use of the data nationally and internationally. A list of approved and proposed projects is in Table 2. Importantly, the OC3 is a highly sought after resource. Twenty projects have been approved to date from 15 different investigators from 11 institutions. Published papers on androgens (Appendix 4) and IGF-1 (Appendix 5) levels and risk have been published and a manuscript on analgesic use has been drafted (Appendix 6); results of other ongoing projects are in Appendix 7.

Table 2. Projects proposed and on-going in the OC3.

Project	Proposed by	Institution	Status
Androgens and risk	Fortner	German Cancer Research Center (DKFZ)	Approved; manuscript published
IGFs and risk	Fortner	DKFZ	Approved; manuscript published
NSAIDs and risk	Trabert	National Cancer Institute (NCI)	Approved; manuscript sent to co-authors
Endometriosis and risk	Wentzensen, Trabert	NCI	Approved; results incorporated into primary histology paper
CRP/inflammatory factors and risk	Tworoger	BWH	Approved; resubmitting R01, Feb 2018
Diabetes and risk	Gapster, Harris	American Cancer Society/ Fred Hutchinson Cancer Research Center	Approved; R03 submitted Feb 2017
OncoArray (GWAS)	Wentzensen, Tworoger	NCI/BWH	Approved; data cleaning on-going
Risk factors by anatomic sites	Schouten	Univ. of Maastricht	Approved; manuscript being drafted
Proportion of subtype associations explained (methods paper)	Poole, Wentzensen	BWH/NCI	Approved; developing statistical approaches
Hypertension and risk	Huang	BWH	Approved; awaiting new data collection
Exposure-wide association study of high-grade serous tumors	Poole	BWH	Approved; awaiting new data collection
Lifecourse adiposity and risk	Fortner, Tworoger	DKFZ/BWH	Approved; awaiting new data collection
Factors associated with long-term survival	Sood	MD Anderson	Approved; P01 submitted Sept. 2016
Telomeres in tumor tissue and survival	Visvanathan	Johns Hopkins	Approved; submitting R01, October 2017
Lifetime ovulatory cycles and risk	Trabert	NCI	Approved; analyses on-going
Talc and risk	Sandler	NIEHS	Approved; permissions being requested
Caffeine/coffee/tea and risk	Harris	Fred Hutchinson Cancer Research Center	Approved; setting up collaboration with the Diet and Cancer Pooling Project
Immuno-proteomics for early detection - tumor-associated Abs (Taabs)	Kaaks	DKFZ	Approved; submitting R01, Feb. 2017
Epigenetics of ovarian cancer	Flanagan	Imperial College London	Approved; submitted European grant Mar. 2017
CARRIERS project of high risk alleles	Couch	Mayo Clinic	Approved; sequencing of cases and controls on-going

KEY RESEARCH ACCOMPLISHMENTS

Below is a list of key research accomplishments/findings during this award.

- Of the 14 established or putative risk factors we examined for ovarian cancer by histologic subtype, 10 risk factors had significant heterogeneity across subtypes. Despite having the smallest number of cases,

every reproductive/hormonal factor was significantly associated with clear cell tumors, except breastfeeding.

- While endometrioid and clear cell carcinomas had qualitatively similar associations for most risk factors (parity, OC use, age at menopause, tubal ligation, endometriosis, height, family history of ovarian cancer, breastfeeding), they differed in associations related to HT use (which went in opposite directions), family history of breast cancer and BMI (associated with endometrioid only), as well as age at menarche, hysterectomy, and smoking (associated with clear cell only).
- Serous and poorly differentiated carcinomas, the most common and aggressive subtype, had only modest associations for parity, OC use, menopausal HT use, and family history of breast cancer, and stronger associations with family history of ovarian cancer. Further HT use was most strongly associated with low-grade serous tumors. Overall, very few strong risk factors are known for high-grade serous tumors.
- Further, supporting the need to examine associations by histology, androgen levels were only positively associated with endometrioid and mucinous tumors, but not serous or clear cell tumors.
- In unexpected findings, IGF-1 was inversely associated with ovarian cancer risk across all subtypes.
- Most reproductive risk factors were associated preferentially with reducing risk of less aggressive disease, but not rapidly fatal tumors. However, lifestyle factors, such as BMI and smoking, were associated with an increased risk of rapidly fatal tumors, although this association varied by histologic type. This suggests that examining multiple tumor characteristics simultaneously may provide additional etiologic insight.
- Reproductive factors were most strongly associated with dominant tumors, that likely have an ovarian origin. This is consistent with the findings by histology.
- Current ovarian cancer risk factors do not have strong predictive capability for identifying specific women at high risk of ovarian cancer, although the AUC is higher for younger women. Given that serous is the most common subtype, but has the least risk factors, it will be critical to identify new risk factors for this type to increase predictive capacity.

CONCLUSION

We are continuing to develop and utilize the OC3 infrastructure by pooling existing cohort data to better elucidate the biology of ovarian cancer. Scientifically, we have evaluated whether associations for putative ovarian cancer risk factors differ by tumor subtypes (histology, cell of origin, aggressiveness), as well as developed risk prediction models overall and now by subtype. This will be beneficial to the entire ovarian cancer research community. Importantly we observed that most established or putative ovarian cancer risk factors showed heterogeneity across tumor subtypes, across multiple metrics to assess tumor heterogeneity, including histology, tumor aggressiveness, and tumor dominance as a surrogate for cell of origin. Notably, all all subtypes had unique patterns of risk factor associations and clustering analysis suggested that aggressive tumors clustered together regardless of histology, while histology was the driving clustering factor for less aggressive disease. Notably, endometrioid and clear cell tumors (which often present as dominant tumors) as well as less aggressive disease phenotypes had the strongest associations for many risk factors, and relatively few associations were observed for high-grade serous as well as highly aggressive tumors, which are the most common types of ovarian cancer. This suggests that risk prediction models of ovarian cancer overall will perform worse for serous tumors than for other types. Further, results strongly suggest that considering multiple facets of tumor heterogeneity adds biologic information that may be useful for prevention.

Our results support that pre-diagnostic factors may influence ovarian cancer development and aggressiveness and that considering multiple tumor characteristics simultaneously may provide a clearer picture of disease etiology. Ultimately, understanding a woman's risk profile with respect to risk of rapidly fatal versus less aggressive disease at diagnosis may aid in determining the most optimal treatment strategy for long term survival. This has several important implications for etiology and prevention of ovarian cancers. The substantial heterogeneity of individual risk factor associations across ovarian cancer subtypes supports the notion that the subtypes are indeed different diseases and that we may need to consider multiple tumor characterizations to adequately stratify tumors. This underscores the importance of evaluating risk factor and biomarkers associations in consortium settings where there is adequate sample size to provide power to assess associations for the more rare tumor types. The research also suggests that we need to identify new

epidemiologic risk factors for serous tumors as the traditional factors are generally most strongly related to endemetrioid and clear cell tumors. Given the higher incidence of serous cancer and its poor survival rates, this is a critical area of future research.

This systematic approach to address ovarian cancer heterogeneity in a large consortial effort will set new standards for evaluating ovarian cancer risk factors and biomarkers and thereby impact understanding of ovarian cancer etiology beyond the work conducted in OC3. Importantly our goal is to continue to expand the data repository of the OC3 by obtaining funding to include dietary factors, updated exposure data from follow-up questionnaires, and biomarker information (both plasma/serum markers and genetics). We also have a grant under review to conduct survival analyses. With 20 projects already proposed in the OC3, the development of OC3 infrastructure will have substantial impact on prevention research in the years to come.

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Two abstracts were accepted as presentations (presenter is bolded):

1. **Elizabeth M. Poole**, Alan A. Arslan, Lesley M. Butler, James V. Lacey, Jr., I-Min Lee, Alpa V. Patel, Kim Robien, Dale P. Sandler, Leo J. Schouten, V. Wendy Setiawan, Kala Visvanathan, Elisabete Weiderpass, Emily White, Nicolas Wentzensen, Shelley S. Tworoger. Ovarian cancer risk factors by histologic type in the Ovarian Cancer Cohort Consortium (OC3). Presented at the 2014 Annual Meeting of the Society for Epidemiologic Research, June 2014, Seattle, WA.
2. **Shelley S. Tworoger**, Elizabeth M. Poole, Alan A. Arslan, Lesley M. Butler, Victoria Kirsh, James V. Lacey, Jr., I-Min Lee, Alpa V. Patel, Kim Robien, Thomas Rohan, Dale P. Sandler, Leo J. Schouten, V.

Wendy Setiawan, Kala Visvanathan, Elisabete Weiderpass, Emily White, Nicolas Wentzensen. Ovarian cancer risk factor associations by tumor aggressiveness in the Ovarian Cancer Cohort Consortium (OC3). Presented at the 10th Biennial Ovarian Cancer Research Symposium sponsored by AACR and the Marsha Rivkin Center for Ovarian Cancer Research, September 2014, Seattle, WA.

Five invited presentations to conferences:

1. Shelley S. Tworoger. The Ovarian Cancer Cohort Consortium (OC3). The 16th Ovarian Cancer Association Consortium Meeting (April 2014).
2. Elizabeth M. Poole. Ovarian cancer risk factors by histologic type in the Ovarian Cancer Cohort Consortium (OC3). Presented at the Society for Epidemiologic Research Annual Meeting (June 2015).
3. Shelley S. Tworoger. Thinking outside the box: New areas in prevention research. Presented at the AACR Advances in Ovarian Cancer Research: Exploiting Vulnerabilities (October 2015).
4. Shelley S. Tworoger, Nicolas Wentzensen. Developing a resource for ovarian cancer research: The OC3. 15th Annual Meeting of the NCI Cohort Consortium (November 2015).
5. Shelley S. Tworoger. Integrating epidemiologic information on heterogeneity into understanding cancer etiology. AACR Annual Meeting (April 2016).

Four poster presentations:

1. Nicolas Wentzensen, Elizabeth M. Poole, Alan Arslan, Alpa Patel, V. Wendy Setiawan, Kala Visvanathan, Elisabete Weiderpass, Emily White, Hans-Olov Adami, Louise A. Brinton, Julie Buring, Lesley M. Butler, Tess V. Clendenen, Renee Fortner, Susan M. Gapstur, Mia Gaudet, Patricia Hartge, Judith Hoffman-Bolton, Michael Jones, Vicki Kirsh, Woon-Puay Koh, James V. Lacey, Jr., I-Min Lee, Ulrike Peters, Jenny Poynter, Kim Robien, Thomas Rohan, Dale P. Sandler, Leo J. Schouten, Louise Sjohölm, Anthony Swerdlow, Britton Trabert, Lynne Wilkens, Alicja Wolk, Hannah P. Yang, Anne Zeleniuch-Jacquotte, Shelley S. Tworoger. Ovarian cancer risk factors by histologic subtypes: Evidence for etiologic heterogeneity. AACR Annual Meeting 2015 (Philadelphia, PA).
2. Britton Trabert, Elizabeth M. Poole, Renée T. Fortner, Kala Visvanathan, Nicolas Wentzensen, Shelley S. Tworoger, on behalf of the Ovarian Cancer Cohort Consortium (OC3). Aspirin use and ovarian cancer risk: an analysis in the Ovarian Cancer Cohort Consortium (OC3). Rivkin Center for Ovarian Cancer Bi-Annual Meeting 2016 (Seattle, WA).
3. Elizabeth M. Poole, Britton L. Trabert, Renée T. Fortner, Nico Wentzensen, and Shelley S. Tworoger on behalf of the OC3. C-reactive protein and ovarian cancer risk: Preliminary results from the Ovarian Cancer Cohort Consortium (OC3). NCI Cohort Consortium Annual Meeting 2016 (Bethesda, MD).
4. Edwin Iversen, Elizabeth Poole, Nicolas Wentzensen, Merlise Clyde, Britton Trabert, Joellen Schildkraut, Shelley Tworoger for the Ovarian Cancer Cohort Consortium. A prospective risk prediction model for ovarian cancer: the Ovarian Cancer Cohort Consortium. Society for Epidemiologic Research Annual Meeting 2016 (Seattle, WA).

INVENTIONS, PATENTS, AND LICENCES

None.

REPORTABLE OUTCOMES

The primary reportable outcome is the development of the OC3 database, which contains data on ovarian cancer risk factors and outcomes from 24 cohort studies with 3 more studies agreeing to participate pending new funds. This resource can be used for the analyses proposed in this grant as well as other analyses.

OTHER ACHIEVEMENTS

None.

REFERENCES

None.

APPENDICES

Appendix 1: Published manuscript on ovarian cancer risk factor associations by histology

Appendix 2: Draft manuscript on ovarian cancer risk factor associations by tumor aggressiveness
Appendix 3: Draft manuscript on ovarian cancer risk factor associations by tumor dominance
Appendix 4: Published manuscript on androgens and risk of ovarian cancer
Appendix 5: Published manuscript on IGF-1 levels and risk of ovarian cancer
Appendix 6: Draft manuscript on aspirin, non-aspirin NSAIDs, and Tylenol with ovarian cancer risk
Appendix 7: Results for other projects in the OC3

Appendix 1: Published manuscript “Ovarian cancer risk factors by histologic subtype: An analysis from the Ovarian Cancer Cohort Consortium”

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ORIGINAL REPORT

Ovarian Cancer Risk Factors by Histologic Subtype: An Analysis From the Ovarian Cancer Cohort Consortium

Nicolas Wentzensen, Elizabeth M. Poole, Britton Trabert, Emily White, Alan A. Arslan, Alpa V. Patel, V. Wendy Setiawan, Kala Visvanathan, Elisabete Weiderpass, Hans-Olov Adami, Amanda Black, Leslie Bernstein, Louise A. Brinton, Julie Buring, Lesley M. Butler, Saioa Chamosa, Tess V. Clendenen, Laure Dossus, Renee Fortner, Susan M. Gapstur, Mia M. Gaudet, Inger T. Gram, Patricia Hartge, Judith Hoffman-Bolton, Annika Idahl, Michael Jones, Rudolf Kaaks, Victoria Kirsh, Woon-Puay Koh, James V. Lacey Jr, I-Min Lee, Eva Lundin, Melissa A. Merritt, N. Charlotte Onland-Moret, Ulrike Peters, Jenny N. Poynter, Sabina Rinaldi, Kim Robien, Thomas Rohan, Dale P. Sandler, Catherine Schairer, Leo J. Schouten, Louise K. Sjöholm, Sabina Sieri, Anthony Swerdlow, Anna Tjonneland, Ruth Travis, Antonia Trichopoulou, Piet A. van den Brandt, Lynne Wilkens, Alicja Wolk, Hannah P. Yang, Anne Zeleniuch-Jacquotte, and Shelley S. Tworoger

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ABSTRACT

Purpose

An understanding of the etiologic heterogeneity of ovarian cancer is important for improving prevention, early detection, and therapeutic approaches. We evaluated 14 hormonal, reproductive, and lifestyle factors by histologic subtype in the Ovarian Cancer Cohort Consortium (OC3).

Patients and Methods

Among 1.3 million women from 21 studies, 5,584 invasive epithelial ovarian cancers were identified (3,378 serous, 606 endometrioid, 331 mucinous, 269 clear cell, 1,000 other). By using competing-risks Cox proportional hazards regression stratified by study and birth year and adjusted for age, parity, and oral contraceptive use, we assessed associations for all invasive cancers by histology. Heterogeneity was evaluated by likelihood ratio test.

Results

Most risk factors exhibited significant heterogeneity by histology. Higher parity was most strongly associated with endometrioid (relative risk [RR] per birth, 0.78; 95% CI, 0.74 to 0.83) and clear cell (RR, 0.68; 95% CI, 0.61 to 0.76) carcinomas (*P* value for heterogeneity [*P*-het] $< .001$). Similarly, age at menopause, endometriosis, and tubal ligation were only associated with endometrioid and clear cell tumors (*P*-het $\leq .01$). Family history of breast cancer (*P*-het = .008) had modest heterogeneity. Smoking was associated with an increased risk of mucinous (RR per 20 pack-years, 1.26; 95% CI, 1.08 to 1.46) but a decreased risk of clear cell (RR, 0.72; 95% CI, 0.55 to 0.94) tumors (*P*-het = .004). Unsupervised clustering by risk factors separated endometrioid, clear cell, and low-grade serous carcinomas from high-grade serous and mucinous carcinomas.

Conclusion

The heterogeneous associations of risk factors with ovarian cancer subtypes emphasize the importance of conducting etiologic studies by ovarian cancer subtypes. Most established risk factors were more strongly associated with nonserous carcinomas, which demonstrate challenges for risk prediction of serous cancers, the most fatal subtype.

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INTRODUCTION

Ovarian cancer is the most lethal gynecologic cancer, with > 152,000 deaths worldwide each year.[1] Most ovarian cancers are detected at a late stage and have a poor prognosis. Screening for ovarian cancer did not reduce mortality in two large screening trials.[2,3] An understanding of the etiologic heterogeneity of ovarian cancer is critical for development of new prevention strategies.

Although multiple carcinogenic mechanisms for ovarian tumorigenesis have been hypothesized, including incessant ovulation, hormonal stimulation, and chronic inflammation,[4-7] the etiology of ovarian cancer is not well understood partly due to its heterogeneous nature. Disease

subtypes have been categorized by putative precursor lesions, mutations, and histology.[8,9] Low-grade serous, mucinous, clear cell, and endometrioid tumors are believed to arise from inclusion cysts or implants in the ovarian surface epithelium and have *KRAS*, *BRAF*, or *PTEN* mutations. High-grade serous tumors, characterized by *TP53* mutations, are believed to arise in the fallopian tube or ovarian epithelium, are more aggressive, and have poorer outcomes than other types.[8-10] Due to limited power, individual epidemiologic and biomarker studies usually have considered risk factor associations for all ovarian tumors together. Individual cohorts and individual-level meta-analyses of primarily case-control studies have reported differential associations by subtype for menopausal hormone therapy (HT) use, oral contraceptive (OC) use, parity, smoking, and body mass index (BMI).[11-17] To establish etiologic models that account for ovarian cancer heterogeneity, a unified prospective evaluation of multiple ovarian cancer risk factors needs to account for heterogeneity. In the Ovarian Cancer Cohort Consortium (OC3), we evaluated associations of 14 key risk factors with invasive epithelial ovarian cancer risk overall and by histologic subtype based on pooled individual-level data from 5,584 invasive ovarian cancer cases from a combined cohort of > 1.3 million women enrolled in 21 prospective studies.

PATIENTS AND METHODS

Study Population

The analysis included women participating in 21 prospective cohort studies from North America, Asia, and Europe (Table 1). Prospective follow-up of ovarian cancer end points through questionnaires, medical records, or cancer registries as well as follow-up for death were required for participation. Minimal required information included age at study entry, OC use, and parity. All studies obtained institutional approval for cohort maintenance as well as participation in the OC3. The OC3 data coordinating center and analytic approaches were approved by the institutional review board of the Brigham and Women's Hospital (Boston, MA).

Exposure Definitions

Full baseline cohort data (19 studies) or case-cohort data sets with weights for subcohort members (two studies) were harmonized centrally. Exposures included parity (ever versus never; number of births: per one birth and one, two, three, four or more births), OC use (ever versus never; duration of use: per 5 years of use and never, ≤ 1, > 1 to 5, > 5 to 10, > 10 years), duration of breastfeeding (per 1 year among parous women), age at menarche (per 1 year and ≤ 11, 12, 13, 14, ≥ 15 years), age at natural menopause (postmenopausal women only: per 5 years and ≤ 40, > 40 to 45, > 45 to 50, > 50 to 55, > 55 years), menopausal HT use (ever versus never; duration of use: per 1 year and never, ≤ 5,

Table 1. Characteristics of Cohorts Participating in the Ovarian Cancer Cohort Consortium

Study Name	Study Acronym	Location	Baseline Enrollment Period	Baseline Cohort Size*	Median Study Participant Age (years)	Median Follow-Up (years)	Last Year of Follow-Up	Invasive Ovarian Cancer Cases
NIH-AARP Diet and Health Study	AARP	US	1995-1997	153,069	62	11	2006	703
Breast Cancer Detection Demonstration Project Follow-Up Study	BCDDP	US	1987-1989	36,212	61	9	1999	159
Breakthrough Generations Study	BGS	UK	2001-2014	101,869	48	6	2014	75
Canadian Study of Diet, Lifestyle, and Health	CSDLH	Canada	1991-1999	2,745†	58	16	2010	90
Campaign Against Cancer and Stroke	CLUEII	US	1989	12,382	46	22	2012	82
Cancer Prevention Study II Nutrition Cohort	CPSII-NC	US	1992-1993	65,884	62	15	2009	533
California Teachers Study	CTS	US	1995-1999	43,778	50	15	2010	185
European Prospective Investigation Into Cancer and Nutrition Study	EPIC	Europe	1992-2000	263,796	51	13	2010	671
Iowa Women's Health Study	IWHS	US	1986	30,537	61	23	2010	263
Multiethnic/Minority Cohort Study‡	MEC	US	1993-1998	16,474	57	11	2011	75
Nurses' Health Study 1980§	NHS80	US	1980-1982	86,608	46	16	1998	351
Nurses' Health Study 1996§	NHS96	US	1996-1998	67,530	62	14	2010	417
Nurses' Health Study II	NHSII	US	1989-1990	111,800	35	20	2011	215
New York University Women's Health Study	NYU	US	1984-1991	12,427	49	24	2012	129
Netherlands Cohort Study on Diet and Cancer	NLCS	Netherlands	1986	2,757†	62	17	2003	448
Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial	PLCO	US	1993-2002	60,191	62	12	2009	358
Singapore Chinese Health Study	SCHS	Singapore	1993-1999	31,939	56	14	2011	95
Sister Study	SS	US	2003-2009	39,195	55	5	2012	39
Swedish Mammography Cohort Study	SMC	Sweden	1997	34,427	60	14	2011	161
Vitamins and Lifestyle Cohort	VITAL	US	2000-2002	28,331	60	10	2011	130
Women's Lifestyle and Health Study	WLHS	Sweden	1991-1992	49,087	40	21	2012	201
Women's Health Study	WHS	US	1993-1996	33,548	53	18	2012	204

Abbreviation: NIH, National Institutes of Health.
*After exclusions for baseline cancers and women with bilateral oophorectomy.
†These cohorts were included as a case-cohort design, which reflected a total cohort population of 39,618 women for the CSDLH and 62,573 women for the NLCS. Appropriate weights for subcohort selection were applied in all analyses.
‡Included only white women.
§The Nurses' Health Study was broken into two study periods (1980 to June 1996 and July 1996 to 2010) because the follow-up was nearly twice as long as any other study. We updated the exposures in 1996 for that follow-up period.

> 5 years), tubal ligation (ever versus never), hysterectomy (ever versus never), endometriosis (ever versus never), first-degree family history of breast cancer (ever versus never), first-degree family history of ovarian cancer (ever versus never), BMI (per 5 kg/m^2 and < 20, 20 to < 25, 25 to < 30, 30 to < 35, ≥ 35 kg/m^2), height (per 0.05 m and < 1.60, 1.60 to < 1.65, 1.65 to 1.70, ≥ 1.70 m), and smoking (ever versus never; per 20 pack-years and ≤ 10, > 10 to 20, > 20 to 35, > 35 pack-years). Studies that did not collect information on a specific risk factor were excluded from the analysis of that factor (Appendix Table A1, online only), which led to different samples sizes for each variable (Appendix Table A2, online only).

Outcome Definitions

Epithelial ovarian or peritoneal cancer cases were confirmed through cancer registries or medical record review (International Classification of Diseases [9th revision codes 183 and 158 and 10th revision code C56]). Ascertainment of incident cancers was ≥ 90% for all studies and ≥ 95% for 17 studies. We evaluated associations of risk factors with all invasive epithelial cancers combined (n = 5,584). Next, we evaluated associations with the four most common histologic types of invasive epithelial ovarian cancers (n = 4,584): serous/poorly differentiated, endometrioid, mucinous, and clear cell. One thousand cases had another histology or were missing histology information. Serous tumors were further divided by grade (well-, moderately, or poorly differentiated or unknown).

Statistical Methods

Women with a history of cancer (other than nonmelanoma skin cancer), with bilateral oophorectomy before study entry, or with missing age at baseline were excluded. We calculated hazard ratios (HRs) and 95% CIs by using competing-risks Cox proportional hazards regression to evaluate associations between exposures and ovarian cancer end points.[18] Follow-up time was time between study entry and date of ovarian cancer diagnosis, death, or end of follow-up, whichever occurred first. Survivor function plots for exposures showed parallel curves, which suggest no relevant deviation from proportional hazards. In primary analyses, we pooled data from all cohorts and stratified by year of birth and cohort to account for potential differences in baseline hazards by these factors. Statistical heterogeneity of associations across subtypes was assessed through a likelihood ratio test that compared a model that allowed for the association for the risk factor of interest to vary by histology versus one that did not allow for the association to vary.[16] We also used random-effects meta-analysis to combine cohort-specific estimates and to assess between-study heterogeneity. All models were adjusted for age at entry, number of children, and duration of OC use, unless the exposure of interest was collinear with one of these factors. Hysterectomy analyses were also adjusted for HT use. For missing data in covariates, we included a missing indicator in the model. The Sister Study was excluded from analyses of family history because all participants had a family history of breast or ovarian cancer. To evaluate whether our primary models sufficiently accounted for confounding, we performed a model that adjusted for all exposures together (by using missing indicators when needed). In 17 studies, grade was available for at least some serous cases. We conducted similar analyses among serous tumors by comparing risk factors for well-, moderately, and poorly differentiated tumors and unknown grade. We performed unsupervised hierarchical clustering of the four subtypes (with and without separating serous tumors by grade) with β-estimates for all exposures, except duration of breastfeeding (not significantly associated with any of the four subtypes), by using complete linkage and uncentered correlation (Pearson coefficient). SAS 9.3 software (SAS Institute, Cary, NC) was used to conduct the analyses. $P < .05$ was considered statistically significant. As a sensitivity analysis, we corrected for multiple comparisons for the test of heterogeneity by using an adjusted α of .004 (.05/13 exposures).

RESULTS

Study Population

Among 1,284,586 participants (1,381,275 with the inclusion of full cohort size for case-cohort studies), 5,584 invasive epithelial ovarian cancers were identified during follow-up. Case numbers ranged from 1,281 for breastfeeding to 5,523 for OC use (Appendix Table A2). There were 3,378 (73.7% of cases with known histology) serous, 606 (13.2%) endometrioid, 331 (7.2%) mucinous, and 269 (5.9%) clear cell carcinomas. Fifteen of 21 cohorts were based in North America, five in Europe, and one in Asia (Table 1); approximately one half of the cohorts started enrollment in the 1990s. The median age at diagnosis was 67.0 years for serous, 63.0 years for endometrioid, 64.0 years for mucinous, and 61.3 years for clear cell carcinomas and 68.9 years for cases of unknown histology.

Associations of Hormonal and Reproductive Factors

Most reproductive and hormonal risk factors, except for breastfeeding, were associated with ovarian cancer risk overall (Table 2). Parous versus nulliparous women had a reduced risk of all ovarian cancer subtypes, with significant heterogeneity by subtype (P value for heterogeneity [P-het] < .001). The strongest risk reduction was observed for clear cell carcinomas (relative risk [RR], 0.35; 95% CI, 0.27 to 0.47), whereas serous cancers had the least risk reduction (RR, 0.81; 95% CI, 0.73 to 0.90). Similar patterns were observed for number of children (P-het < .001). In subtype-specific analyses, a 5-year increase in duration of OC use was associated with a significant 14% to 15% lower risk of serous, endometrioid, and clear cell carcinomas but not with mucinous tumors (P-het = .04). Similarly, OC use for > 10 years was associated with a 36% to 49% reduction in risk for serous, endometrioid, and clear cell tumors.

A 5-year later menopause was associated with endometrioid and clear cell carcinomas (RR, 1.19 [95% CI, 1.05 to 1.34] and 1.37 [95% CI, 1.15 to 1.64], respectively), with no association for serous and mucinous carcinomas (P-het = .009). A 5-year increase in menopausal HT use was associated with an increased risk of serous (RR, 1.21; 95% CI, 1.17 to 1.25) and endometrioid (RR, 1.25; 95% CI, 1.15 to 1.36) carcinomas but a reduced risk of clear cell carcinoma (RR, 0.69; 95% CI, 0.52 to 0.92; P-het < .001). Tubal ligation was only associated with reduced risk of endometrioid (RR, 0.60; 95% CI, 0.41 to 0.88) and clear cell (RR, 0.35; 95% CI, 0.18 to 0.69; P-het < .001) carcinomas, whereas hysterectomy was associated with decreased risk of clear cell carcinomas (RR, 0.57; 95% CI, 0.36 to 0.88; P-het = .006). Self-reported endometriosis was significantly associated only with endometrioid (RR, 2.32; 95% CI, 1.36 to 3.95) and clear cell (RR, 2.87; 95% CI, 1.53 to 5.39; P-het = .01) carcinomas. There was no significant heterogeneity in associations by histology for breastfeeding or age at menarche, although the latter was significantly inversely associated with clear cell carcinomas.

Associations of Other Risk Factors

Height and family history of both breast and ovarian cancer, but not smoking or BMI, were significantly associated with ovarian cancer risk overall (Table 3). A first-degree family history of breast

Table 2. Associations of Hormonal and Reproductive Factors With Invasive Epithelial Ovarian Cancer Overall and by Subtypes in the Ovarian Cancer Cohort Consortium

Exposure	All Invasive RR (95% CI)	Serous RR (95% CI)	Endometrioid RR (95% CI)	Mucinous RR (95% CI)	Clear Cell RR (95% CI)	*P*-het (between histologic types)*
No. of patients	5,584	3,378	606	331	269	
Parity						
Ever/never	0.69 (0.64 to 0.74)	0.81 (0.73 to 0.90)	0.48 (0.39 to 0.58)	0.56 (0.42 to 0.74)	0.35 (0.27 to 0.47)	< .001†
No. of children, per one child	0.90 (0.89 to 0.92)	0.93 (0.92 to 0.95)	0.78 (0.74 to 0.83)	0.91 (0.84 to 0.99)	0.68 (0.61 to 0.76)	< .001†
No. of children						
0	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	< .001†
1	0.82 (0.43 to 0.91)	0.86 (0.75 to 1.00)	0.78 (0.60 to 1.03)	0.59 (0.38 to 0.92)	0.67 (0.46 to 0.98)	
2	0.74 (0.68 to 0.81)	0.87 (0.78 to 0.97)	0.49 (0.39 to 0.62)	0.61 (0.44 to 0.86)	0.38 (0.27 to 0.53)	
3	0.67 (0.62 to 0.74)	0.82 (0.73 to 0.92)	0.41 (0.32 to 0.54)	0.52 (0.36 to 0.74)	0.29 (0.19 to 0.43)	
≥ 4	0.58 (0.53 to 0.64)	0.72 (0.63 to 0.81)	0.34 (0.25 to 0.45)	0.55 (0.38 to 0.80)	0.14 (0.08 to 0.25)	
Oral contraceptive use						
Ever/never	0.84 (0.79 to 0.89)	0.82 (0.76 to 0.89)	0.89 (0.73 to 1.07)	1.02 (0.80 to 1.31)	0.72 (0.55 to 0.94)	.25
Duration of use, per 5-year increase	0.87 (0.84 to 0.90)	0.85 (0.81 to 0.89)	0.86 (0.77 to 0.95)	1.54 (0.93 to 1.19)	0.86 (0.74 to 1.00)	.04
Duration of use, years						
Never	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	.35
≤ 1	0.98 (0.87 to 1.05)	0.99 (0.88 to 1.12)	1.01 (0.76 to 1.35)	0.98 (0.66 to 1.45)	0.68 (0.42 to 1.09)	
> 1-5	0.86 (0.78 to 0.92)	0.85 (0.77 to 0.95)	0.82 (0.64 to 1.05)	0.84 (0.58 to 1.21)	0.88 (0.62 to 1.24)	
> 5-10	0.77 (0.67 to 0.84)	0.72 (0.64 to 0.83)	0.85 (0.64 to 1.13)	0.91 (0.61 to 1.37)	0.80 (0.54 to 1.20)	
> 10	0.67 (0.58 to 0.75)	0.64 (0.54 to 0.74)	0.64 (0.44 to 0.93)	1.18 (0.77 to 1.81)	0.51 (0.29 to 0.87)	
Duration of breastfeeding, per 1 year‡	0.96 (0.89 to 1.03)	0.94 (0.86 to 1.03)	0.85 (0.69 to 1.05)	0.88 (0.63 to 1.23)	1.03 (0.81 to 1.33)	.64
Age at menarche						
Per 1-year increase	0.99 (0.97 to 1.00)	0.99 (0.97 to 1.02)	1.00 (0.94 to 1.05)	1.00 (0.93 to 1.07)	0.92 (0.85 to 0.99)	.31
Age, years						
≤ 11	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference.)	.66
12	0.92 (0.84 to 1.00)	0.95 (0.85 to 1.05)	1.02 (0.80 to 1.31)	1.15 (0.81 to 1.65)	0.78 (0.54 to 1.12)	
13	0.94 (0.87 to 1.02)	0.99 (0.90 to 1.09)	0.97 (0.79 to 1.22)	1.06 (0.76 to 1.48)	0.79 (0.56 to 1.11)	
14	0.93 (0.85 to 1.03)	0.99 (0.88 to 1.12)	0.84 (0.62 to 1.13)	0.97 (0.64 to 1.47)	0.80 (0.52 to 1.23)	
≥ 15	0.88 (0.80 to 0.97)	0.92 (0.81 to 1.05)	0.98 (0.73 to 1.31)	1.13 (0.76 to 1.66)	0.55 (0.34 to 0.90)	
Age at menopause§						
Per 5-year increase	1.06 (1.02 to 1.10)	1.05 (1.01 to 1.10)	1.19 (1.05 to 1.34)	0.95 (0.81 to 1.11)	1.37 (1.15 to 1.64)	.009
Age, years						
≤ 40	0.89 (0.77 to 1.03)	0.87 (0.73 to 1.04)	0.59 (0.34 to 1.00)	1.31 (0.78 to 2.20)	0.15 (0.03 to 0.71)	.11
> 40-45	0.80 (0.70 to 0.91)	0.85 (0.73 to 1.00)	0.76 (0.51 to 1.14)	0.77 (0.44 to 1.33)	0.43 (0.20 to 0.94)	
> 45-50	0.93 (0.86 to 1.00)	0.96 (0.87 to 1.06)	0.86 (0.67 to 1.09)	0.95 (0.68 to 1.31)	0.95 (0.64 to 1.39)	
> 50-55	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	
> 55	1.02 (0.89 to 1.17)	1.01 (0.85 to 1.21)	1.19 (0.78 to 1.80)	0.91 (0.49 to 1.68)	1.03 (0.50 to 2.09)	
Hormone therapy use§						
Ever/never	1.36 (1.28 to 1.46)	1.41 (1.30 to 1.53)	1.67 (1.36 to 2.05)	1.00 (0.75 to 1.34)	0.90 (0.64 to 1.28)	.004
Duration of use, per 5-year increase	1.20 (1.16 to 1.23)	1.21 (1.17 to 1.25)	1.25 (1.15 to 1.36)	1.09 (0.94 to 1.26)	0.69 (0.52 to 0.92)	< .001†
Duration of use, years						
Never	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	< .001†
≤ 5	1.17 (1.07 to 1.27)	1.22 (1.09 to 1.36)	1.46 (1.11 to 1.91)	1.13 (0.78 to 1.63)	0.94 (0.61 to 1.44)	
> 5	1.60 (1.47 to 1.74)	1.75 (1.58 to 1.94)	1.90 (1.44 to 2.51)	1.06 (0.69 to 1.65)	0.51 (0.27 to 0.96)	
Tubal ligation, ever/never	0.82 (0.73 to 0.93)	0.91 (0.79 to 1.06)	0.60 (0.41 to 0.88)	1.01 (0.60 to 1.71)	0.35 (0.18 to 0.69)	.005
Hysterectomy,‖ ever/never	0.96 (0.89 to 1.03)	1.03 (0.94 to 1.13)	0.84 (0.66 to 1.07)	0.72 (0.51 to 1.02)	0.57 (0.36 to 0.88)	.006
Endometriosis, ever/never	1.35 (1.07 to 1.71)	1.03 (0.74 to 1.46)	2.32 (1.36 to 3.95)	1.62 (0.58 to 4.51)	2.87 (1.53 to 5.39)	.01

NOTE. Associations stratified by birth year and cohort and adjusted for age at study entry, parity, and duration of oral contraceptive use (except when parity or oral contraceptive use was the primary exposure of interest, and then we adjusted only for the other risk factor) by using pooled analyses of all cohorts combined. Abbreviations: *P*-het, *P* value for heterogeneity; RR, relative risk.

*Assessed by using a likelihood ratio test that compared a Cox proportional hazards competing-risks model to allow for the association to vary by histologic subtype to a model that forced the association to be the same across subtypes.

†Significant at a Bonferroni threshold.

‡Parous women only.

§Postmenopausal women only.

‖Also adjusted for duration of hormone therapy use.

and ovarian cancer was associated with an increased risk of serous tumors (RR, 1.13 [95% CI, 1.02 to 1.26; *P*-het = .008] and 1.61 [95% CI, 1.32 to 1.97; *P*-het = .31], respectively). Family history of breast cancer was also associated with endometrioid carcinomas (RR, 1.47; 95% CI, 1.15 to 1.87). BMI was not significantly associated with ovarian carcinomas overall or with any subtype, although there was a borderline association with endometrioid carcinomas (RR per 5 kg/m^2, 1.07; 95% CI, 0.99 to 1.16). Ever smoking was associated with mucinous carcinomas only (RR, 1.27; 95% CI, 1.01 to 1.59); each 20 pack-years of smoking was

associated with an increased risk of mucinous and a decreased risk of clear cell carcinomas (*P*-het = .002).

Associations by Subtype of Serous Carcinomas

Among serous tumors, moderately and poorly differentiated carcinomas had similar associations, whereas associations for well-differentiated carcinomas were qualitatively different. However, the heterogeneity was not significant for most individual factors (Table 4; Appendix Table A3, online only) for high-/moderate- versus low-grade serous carcinomas. For example, endometriosis was significantly associated with well-differentiated carcinomas (RR, 3.77; 95% CI, 1.24 to 11.48) but not poorly differentiated carcinomas (RR, 1.11; 95% CI, 0.70 to 1.74; *P*-het = .12). Similarly, > 5 years of HT use versus never use was associated with a 2.9-fold higher risk of well-differentiated carcinomas but only an 80% higher risk of poorly differentiated carcinomas (*P*-het = .45).

Meta-Analysis and Heterogeneity Across Studies

Results for meta-analyses were similar to the pooled analyses (Appendix Table A4, online only). We observed little heterogeneity in associations across studies ($P < .01$ for only 13 of 188 comparisons). All of these were for continuous variables, but the categorical associations did not show heterogeneity. Family history of ovarian cancer showed heterogeneity for all four subtypes across studies likely because of the small number of exposed cases in many studies. Results were similar when including women with a history of cancer at baseline or when all exposures were included in the model (data not shown).

Integrated Analysis of Risk Factors in Ovarian Cancer Subtypes

Each subtype had unique patterns of risk factor associations (Fig 1). The strongest associations for most factors were observed for endometrioid and clear cell tumors. Unsupervised clustering divided the four histologic subtypes into two major groups (Fig 1A). Serous carcinomas were separate from the other three subtypes (Pearson correlation, 0.19). Endometrioid and clear cell carcinomas had the most similar risk factor associations (Pearson correlation, 0.71). Serous cancers divided by grade (Fig 1B) were split into two distinct groups: well-differentiated serous carcinomas clustered with endometrioid carcinomas (Pearson correlation, 0.75), whereas moderately and poorly differentiated serous carcinomas clustered together (Pearson correlation, 0.90).

Table 3. Associations of Family History, Demographic, and Lifestyle Factors With Invasive Epithelial Ovarian Cancer Overall and by Subtypes in the Ovarian Cancer Cohort Consortium

Exposure	All Invasive RR (95% CI)	Serous RR (95% CI)	Endometrioid RR (95% CI)	Mucinous RR (95% CI)	Clear Cell RR (95% CI)	*P*-het (between histologic types)*
No. of patients	5,584	3,378	606	331	269	
First-degree family history of breast cancer, ever/never	1.09 (1.00 to 1.19)	1.13 (1.02 to 1.26)	1.47 (1.15 to 1.87)	0.73 (0.47 to 1.13)	0.75 (0.46 to 1.22)	.008
First-degree family history of ovarian cancer, ever/never	1.48 (1.26 to 1.75)	1.61 (1.32 to 1.97)	0.97 (0.52 to 1.82)	1.33 (0.59 to 3.00)	0.96 (0.36 to 2.57)	.31
Body mass index						
Per 5 kg/m^2	1.01 (0.98 to 1.04)	0.97 (0.93 to 1.01)	1.07 (0.99 to 1.16)	1.08 (0.96 to 1.20)	1.04 (0.92 to 1.17)	.06
Categorical, kg/m^2						
< 20	1.02 (0.91 to 1.13)	1.06 (0.92 to 1.21)	0.85 (0.60 to 1.19)	1.36 (0.90 to 2.04)	0.96 (0.60 to 1.53)	.10
20 to < 25	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	
25 to < 30	0.97 (0.91 to 1.03)	0.91 (0.84 to 0.99)	0.97 (0.80 to 1.18)	1.42 (1.10 to 1.83)	1.21 (0.91 to 1.61)	
30 to < 35	0.99 (0.90 to 1.08)	0.92 (0.82 to 1.04)	1.09 (0.83 to 1.43)	1.23 (0.83 to 1.82)	0.97 (0.62 to 1.51)	
≥ 35	1.09 (0.97 to 1.24)	0.97 (0.83 to 1.14)	1.26 (0.88 to 1.80)	1.24 (0.69 to 2.21)	1.23 (0.70 to 2.15)	
Height						
Per 0.5 m	1.06 (1.04 to 1.08)	1.06 (1.03 to 1.09)	1.06 (1.00 to 1.13)	1.04 (0.95 to 1.13)	1.08 (0.98 to 1.19)	.94
Categorical, m						
< 1.60	0.89 (0.83 to 0.96)	0.86 (0.78 to 0.95)	1.03 (0.82 to 1.29)	0.87 (0.64 to 1.18)	0.92 (0.65 to 1.30)	.27
1.60 to < 1.65	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	
1.65 to < 1.70	1.02 (0.95 to 1.10)	1.04 (0.95 to 1.14)	0.93 (0.74 to 1.17)	0.83 (0.61 to 1.13)	0.97 (0.70 to 1.36)	
≥ 1.70	1.12 (1.03 to 1.21)	1.06 (0.96 to 1.17)	1.27 (1.01 to 1.60)	1.12 (0.82 to 1.52)	1.24 (0.88 to 1.73)	
Smoking						
Ever/never	0.99 (0.94 to 1.05)	0.99 (0.92 to 1.06)	0.93 (0.79 to 1.09)	1.27 (1.01 to 1.59)	0.95 (0.74 to 1.21)	.14
Per 20 pack-years	0.98 (0.94 to 1.02)	1.01 (0.96 to 1.06)	0.92 (0.80 to 1.06)	1.20 (1.04 to 1.39)	0.68 (0.53 to 0.89)	.002†
Categorical, pack-years						
Never	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	.09
≤ 10	1.07 (0.97 to 1.17)	1.07 (0.96 to 1.21)	1.02 (0.78 to 1.32)	1.14 (0.78 to 1.68)	0.95 (0.64 to 1.40)	
> 10 to 20	1.02 (0.90 to 1.15)	1.04 (0.89 to 1.21)	0.72 (0.49 to 1.07)	1.40 (0.89 to 2.20)	0.88 (0.52 to 1.48)	
> 20 to 35	0.96 (0.85 to 1.08)	0.99 (0.85 to 1.15)	0.92 (0.65 to 1.30)	1.16 (0.72 to 1.88)	0.44 (0.22 to 0.91)	
> 35	0.99 (0.88 to 1.12)	1.08 (0.93 to 1.24)	0.85 (0.57 to 1.26)	1.60 (1.02 to 2.51)	0.42 (0.18 to 0.94)	

NOTE. Associations stratified by birth year and cohort and adjusted for age at study entry, parity, and duration of oral contraceptive use (except when parity or oral contraceptive use was the primary exposure of interest, and then we adjusted only for the other risk factor) by using a pooled analysis of all cohorts combined. Abbreviations: *P*-het, *P* value for heterogeneity; RR, relative risk.

*Assessed by using a likelihood ratio test that compared a Cox proportional hazards competing-risks model to allow for the association to vary by histologic subtype to a model that forced the association to be the same across subtypes.

†Significant at a Bonferroni threshold.

Table 4. Associations of Risk Factors Among Serous Ovarian Carcinomas by Grade in the Ovarian Cancer Cohort Consortium

Exposure	Grade, RR (95% CI)				*P*-het†
	Well Differentiated*	Moderately Differentiated	Poorly Differentiated	Unknown	
Parity					
Ever/never	0.78 (0.47 to 1.29)	0.77 (0.60 to 0.99)	0.83 (0.72 to 0.96)	0.88 (0.71 to 1.09)	.87
No. of children, per one child	0.89 (0.80 to 1.00)	0.90 (0.85 to 0.95)	0.94 (0.91 to 0.96)	0.96 (0.93 to 1.01)	.20
No. of children					
0	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	
1	0.84 (0.41 to 1.73)	0.90 (0.64 to 1.27)	0.85 (0.69 to 1.05)	0.94 (0.70 to 1.26)	
2	0.88 (0.50 to 1.55)	0.86 (0.65 to 1.13)	0.89 (0.76 to 1.05)	0.89 (0.70 to 1.13)	.42
3	0.88 (0.50 to 1.54)	0.68 (0.51 to 0.91)	0.87 (0.74 to 1.03)	0.86 (0.67 to 1.10)	
≥ 4	0.45 (0.22 to 0.91)	0.68 (0.50 to 0.92)	0.69 (0.58 to 0.82)	0.89 (0.69 to 1.14)	
Oral contraceptive use					
Ever/never	1.11 (0.72 to 1.72)	0.80 (0.65 to 0.98)	0.85 (0.76 to 0.95)	0.77 (0.66 to 0.90)	.36
Duration of use, per 5-year increase	0.79 (0.62 to 1.00)	0.82 (0.73 to 0.92)	0.90 (0.84 to 0.96)	0.77 (0.69 to 0.87)	.09
Duration of use, years					
Never	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	
≤ 1	1.80 (0.98 to 3.30)	0.90 (0.63 to 1.29)	1.01 (0.84 to 1.20)	0.96 (0.74 to 1.24)	
> 1-5	1.12 (0.65 to 1.94)	0.95 (0.72 to 1.25)	0.86 (0.74 to 1.00)	0.85 (0.68 to 1.06)	.25
> 5-10	0.94 (0.48 to 1.83)	0.82 (0.60 to 1.13)	0.77 (0.65 to 0.92)	0.59 (0.44 to 0.79)	
> 10	0.56 (0.22 to 1.42)	0.45 (0.28 to 0.73)	0.76 (0.61 to 0.94)	0.49 (0.34 to 0.71)	
Duration of breastfeeding, per 1 year‡	1.06 (0.68 to 1.66)	0.93 (0.75 to 1.15)	0.95 (0.83 to 1.08)	0.89 (0.74 to 1.08)	.86
Age at menarche					
Per 1-year increase	1.01 (0.91 to 1.11)	1.00 (0.94 to 1.06)	1.01 (0.98 to 1.04)	0.95 (0.91 to 1.00)	.21
Age, years					
≤ 11	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	
12	1.26 (0.70 to 2.28)	0.86 (0.64 to 1.14)	1.06 (0.91 to 1.23)	0.86 (0.69 to 1.06)	
13	1.37 (0.83 to 2.28)	0.94 (0.73 to 1.20)	1.10 (0.96 to 1.26)	0.76 (0.62 to 0.92)	.22
14	1.20 (0.62 to 2.34)	0.86 (0.62 to 1.18)	1.16 (0.97 to 1.38)	0.83 (0.65 to 1.05)	
≥ 15	1.00 (0.49 to 2.05)	0.99 (0.72 to 1.36)	0.94 (0.78 to 1.14)	0.80 (0.62 to 1.02)	
Age at menopause					
Per 5-year increase	1.54 (1.23 to 1.91)	1.04 (0.93 to 1.16)	1.03 (0.97 to 1.10)	1.05 (0.95 to 1.16)	.06
Age, years					
≤ 45	0.20 (0.07 to 0.56)	0.92 (0.66 to 1.28)	0.91 (0.77 to 1.09)	0.89 (0.69 to 1.17)	
> 45-50	0.49 (0.29 to 0.84)	1.21 (0.94 to 1.56)	0.96 (0.83 to 1.10)	0.98 (0.80 to 1.21)	.02
> 50-55	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	
> 55	0.41 (0.13 to 1.32)	1.16 (0.73 to 1.84)	0.97 (0.75 to 1.24)	1.23 (0.87 to 1.73)	
Hormone therapy use§					
Ever/never	1.80 (1.15 to 2.83)	1.57 (1.27 to 1.95)	1.49 (1.33 to 1.67)	1.23 (1.04 to 1.45)	.15
Duration of use, per 5-year increase	1.35 (1.18 to 1.53)	1.26 (1.17 to 1.36)	1.21 (1.16 to 1.26)	1.20 (1.12 to 1.29)	.54
Duration of use, years					
Never	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	
≤ 5	1.33 (0.71 to 2.48)	1.26 (0.94 to 1.69)	1.27 (1.09 to 1.48)	1.12 (0.90 to 1.41)	.42
> 5	2.91 (1.72 to 4.92)	2.10 (1.60 to 2.76)	1.80 (1.56 to 2.07)	1.57 (1.27 to 1.95)	
Tubal ligation, ever/never	1.25 (0.66 to 2.36)	1.05 (0.71 to 1.57)	0.92 (0.76 to 1.11)	0.62 (0.43 to 0.88)	.10
Hysterectomy, ever/never‖	0.87 (0.53 to 1.43)	1.05 (0.84 to 1.33)	1.01 (0.89 to 1.14)	1.04 (0.87 to 1.25)	.90
Endometriosis, yes/no	3.77 (1.24 to 11.48)	1.54 (0.72 to 3.30)	1.11 (0.70 to 1.74)	0.57 (0.18 to 1.80)	.12
First-degree family history of breast cancer, yes/no	1.23 (0.71 to 2.15)	1.20 (0.91 to 1.58)	1.12 (0.97 to 1.30)	0.96 (0.76 to 1.21)	.58
First-degree family history of ovarian cancer, yes/no	0.90 (0.22 to 3.70)	1.46 (0.83 to 2.54)	1.63 (1.25 to 2.13)	1.64 (1.08 to 2.47)	.82
Body mass index					
Per 5 kg/m^2	0.92 (0.74 to 1.14)	0.99 (0.90 to 1.08)	0.92 (0.87 to 0.97)	1.05 (0.97 to 1.13)	.03
Categorical, kg/m^2					
< 20	1.33 (0.67 to 2.62)	0.78 (0.51 to 1.19)	1.15 (0.95 to 1.39)	1.11 (0.83 to 1.49)	
20 to < 25	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	
25 to < 30	1.02 (0.65 to 1.59)	1.08 (0.88 to 1.33)	0.84 (0.74 to 0.94)	0.89 (0.75 to 1.05)	.22
30 to < 35	0.85 (0.44 to 1.66)	0.98 (0.73 to 1.32)	0.85 (0.72 to 1.00)	1.04 (0.83 to 1.32)	
≥ 35	1.15 (0.51 to 2.59)	0.88 (0.56 to 1.39)	0.88 (0.70 to 1.10)	1.25 (0.92 to 1.70)	
Height					
Per 0.5 m	1.05 (0.93 to 1.18)	1.06 (0.99 to 1.14)	1.07 (1.03 to 1.11)	1.03 (0.97 to 1.08)	.72
Categorical, m					
< 1.60	0.83 (0.49 to 1.39)	0.92 (0.72 to 1.17)	0.82 (0.72 to 0.95)	1.00 (0.82 to 1.21)	
1.60 to < 1.65	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	.70
1.65 to < 1.70	1.21 (0.75 to 1.95)	1.03 (0.81 to 1.30)	1.03 (0.91 to 1.18)	1.15 (0.95 to 1.39)	
≥ 1.70	0.96 (0.55 to 1.69)	1.08 (0.83 to 1.41)	1.06 (0.92 to 1.22)	0.96 (0.77 to 1.20)	

(continued on following page)

Table 4. Associations of Risk Factors Among Serous Ovarian Carcinomas by Grade in the Ovarian Cancer Cohort Consortium (continued)

Exposure	Grade, RR (95% CI)				*P*-het†
	Well Differentiated*	Moderately Differentiated	Poorly Differentiated	Unknown	
Smoking					
Ever/never	1.10 (0.85 to 1.41)	0.95 (0.84 to 1.07)	0.96 (0.90 to 1.03)	1.04 (0.95 to 1.15)	.38
Continuous pack-years, per 20 pack-years	0.87 (0.59 to 1.26)	1.00 (0.87 to 1.15)	0.98 (0.92 to 1.05)	1.07 (0.97 to 1.18)	.44
Categorical, pack-years					
Never	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)	
≤ 20	1.20 (0.70 to 2.08)	1.00 (0.76 to 1.32)	1.08 (0.94 to 1.24)	1.10 (0.88 to 1.36)	.91
> 20	0.72 (0.34 to 1.52)	0.97 (0.71 to 1.31)	1.03 (0.89 to 1.21)	1.09 (0.87 to 1.38)	

NOTE. Associations stratified by birth year and cohort and adjusted for age at study entry, parity, and duration of oral contraceptive use (except when parity or oral contraceptive use was the primary exposure of interest, and then we adjusted only for the other risk factor) by using pooled analyses of all cohorts combined. Five cohorts with no information on grade for any ovarian cancer cases were excluded. Abbreviation: *P*-het, *P* value for heterogeneity; RR, relative risk.

*Number of cases ranged from 28 (breastfeeding) to 121 (oral contraceptive use) for well-differentiated, 113 (endometriosis) to 496 (oral contraceptive use) for moderately differentiated, 338 (breastfeeding) to 1,637 (oral contraceptive use) for poorly differentiated, and 141 (endometriosis) to 773 (oral contraceptive use) for unknown grade.

†Assessed by using a likelihood ratio test that compared a Cox proportional hazards competing-risks model to allow for the association to vary by grade to a model that forced the association to be the same across grades.

‡Parous women only.

§Postmenopausal women only.

‖Also adjusted for duration of hormone therapy use.

DISCUSSION

In a large pooled analysis of > 1.3 million women, we investigated 14 established or putative risk factors in ovarian cancer subtypes. Nine risk factors had significant heterogeneity across subtypes. Most reproductive and hormonal risk factors had stronger associations with endometrioid and clear cell carcinomas compared with the other types. Serous and poorly differentiated carcinomas, the most common and aggressive subtypes, had modest associations only with parity, OC use, menopausal HT use, and family history of breast cancer and stronger associations with family history of ovarian cancer.

The current analysis represents, to our knowledge, the largest comprehensive and prospective evaluation of ovarian cancer risk factors by histologic subtypes. The results are consistent with previous reports from individual prospective studies within the OC3 (ie, Nurses' Health Study/Nurses' Health Study II, National Institutes of Health-AARP Diet and Health Study, European Prospective Investigation Into Cancer).[15-17] However, individually, these studies were underpowered to assess subtype-specific associations, particularly for rare types. Previously, other consortia, largely based on case-control studies, reported subtype-specific associations for individual risk factors[12-14,19-21] similar to what we observed.

Models of ovarian carcinogenesis have separated epithelial tumors into major pathways with distinct cells of origin, carcinogenic pathways, and histology with different clinical behavior.[8,10] An integrated evaluation of ovarian cancer risk factors by subtypes is important to understand factors that drive these etiologic pathways on the population level. Each subtype had a qualitatively unique pattern of associations, and serous carcinomas were clearly separated from endometrioid, clear cell, and mucinous carcinomas. Although endometrioid and clear cell carcinomas had qualitatively similar associations for 10 risk factors, they differed in associations related to HT use (which went in opposite directions), family history of breast cancer (associated with endometrioid only), as well as age at menarche and smoking (associated with clear cell only). Every reproductive/hormonal factor, except breastfeeding, was significantly associated with clear cell tumors.

The present results suggest that currently hypothesized, unifying mechanisms, such as incessant ovulation,[4] do not apply equally to ovarian cancers. Several variables that determine a woman's lifetime number of ovulations had significant heterogeneity across subtypes. Only parity and height were associated with all subtypes, which suggests a common biologic effect.[22] Of note, mucinous tumors were not associated with any ovulation-related factors except parity, which suggests a more distinct etiology.

Ovarian cancer subtypes share some risk factors with other cancer sites. The inverse association between smoking and clear cell ovarian carcinomas is similar to that for endometrial cancer.[23] Mucinous ovarian cancers share histologic appearance and an association with smoking with colorectal cancers.[24] Serous ovarian cancers had weaker associations with most hormonal and reproductive factors compared with nonserous cancers (with the exception of OC use), which is similar to associations for hormone receptor–negative breast cancers.[25] These similarities of risk factor associations across cancers mirror molecular data that showed that tumor subtypes from different organs may be more similar to one another on the molecular level compared with other subtypes at the same site (eg, high-grade serous ovarian cancer, basal-like breast cancer).[26]

Although the subtype-specific associations observed in the current study strongly corroborate the etiologic heterogeneity of ovarian cancers, a purely histology-based classification of end points may have limitations.[27] Histologic evaluation is subjective, and pathology practice changes over time, which could affect subtype distributions by location and year of diagnosis. We observed heterogeneity among studies for four risk factors among mucinous tumors, which were possibly related to temporal and geographic differences in defining mucinous tumors. However, overall, we did not observe significant differences in subtype

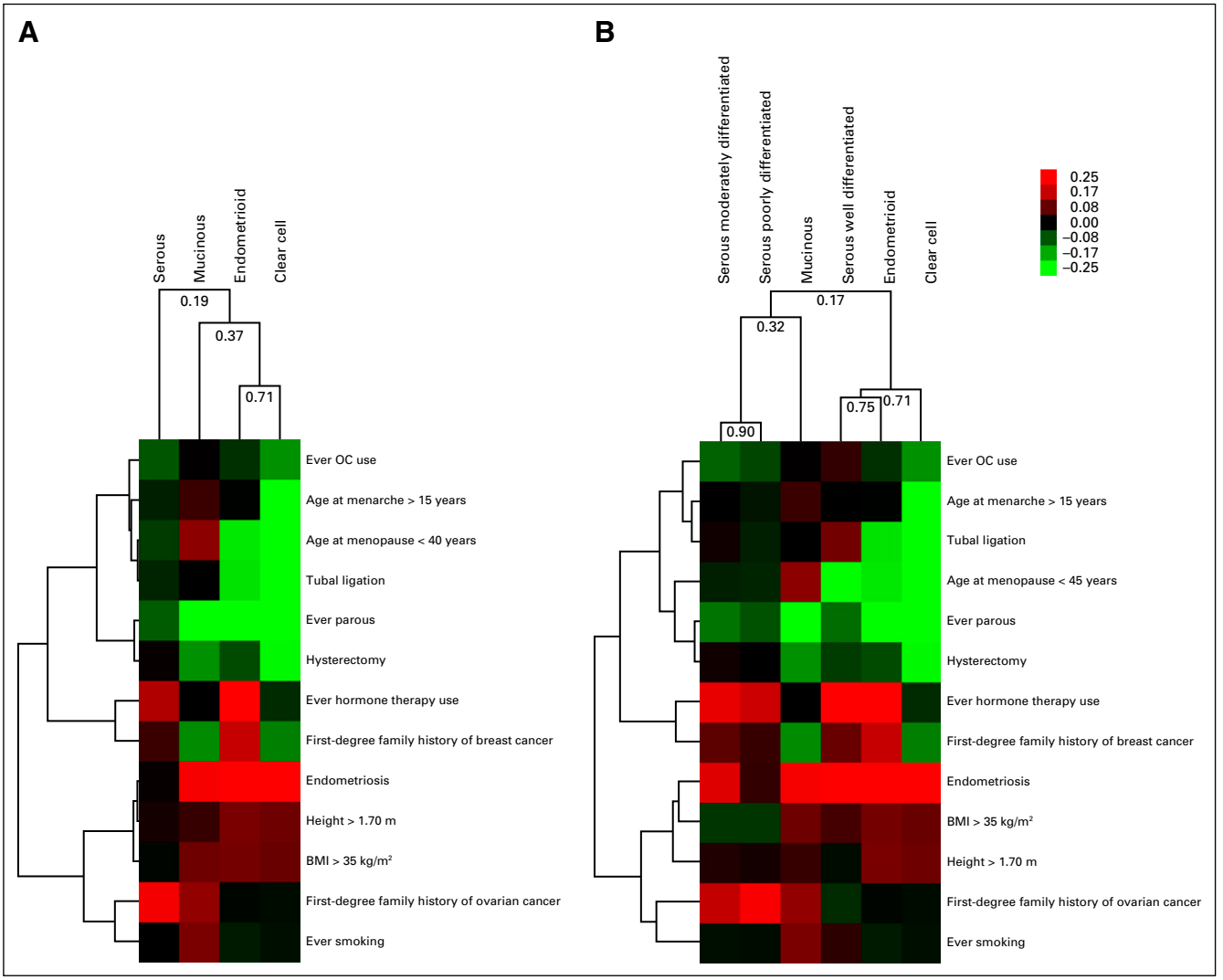

Fig 1. Unsupervised hierarchical clustering of ovarian cancer histologic subtypes by their associations with risk factors. Unsupervised hierarchical clustering of the (A) four subtypes and (B) that includes the serous subtype divided into well-, moderately, and poorly differentiated carcinomas by using β-estimates, complete linkage, and an uncentered correlation similarity metric. The categories used in the cluster analysis were ever versus never parous, ever versus never oral contraceptive (OC) use, ever versus never tubal ligation, ever versus never endometriosis, age at menarche $>$ 15 *v* $\leq$ 11 years, age at menopause $<$ 40 versus 50 to 55 years, ever versus never menopausal hormone therapy use, ever versus never hysterectomy, family history of breast cancer (yes *v* no), family history of ovarian cancer (yes *v* no), body mass index (BMI) $>$ 35 versus 20 to 25 kg/m^2, height (per 5-cm increase), and ever versus never smoking. The color scale shows the range of β-values for each exposure.

proportions across studies or over time (data not shown). Unsupervised clustering demonstrated that well-differentiated serous carcinomas were distinct from higher grade serous carcinomas and grouped with endometrioid carcinomas. This is important etiologically and further supports the differentiation of these two groups of serous carcinomas as proposed in models based on somatic mutations.[8,9] However, in population-based studies, the grade reported on pathology reports may not be reliable, and low-grade serous carcinomas account for only approximately 5% of all serous cancers,[28] which limits potential misclassification when associations for all serous carcinomas are considered together.[29] Analyses by tumor aggressiveness and tumor dominance have also shown differences in risk factor associations, which indicates important biologic heterogeneity beyond histologic subtypes.[30,31] Furthermore, additional molecular subgroups have been described within high-grade serous ovarian cancers,[32,33] but thus far, based on small studies, these subtypes have shown only limited heterogeneity in risk factor associations.[34]

In summary, we conducted the largest integrated prospective analysis of ovarian cancer risk factors to date. Most factors showed heterogeneity across histologic subtypes, and each subtype had unique patterns of risk factor associations. The results have important implications with respect to etiology and prevention of ovarian cancers. OCs continue to be an important preventive factor for most types of ovarian cancer. Few other risk factors for ovarian cancer are modifiable, and those that are, such as smoking and obesity, did not show clear associations with serous carcinomas, the most common and fatal subtype. The substantial heterogeneity of individual risk factor associations across ovarian cancer subtypes supports that subtypes are indeed different diseases and underscores the importance of evaluating risk factors and biomarkers

by ovarian cancer subtypes.[35-37] Our work has implications for the development of risk prediction models, which generally consider ovarian cancer as a whole.[38] Due to weaker associations observed for high-grade serous carcinomas, prediction of the clinically most important subtype may perform worse than for other types, which underscores the importance of finding better risk factors for serous carcinomas. Evaluation of subtype-specific risk factor associations is important to gain a better understanding of ovarian cancer etiology and for targeted development of novel prevention approaches; these analyses require pooling of data across many studies in consortia. To this end, future work in the OC3 will include an evaluation of circulating biomarkers, such as inflammation markers, by ovarian cancer subtypes and the development of risk prediction models that integrate risk factor information and genetic data that account for the heterogeneity of ovarian cancer. Furthermore, we and others should explore potential risk factors for high-grade serous cancers, which showed the weakest associations for most established ovarian cancer risk factors.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

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Ovarian Cancer Risk Factors by Histologic Subtype: An Analysis From the Ovarian Cancer Cohort Consortium

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Appendix

Table A1. Studies in the Ovarian Cancer Cohort Consortium That Contributed to Each Exposure Analysis

Variable	Studies
Ever/never parous	AARP, BCDDP, BGS, CLUEII, CPSII-NC, CSDLH, CTS, EPIC, IWHS, MEC, NHS, NHSII, NLCS, NYU, PLCO, SCHS, SMC, SS, VITAL, WHS, WLHS
No. of children (continuous or categorical)	AARP, BCDDP, BGS, CLUEII, CPSII-NC, CSDLH, CTS, EPIC, IWHS, MEC, NHS, NHSII, NLCS, NYU, PLCO, SMC, SS, VITAL, WHS, WLHS
Ever/never OC use	AARP, BCDDP, BGS, CLUEII, CPSII-NC, CSDLH, CTS, EPIC, IWHS, MEC, NHS, NHSII, NLCS, NYU, PLCO, SCHS, SMC, SS, VITAL, WHS, WLHS
Duration of OC use (continuous or categorical)	AARP, BCDDP, BGS, CLUEII, CPSII-NC, CSDLH, CTS, EPIC, IWHS, MEC, NHS, NHSII, NLCS, NYU, PLCO, SCHS, SMC, SS, VITAL, WHS, WLHS
Duration of breastfeeding (continuous)	BGS, CTS, EPIC, NHS, NHSII, SS, WLHS
Age at menarche (continuous or categorical)	AARP, BCDDP, BGS, CLUEII, CPSII-NC, CSDLH, CTS, EPIC, IWHS, MEC, NHS, NHSII, NLCS, NYU, PLCO, SCHS, SMC, SS, VITAL, WHS, WLHS
Age at menopause (continuous and categorical)	AARP, BCDDP, BGS, CLUEII, CPSII-NC, CSDLH, CTS, EPIC, IWHS, MEC, NHS, NLCS, NYU, PLCO, SCHS, SMC, SS, VITAL, WHS
Ever use of HT	AARP, BCDDP, BGS, CLUEII, CPSII-NC, CSDLH, CTS, EPIC, IWHS, MEC, NHS, NLCS, NYU, PLCO, SCHS, SMC, SS, VITAL, WHS, WLHS
Duration of HT use (continuous and categorical)	AARP, BCDDP, BGS, CPSII-NC, CSDLH, EPIC, IWHS, MEC, NHS, NLCS, NYU, PLCO, SCHS, SMC, SS, VITAL, WHS
Tubal ligation	CPSII-NC, CTS, EPIC, MEC, NHS, NHSII, NLCS, NYU, PLCO, SMC, SS, VITAL, WHS
Hysterectomy	AARP, BCDDP, BGS, CLUEII, CPSII-NC, CSDLH, EPIC, IWHS, MEC, NHS, NHSII, NLCS, NYU, PLCO, SCHS, SMC, SS, VITAL, WHS
Endometriosis	BGS, CTS, IWHS, NHSII, PLCO, SS
Family history of breast cancer	AARP, BCDDP, BGS, CLUEII, CPSII-NC, CSDLH, CTS, EPIC, IWHS, MEC, NHS, NHSII, NLCS, NYU, PLCO, SCHS, SMC, VITAL, WHS
Family history of ovarian cancer	AARP, BCDDP, BGS, CLUEII, CPSII-NC, CTS, IWHS, MEC, NHS, NHSII, NLCS, PLCO, SCHS, SS, VITAL, WHS
BMI (continuous and categorical)	AARP, BCDDP, BGS, CLUE, CPSII-NC, CSDLH, CTS, EPIC, IWHS, MEC, NHS, NHSII, NLCS, NYU, PLCO, SCHS, SMC, SS, VITAL, WHS, WLHS
Height (continuous and categorical)	AARP, BCDDP, BGS, CLUE, CPSII-NC, CSDLH, CTS, EPIC, IWHS, MEC, NHS, NHSII, NLCS, NYU, PLCO, SCHS, SMC, SS, VITAL, WHS, WLHS
Ever/never smoker	AARP, BCDDP, BGS, CLUEII, CPSII-NC, CSDLH, CTS, EPIC, IWHS, MEC, NHS, NHSII, NLCS, NYU, PLCO, SCHS, SMC, SS, VITAL, WHS, WLHS
Pack-years of smoking (continuous and categorical)	BCDDP, BGS, CPSII-NC, CSDLH, IWHS, MEC, NHS, NHSII, NLCS, NYU, PLCO, SCHS, SMC, SS, VITAL, WHS

Abbreviations: AARP, National Institutes of Health-AARP Diet and Health Study; BCDDP, Breast Cancer Detection Demonstration Project Follow-Up Study; BGS, Breakthrough Generations Study; BMI, body mass index; CSDLH, Canadian Study of Diet, Lifestyle, and Health; CLUEII, Campaign Against Cancer and Stroke; CPSII-NC, Cancer Prevention Study II Nutrition Cohort; CTS, California Teachers Study; EPIC, European Prospective Investigation Into Cancer and Nutrition Study; HT, hormone therapy; IWHS, Iowa Women's Health Study; MEC, Multiethnic/Minority Cohort Study; NHS, Nurses' Health Study; NHSII, Nurses' Health Study II; NYU, New York University Women's Health Study; NLCS, Netherlands Cohort Study on Diet and Cancer; OC, oral contraceptive; PLCO, Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial; SCHS, Singapore Chinese Health Study; SS, Sister Study; SMC, Swedish Mammography Cohort Study; VITAL, Vitamins and Lifestyle Cohort; WLHS, Women's Lifestyle and Health Study; WHS, Women's Health Study.

Table A2. Number of Invasive Epithelial Ovarian Cancer Cases Overall and by Histologic Subtype for each Exposure

Exposure	No. of Cases for Each Exposure				
	Serous	Endometrioid	Mucinous	Clear Cell	All Invasive
Parity					
Ever/never	3,300	598	318	254	5,429
No. of children (continuous or categorical)	3,268	587	303	241	5,351
Oral contraceptive use					
Ever/never	3,347	604	326	265	5,523
Duration of use (continuous or categorical)	3,287	587	318	263	5,418
Duration of breastfeeding	831	157	70	63	1,281
Age at menarche (continuous or categorical)	3,331	602	327	266	5,489
Age at menopause (postmenopausal only; continuous or categorical)	2,162	345	207	132	3,494
HT use (postmenopausal only)					
Ever/never	2,682	411	238	157	4,319
Duration of use (continuous or categorical)	2,394	347	216	138	3,802
Tubal ligation	2,387	435	213	193	3,914
Hysterectomy	3,146	550	301	230	5,486
Endometriosis	900	169	73	86	1,503
First-degree family history of breast cancer	3,291	589	316	262	5,383
First-degree family history of ovarian cancer	2,634	459	238	205	4,332
Body mass index (continuous or categorical)	3,234	578	319	262	5,354
Height (continuous or categorical)	3,277	592	322	267	5,433
Smoking					
Ever/never	3,335	605	328	268	5,514
Pack-years (continuous or categorical)	2,257	416	223	191	4,690

Abbreviation: HT, hormone therapy.

Table A3. Associations of Risk Factors for Low- and High-Grade Serous Ovarian Carcinomas in the Ovarian Cancer Cohort Consortium

Exposure	Low-Grade Serous*	High-Grade Serous	*P*-het†
Parity			
Ever/never	0.78 (0.47 to 1.28)	0.81 (0.72 to 0.92)	.87
No. of children, per one child	0.90 (0.80 to 1.00)	0.93 (0.90 to 0.95)	.58
No. of children			
0	1.00 (reference)	1.00 (reference)	
1	0.83 (0.41 to 1.65)	0.85 (0.72 to 1.01)	
2	0.87 (0.51 to 1.50)	0.87 (0.76 to 1.00)	.66
3	0.87 (0.51 to 1.49)	0.81 (0.71 to 0.93)	
≥ 4	0.45 (0.23 to 0.89)	0.67 (0.58 to 0.78)	
Oral contraceptive use			
Ever/never	1.12 (0.72 to 1.72)	0.84 (0.76 to 0.93)	.19
Duration of use, per 5-year increase	0.79 (0.62 to 1.00)	0.88 (0.83 to 0.94)	.40
Duration of use, years			
Never	1.00 (reference)	1.00 (reference)	
≤ 1	1.80 (0.98 to 3.31)	0.99 (0.84 to 1.16)	
> 1 to 5	1.13 (0.65 to 1.94)	0.88 (0.77 to 1.01)	.36
> 5 to 10	0.94 (0.48 to 1.83)	0.78 (0.67 to 0.92)	
> 10	0.56 (0.22 to 1.42)	0.68 (0.56 to 0.83)	
Duration of breastfeeding, per 1-year increase‡	1.07 (0.69 to 1.66)	0.95 (0.85 to 1.06)	.55
Age at menarche			
Per 1-year increase	1.01 (0.91 to 1.11)	1.01 (0.98 to 1.03)	.98
Age, years			
≤ 11	1.00 (reference)	1.00 (reference)	
12	1.26 (0.70 to 2.28)	1.01 (0.88 to 1.15)	
13	1.38 (0.83 to 2.28)	1.06 (0.94 to 1.19)	.86
14	1.21 (0.62 to 2.34)	1.08 (0.93 to 1.26)	
≥ 15	1.00 (0.49 to 2.05)	0.96 (0.82 to 1.13)	
Age at menopause			
Per 5-year increase	1.54 (1.23 to 1.92)	1.03 (0.98 to 1.09)	.006
Age, years			
≤ 45	0.20 (0.07 to 0.56)	0.91 (0.78 to 1.07)	
> 45 to 50	0.49 (0.29 to 0.84)	1.01 (0.90 to 1.14)	.001
> 50 to 55	1.00 (reference)	1.00 (reference)	
> 55	0.41 (0.13 to 1.33)	1.01 (0.81 to 1.25)	
Hormone therapy use§			
Ever/never	1.87 (1.17 to 2.97)	1.48 (1.34 to 1.65)	.36
Duration of use, per 5-year increase	1.34 (1.18 to 1.53)	1.22 (1.18 to 1.27)	.26
Duration of use, years			
Never	1.00 (reference)	1.00 (reference)	
≤ 5	1.27 (0.68 to 2.37)	1.27 (1.11 to 1.46)	.43
> 5	2.67 (1.57 to 4.55)	1.86 (1.64 to 2.11)	
Tubal ligation, ever/never	1.30 (0.69 to 2.46)	0.98 (0.83 to 1.15)	.42
Hysterectomy, ever/never‖	0.87 (0.53 to 1.43)	1.02 (0.91 to 1.14)	.55
Endometriosis, yes/no	3.74 (1.23 to 11.38)	1.19 (0.80 to 1.76)	.08
First-degree family history of breast cancer, yes/no	1.23 (0.71 to 2.14)	1.13 (1.00 to 1.29)	.78
First-degree family history of ovarian cancer, yes/no	0.90 (0.22 to 3.71)	1.60 (1.26 to 2.03)	.38
Body mass index			
Per 5 kg/m^2	0.92 (0.74 to 1.14)	0.94 (0.89 to 0.98)	.85
Categorical, kg/m^2			
< 20	1.33 (0.67 to 2.62)	1.07 (0.90 to 1.27)	
20 to < 25	1.00 (reference)	1.00 (reference)	
25 to < 30	1.02 (0.65 to 1.59)	0.89 (0.81 to 0.99)	.93
30 to < 35	0.86 (0.44 to 1.67)	0.88 (0.76 to 1.02)	
≥ 35	1.16 (0.52 to 2.60)	0.89 (0.73 to 1.09)	
Height			
Per 0.5 m	1.05 (0.93 to 1.18)	1.06 (1.03 to 1.10)	.81
Categorical, m			
< 1.60	0.83 (0.49 to 1.39)	0.85 (0.75 to 0.96)	
1.60 to < 1.65	1.00 (reference)	1.00 (reference)	.81
1.65 to < 1.70	1.21 (0.75 to 1.95)	1.03 (0.92 to 1.16)	
≥ 1.70	0.96 (0.55 to 1.69)	1.07 (0.94 to 1.21)	

(continued on following page)

Table A3. Associations of Risk Factors for Low- and High-Grade Serous Ovarian Carcinomas in the Ovarian Cancer Cohort Consortium (continued)

Exposure	Low-Grade Serous*	High-Grade Serous	*P*-het†
Smoking			
Ever/never	1.11 (0.76 to 1.63)	0.95 (0.87 to 1.04)	.42
Continuous, per 20 pack-years	0.86 (0.59 to 1.26)	0.98 (0.93 to 1.04)	.45
Categorical, pack-years			
Never	1.00 (reference)	1.00 (reference)	
≤ 20	1.20 (0.69 to 2.07)	1.06 (0.94 to 1.20)	.49
> 20	0.72 (0.34 to 1.51)	1.01 (0.88 to 1.16)	

NOTE. Associations stratified by birth year and cohort and adjusted for age at study entry, parity, and duration of oral contraceptive use (except when parity or oral contraceptive use was the primary exposure of interest, and then we adjusted only for the other risk factor) by using pooled analyses of all cohorts combined. Five cohorts with no information on grade for any ovarian cancer cases were excluded.

Abbreviation: *P*-het, *P* value for heterogeneity.

*Number of cases ranged from 28 (breastfeeding) to 121 (oral contraceptive use) for low-grade serous and 460 (breastfeeding) to 2,133 (oral contraceptive use) for high-grade serous carcinomas; serous cases with unknown grade were excluded.

†Assessed by a likelihood ratio test that compared a Cox proportional hazards competing-risks model to allow for the association to vary by grade to a model that forced the association to be the same across grades.

‡Parous women only.

§Postmenopausal women only.

‖Also adjusted for duration of hormone therapy use.

Table A4. Associations of Risk Factors With Ovarian Cancer Subtypes Based on Meta-analysis by Pooling the Results of Individual Studies in the Ovarian Cancer Cohort Consortium

Exposure	Serous	Endometrioid	Mucinous	Clear cell
Parity				
Ever/never	0.80 (0.73 to 0.89)	0.44 (0.36 to 0.55)	0.45 (0.31 to 0.64)	0.32 (0.24 to 0.43)
No. of children, per one child	0.94 (0.92 to 0.96)	0.78 (0.72 to 0.84)	0.84 (0.75 to 0.95)*	0.65 (0.57 to 0.73)
No. of children				
0	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
1	0.87 (0.74 to 1.02)	0.79 (0.58 to 1.07)	0.83 (0.48 to 1.45)	0.57 (0.36 to 0.91)
2	0.87 (0.77 to 0.97)	0.47 (0.37 to 0.59)	0.52 (0.33 to 0.82)	0.41 (0.27 to 0.63)
3	0.80 (0.71 to 0.90)	0.41 (0.32 to 0.54)	0.53 (0.34 to 0.80)	0.32 (0.19 to 0.52)
≥ 4	0.72 (0.63 to 0.83)	0.33 (0.24 to 0.46)	0.60 (0.39 to 0.91)	0.31 (0.14 to 0.67)
Oral contraceptive use				
Ever/never	0.82 (0.75 to 0.89)	0.88 (0.73 to 1.05)	1.04 (0.81 to 1.34)	0.74 (0.54 to 1.01)
Duration of use, per 5-year increase	0.84 (0.78 to 0.90)	0.89 (0.77 to 1.02)	1.19 (0.99 to 1.43)	0.96 (0.82 to 1.12)
Duration of use, years				
Never	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
≤ 1	1.01 (0.88 to 1.16)	1.15 (0.86 to 1.55)	1.22 (0.77 to 1.91)	1.24 (0.74 to 2.06)
> 1 to 5	0.88 (0.78 to 0.99)	0.95 (0.74 to 1.23)	1.15 (0.77 to 1.71)	1.25 (0.78 to 2.01)
> 5 to 10	0.76 (0.65 to 0.89)	0.90 (0.67 to 1.21)	1.28 (0.84 to 1.95)	1.06 (0.67 to 1.68)
> 10	0.67 (0.57 to 0.79)	0.75 (0.97 to 1.16)	1.67 (1.06 to 2.64)	0.73 (0.36 to 1.45)
Duration of breastfeeding, per 1 year†	1.01 (0.87 to 1.18)*	0.93 (0.78 to 1.11)	0.94 (0.68 to 1.31)	1.13 (0.93 to 1.36)
Age at menarche				
Per 1-year increase	0.99 (0.96 to 1.02)	1.00 (0.95 to 1.05)	1.00 (0.94 to 1.07)	0.94 (0.87 to 1.02)
Age, years				
≤ 11	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
12	0.90 (0.75 to 1.08)	0.97 (0.74 to 1.27)	1.13 (0.75 to 1.70)	0.81 (0.54 to 1.22)
13	0.99 (0.88 to 1.10)	1.00 (0.75 to 1.33)	1.05 (0.74 to 1.49)	0.84 (0.47 to 1.49)
14	0.97 (0.85 to 1.12)	0.88 (0.63 to 1.23)	1.05 (0.65 to 1.68)	0.77 (0.46 to 1.27)
≥ 15	0.91 (0.79 to 1.05)	1.02 (0.73 to 1.42)	1.37 (0.87 to 2.17)	0.80 (0.46 to 1.40)
Age at menopause				
Per 5-year increase	1.05 (1.00 to 1.10)	1.44 (1.08 to 1.93)*	1.04 (0.80 to 1.37)*	1.96 (1.37 to 2.81)*
Age, years				
≤ 40	1.02 (0.82 to 1.27)	0.79 (0.45 to 1.40)	2.02 (0.67 to 6.04)	0.64 (0.14 to 2.89)
> 40 to 45	0.88 (0.75 to 1.04)	1.03 (0.64 to 1.66)	1.10 (0.54 to 2.25)	0.95 (0.37 to 2.48)
> 45 to 50	0.96 (0.86 to 1.06)	0.86 (0.65 to 1.13)	0.96 (0.68 to 1.35)	1.06 (0.69 to 1.63)
> 50 to 55	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
> 55	1.05 (0.88 to 1.25)	1.35 (0.88 to 2.08)	1.66 (0.83 to 3.34)	1.93 (0.88 to 4.23)
Hormone therapy use‡				
Ever/never	1.40 (1.27 to 1.55)	1.81 (1.41 to 2.32)	1.04 (0.77 to 1.41)	0.90 (0.57 to 1.42)
Duration of use, per 5-year increase	1.22 (1.15 to 1.29)	1.33 (1.17 to 1.51)	1.08 (0.86 to 1.36)	0.69 (0.49 to 0.98)*
Duration of use, years				
Never	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
≤ 5	1.24 (1.11 to 1.38)	1.71 (1.20 to 2.43)	1.27 (0.87 to 1.85)	1.06 (0.63 to 1.75)
> 5	1.75 (1.55 to 1.98)	2.32 (1.59 to 3.38)	1.43 (0.89 to 2.30)	0.83 (0.55 to 1.25)
Tubal ligation, ever/never	0.97 (0.81 to 1.16)	0.79 (0.53 to 1.18)	1.43 (0.80 to 2.56)	0.63 (0.27 to 1.46)
Hysterectomy, ever/never§	0.99 (0.88 to 1.12)	0.90 (0.70 to 1.16)	0.82 (0.57 to 1.16)	0.89 (0.54 to 1.46)
Endometriosis, yes/no	1.14 (0.81 to 1.61)	2.84 (1.56 to 5.18)	5.06 (1.51 to 16.9)	3.43 (1.52 to 7.75)
First-degree family history of breast cancer, yes/no	1.19 (1.02 to 1.39)	1.56 (1.22 to 1.99)	1.04 (0.67 to 1.61)	1.29 (0.78 to 2.13)
First-degree family history of ovarian cancer, yes/no	1.16 (0.43 to 3.18)*	0.29 (0.01 to 5.89)*	0.01 (0.00 to 1.13)*	0.02 (0.00 to 1.68)*
Body mass index				
Per 5 kg/m^2	0.97 (0.93 to 1.01)	1.03 (0.92 to 1.15)*	1.08 (0.97 to 1.20)	0.95 (0.80 to 1.14)*
Categorical, kg/m^2				
< 20	1.08 (0.94 to 1.24)	1.18 (0.83 to 1.67)	1.97 (1.28 to 3.02)	1.50 (0.92 to 2.44)
20 to < 25	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
25 to < 30	0.93 (0.84 to 1.03)	1.00 (0.82 to 1.23)	1.44 (1.11 to 1.87)	1.37 (1.01 to 1.84)
30 to < 35	0.94 (0.83 to 1.06)	1.28 (0.97 to 1.70)	1.86 (1.22 to 2.86)	1.77 (1.04 to 3.00)
≥ 35	1.07 (0.84 to 1.35)	1.73 (1.20 to 2.50)	2.18 (1.09 to 4.36)	2.26 (1.19 to 4.29)
Height				
Per 0.5m	1.06 (1.03 to 1.10)	1.06 (0.99 to 1.13)	1.08 (0.96 to 1.19)*	1.08 (0.98 to 1.17)
Categorical, m				
< 1.60	0.87 (0.79 to 0.96)	1.05 (0.83 to 1.32)	0.98 (0.71 to 1.34)	1.02 (0.71 to 1.46)
1.60 to < 1.65	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
1.65 to < 1.70	1.05 (0.94 to 1.19)	1.00 (0.79 to 1.27)	1.02 (0.73 to 1.41)	1.02 (0.67 to 1.58)
≥ 1.70	1.06 (0.96 to 1.17)	1.28 (1.01 to 1.63)	1.23 (0.88 to 1.71)	1.23 (0.85 to 1.78)

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Table A4. Associations of Risk Factors With Ovarian Cancer Subtypes Based on Meta-analysis by Pooling the Results of Individual Studies in the Ovarian Cancer Cohort Consortium (continued)

Exposure	Serous	Endometrioid	Mucinous	Clear cell
Smoking				
Ever/never	1.02 (0.92 to 1.12)	0.95 (0.80 to 1.12)	1.25 (0.99 to 1.57)	0.92 (0.70 to 1.21)
Continuous, per 20 pack-years	1.03 (0.97 to 1.10)	0.98 (0.84 to 1.15)	1.21 (1.04 to 1.40)	0.79 (0.59 to 1.05)
Categorical, pack-years				
Never	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
≤ 10	1.12 (0.99 to 1.27)	1.21 (0.91 to 1.59)	1.29 (0.86 to 1.93)	1.04 (0.67 to 1.63)
> 10 to 20	1.09 (0.92 to 1.28)	0.91 (0.61 to 1.37)	1.62 (0.96 to 2.72)	1.25 (0.66 to 2.37)
> 20 to 35	1.08 (0.87 to 1.32)	1.12 (0.77 to 1.63)	1.53 (0.89 to 2.61)	0.94 (0.42 to 2.11)
> 35	1.13 (0.94 to 1.35)	1.20 (0.78 to 1.85)	2.13 (1.27 to 3.55)	0.98 (0.40 to 2.40)

NOTE. Associations stratified by birth year and adjusted for age at study entry, parity, and duration of oral contraceptive use (except when parity or oral contraceptive use was the primary exposure of interest, and then we adjusted only for the other risk factor).
*Meta-analysis *P* value for heterogeneity across studies < .01 by using the *q* statistic from a random-effects meta-analysis.
†Parous women only.
‡Postmenopausal women only.
§Also adjusted for duration of hormone therapy use.

Appendix 2: Draft manuscript "Ovarian cancer risk factors by tumor aggressiveness: An analysis from the Ovarian Cancer Cohort Consortium"

Ovarian cancer risk factors by tumor aggressiveness: An analysis from the Ovarian Cancer Cohort Consortium

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Key words: ovarian cancer, risk factors, subtypes, aggressiveness, cohort

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Abstract:

Ovarian cancer is a heterogeneous disease, with established risk factor differences by histologic subtype; however, within subtype there is substantial variability in outcomes and we hypothesized that risk factor profiles may influence tumor aggressiveness, as defined by time between diagnosis and death. Among 1.3 million women from 21 prospective cohort studies, 5,576 invasive epithelial ovarian cancers were identified and classified as highly aggressive (death in <1 year, n=864), very aggressive (death in 1-<3 years, n=1,390), moderately aggressive (death in 3-<5 years, n=639), and less aggressive (lived 5+ years, n=1,691); other cases did not have sufficient follow-up to classify. Using competing risks Cox proportional hazards regression, we assessed heterogeneity of associations by tumor aggressiveness for all invasive cancers and separately among serous and endometrioid/clear cell tumors. We observed significant differences in the associations between parity (p_{het}=0.01), family history of ovarian cancer (p_{het}=0.02), body mass index (BMI, kg/m^2; p_{het}<0.04) and smoking status (p_{het}=0.01) and ovarian cancer risk by disease aggressiveness. A first pregnancy was inversely associated with highly aggressive disease (HR: 0.72; 95%CI [0.58-0.88]), but no association was observed for subsequent pregnancies (HR: 0.97 [0.92-1.02]). In contrast, both first and subsequent pregnancies were similarly associated with less aggressive disease (HR: 0.87 for both). Family history of ovarian cancer was only associated with risk of less aggressive disease (HR: 1.94 [1.47-2.55]). Both high BMI and current smoking were associated with highest risk of highly aggressive disease (BMI ≥35 vs. 20-<25 kg/m^2, HR: 1.93 [1.46-2.56]; current vs. never smoking, HR: 1.14 [1.04-1.25]). Results were similar within histotypes. Our results suggest that risk factor profiles may drive tumor aggressiveness. Additional work to assess biological pathways for these relationships is warranted.

Introduction

Ovarian cancer is one of the most fatal cancers in women, with over 150,000 deaths globally per year (1). The five-year relative survival for ovarian cancer patients is about 45%, while the ten-year relative survival is only slightly lower at 35% (2, 3); further, across all stages of disease, the probability of surviving the next five years increases with longer survival (4). This, in conjunction with data showing worse outcomes for high-grade serous tumors compared to other types (5-7), suggests that some tumors may be innately more aggressive than others. While differences in survival across tumor subtypes can be explained, in part, by surgical outcomes (8), a recent study noted that changes in chemotherapy did not substantially influence long-term survival (9). More recently, studies have shown that exposures before diagnosis are differently associated with ovarian cancer subtypes (10-14), with each histologic type showing a distinct pattern of risk factor associations (10). However, few studies have considered whether risk factor profiles may be associated with more versus less aggressive ovarian cancers.

One prior study including two prospective cohort studies and two case-control studies used time to death as a surrogate for characterizing more versus less aggressive disease (i.e., death within 3 years versus not, respectively) (15). Multiple established ovarian cancer risk factors, including age, parity, oral contraceptive (OC) use, and menopausal status, were differentially associated with risk by tumor aggressiveness for all invasive and serous tumors. For example, each birth was associated with a significant 13% lower risk of less aggressive disease but only a 2% lower risk for more aggressive tumors, although the first birth was associated with a similar ~20% lower risk of both tumor types. We undertook a replication and expansion of this analysis within the Ovarian Cancer Cohort Consortium (OC3), which included 21 prospective cohort studies across Europe, Asia, and North America. With more than 5,500

invasive ovarian cancer cases, we examined the relationship of 17 established and putative risk factors by tumor aggressiveness, defined by time to death (<1, 1-<3, 3-<5, 5+ years) for all invasive tumors as well as within specific histologic subtypes.

Methods

Study population

The OC3 includes women participating in 23 prospective cohort studies, 21 of which have sufficient cases and follow-up for death (at least 3 years of follow-up for >50 cases) to be included in this analysis (Table 1). Studies were required to have prospective follow-up of ovarian cancer endpoints through questionnaires, medical records or cancer registries, as well as follow-up for death, along with data on age at study entry, OC use, and parity. All studies obtained institutional approval for cohort maintenance as well as participation in the OC3. The OC3 Data Coordinating Center and analytic approaches were approved by the institutional review board of the Brigham and Women's Hospital (BWH).

Exposure assessment

Either full baseline cohort data (19 studies) or case-cohort datasets with weights for subcohort members (2 studies) were obtained and centrally harmonized. We examined multiple putative and known ovarian cancer risk factors, including parity (no children, first child, linear term for subsequent children), age at first birth (per 1 year; <20, 20-<25, 25-<30, 30+ years), age at last birth (per 1 year; <25, 25-<30, 30-<35, 35+ years), duration of OC use (per 5 years of use; never, ≤1, >1-≤5, >5-≤10, >10 years), duration of breastfeeding (per 1 year; ever vs. never among parous women), age at menarche (per 1 year; ≤11, 12, 13, 14, ≥15 years), age at natural menopause (postmenopausal women only: per 5 years; ≤40, >40-≤45, >45-≤50, >50-≤55, >55

years), duration of menopausal hormone therapy (HT) use (postmenopausal women only: per 1 year; never, ≤5, >5 years), tubal ligation (ever vs. never), hysterectomy (ever vs. never), endometriosis (ever vs. never), first degree family history of breast cancer (ever vs. never), first degree family history of ovarian cancer (ever vs. never), BMI at baseline (per 5 kg/m^2; <20, 20-<25, 25-<30, 30-<35, ≥35 kg/m^2), BMI at age 18-20 years (per 5 kg/m^2; <18, 18-<20, 20-<22, ≥22 kg/m^2), height (per 0.05m; <1.60, 1.60-<1.65, 1.65-1.70, ≥1.70 m), and smoking at baseline (never, former, current). Studies that did not provide data on a specific risk factor were excluded from the analysis of that factor, leading to different numbers of cases for each exposure (Supplemental Table 1).

Outcome definition

Epithelial ovarian or peritoneal cancer cases were confirmed through cancer registries or medical record review (ICD9: 183, 158; ICD10: C56); details were described previously (10). For each case, we requested information on date of or age at diagnosis, histology (classified as serous/poorly differentiated, endometrioid, mucinous, clear cell, other/unknown), and date of or age at death (if applicable). All studies obtained information on deaths during the course of follow-up, primarily through mortality registries and family members; all studies have >95% mortality follow-up. We calculated the time between diagnosis and death for all cases who died and classified tumors as highly aggressive (death in <1 year, n=864), very aggressive (death in 1-<3 years, n=1,390), moderately aggressive (death in 3-<5 years, n=639), and less aggressive (lived 5+ years, n=1,691). For cases who did not die during follow-up, we excluded those who had less than 5 years of follow-up time after diagnosis (n=992).

Statistical methods

Women with a history of cancer (other than non-melanoma skin cancer), with bilateral oophorectomy prior to study entry, or missing age at baseline were excluded. We calculated hazard ratios (HR) and 95% confidence intervals (95% CI) using competing risks Cox proportional hazards regression to evaluate associations between exposures and ovarian cancer based on aggressiveness (16). Follow-up time was time between study entry and date of i) ovarian cancer diagnosis, ii) death, or iii) end of follow-up, whichever occurred first. Survivor function plots for exposures generally showed parallel curves, suggesting no relevant deviation from proportional hazards; deviations were due to small numbers of exposed cases within a specific category of aggressiveness. In primary analyses, we pooled data from all cohorts, and stratified on year of birth and cohort to account for potential differences in baseline hazards by these factors; associations were similar to those using random effects meta-analysis to combine cohort-specific estimates (data not shown). Statistical heterogeneity of associations across tumor aggressiveness categories was assessed via a likelihood ratio test comparing a model allowing the association for the risk factor of interest to vary by aggressiveness versus one not allowing the association to vary (17). A trend test was calculated across the ordinal aggressiveness subtype beta coefficients using meta-regression. All models were adjusted for age at entry, number of children, and duration of OC use, unless the exposure of interest was collinear with one of these factors. Hysterectomy analyses were additionally adjusted for HT use. For missing data in covariates, we included a missing indicator in the model.

We considered all invasive cases together and conducted analyses among serous tumors only and endometrioid/clear cell tumors; we combined these latter subtypes due to their similar risk factor profiles, as observed in our prior analysis (10). We evaluated known high-grade

serous tumors in a secondary analysis. For BMI and smoking, we conducted sensitivity analyses excluding cases diagnosed within 2 years of baseline (to address potential for reverse causation), excluding all women with cardiovascular disease (CVD) or diabetes at baseline, and stratified by stage at diagnosis; for BMI, we also stratified by menopausal status and HT use. We also performed unsupervised hierarchical clustering of the four aggressiveness categories alone and further separated by histology (serous and endometrioid/clear cell) using beta estimates for exposures that had differential associations by tumor aggressiveness overall invasive cases or within the serous or endometrioid/clear cell subsets using complete linkage and uncentered correlation (Pearson's coefficient). SAS 9.4 was used to conduct the analyses. A p-value of <0.05 was considered statistically significant. As a sensitivity analysis, we corrected for multiple comparisons for the test of heterogeneity using an adjusted alpha of 0.003 (0.05/17 exposures).

Results

Study population

Among 1,203,353 participants (1,300,044 including full cohort size for case-cohort studies), 5,576 invasive epithelial ovarian cancers were identified during follow-up. Case numbers ranged from 1,009 for breastfeeding to 4,530 for smoking status (Supplemental Table 1). This study included 3,378 (73.7% of cases with known histology) serous, 606 (13.2%) endometrioid, 331 (7.2%) mucinous, and 269 (5.9%) clear cell carcinomas. Fifteen of 21 cohorts were based in North America, five in Europe, and one in Asia (Table 1); about half of the cohorts started enrollment in the 1990s. The median age at diagnosis was 71.0 years for highly aggressive (death <1 years), 67.5 years for very aggressive (death 1-<3 years), 65.6 years for moderately aggressive (death 3-<5 years), and 62.7 years for less aggressive (lived at least 5 years).

Associations of putative and established risk factors

Parity (p_{het}=0.01), family history of ovarian cancer (p_{het}=0.02), BMI (p_{het}<0.04), and smoking status (p_{het}<0.01) were differentially associated with ovarian cancer by aggressiveness (Table 2). Both higher parity and family history of ovarian cancer were most strongly associated with less aggressive disease, though in opposing directions, whereas very high and very low BMI and smoking were both more strongly associated with increased risk of highly aggressive disease.

A first child (i.e., parity of 1) conferred significant protection against highly and very aggressive disease (e.g., highly aggressive (HR: 0.72 [95% CI: 0.58-0.88]); subsequent pregnancies conferred no additional protection (HR: 0.97 [0.92-1.02]). For less aggressive disease, both the first and subsequent pregnancies were associated with lower risk (first pregnancy, HR: 0.87 [0.74-1.01]; subsequent pregnancies, HR: 0.87 [0.83-0.91]]; p_{trend} across aggressiveness categories=0.002). Family history of ovarian cancer was associated with higher risk of all but the highly aggressive ovarian cancers, with risk increasing stepwise with lower aggressiveness (e.g., highly aggressive, HR: 0.70 [0.38-1.32]); less aggressive, HR: 1.94 [1.47-2.55]; $p_{trend\_aggressiveness}$ =0.01).

In contrast higher BMI and current smoking were associated with higher risk of highly aggressive, but not less aggressive, disease ($p_{trend\_aggressiveness}$, BMI ≥35 kg/m^2 category=0.002; current smoking=0.002). Notably, relative to women in the normal weight category (BMI 20-<25 kg/m^2), higher risk of highly aggressive disease was observed in women in both the lowest (<20 kg/m^2; HR: 1.36 [1.04-1.77]) and highest (≥35 kg/m^2; HR: 1.93 [1.46-2.56]) BMI categories.

We also observed a significant trend across aggressiveness categories for duration of HT use (>5 years; p=0.03) and family history of breast cancer (p=0.03), both suggestive of higher risk with

less aggressive disease, and tubal ligation (p=0.02), suggestive of lower risk with less aggressive disease. However, the p for heterogeneity overall using the likelihood ratio test was not statistically significant (all p=0.12). No heterogeneity was observed for the other examined risk factors.

Analyses in Histologic Subgroups

We next evaluated the associations separately for (i) serous (n=3,378; Supplemental Table 2) and (ii) endometrioid /clear cell (n=875; Supplemental Table 3) disease. Overall, results were of similar magnitude and in the same direction as those observed for invasive ovarian cancer overall. Among cases of endometrioid/clear cell disease, age at last birth appeared to be more strongly associated with risk of less aggressive disease (p-trend across aggressiveness≤0.04). Further, we observed a significant trend across aggressiveness categories for tubal ligation (p=0.02), and height (p=0.01) in this subset of cases; however, the p for heterogeneity was not statistically significant (p≥0.08). Among serous tumors, breastfeeding was suggestively associated more strongly with highly and very aggressive tumors compared to the less aggressive phenotypes (p-trend across aggressiveness=0.09).

Sensitivity Analyses

We conducted sensitivity analyses for both BMI and smoking to evaluate associations excluding cases diagnosed within 2 years of baseline, diagnosed with CVD or diabetes at baseline, and stratified by stage at diagnosis (Supplemental Tables 5-6); we further evaluated BMI associations by menopausal status at baseline and among postmenopausal women by HT use (data not shown). Patterns of association were similar in these subgroups, with the exception of analyses restricted to women diagnosed at stages 1 or 2, in which the associations between both BMI and

smoking and highly aggressive disease were attenuated. After adjusting for multiple comparisons, none of the p_{het} remained statistically significant. However, the p_{trend} across aggressiveness categories for parity, BMI ($\geq$35 kg/m^2 category), and smoking met the stricter $p<0.003$ criterion.

We further considered clustering of risk factor associations by tumor aggressiveness alone and when further stratifying by histology (Figure 1). Overall, the risk factor profile for highly aggressive disease was clearly distinct from the other tumor types (Figure 1a), which was independent of histotype (Figure 1b). Further, risk factor associations tended to cluster by tumor aggressiveness rather than histotype, such that highly aggressive serous and non-serous tumors clustered together, then very aggressive serous and non-serous tumors, and so on. However, certain risk factors, such as endometriosis and number of children, tended to only be associated with one histotype (e.g., non-serous tumors) regardless of tumor aggressiveness.

Discussion

We identified parity, family history of ovarian cancer, BMI, and smoking as risk factors that were differentially associated with ovarian cancer by tumor aggressiveness, overall and within specific histotypes, in this first large-scale, prospective investigation on the topic. Notably, high BMI and smoking, two relatively readily modifiable risk factors, were most strongly associated with higher risk of the most aggressive, rapidly fatal, ovarian cancers. Further, clustering analysis suggested that risk factor associations largely tracked with tumor aggressiveness rather than by histology.

To date, only one pooled study, including two retrospective and two prospective studies, has investigated risk differences by ovarian cancer aggressiveness defined using time to death. Poole

et al. compared risk of “rapidly fatal” (death from ovarian cancer within 3 years of diagnosis) and “less aggressive” disease (alive or did not die from ovarian cancer within 3 years of diagnosis) (15), observing significant heterogeneity in the associations between select ovarian cancer risk factors and risk of disease by aggressiveness. In both that study and ours, cases with the most aggressive disease in our study were, on average, nearly 8 years older than the least aggressive phenotype. This is consistent with registry data showing higher risk of death among women with older age at diagnosis (e.g., (4, 18)). Consistent with the prior study (15), we observed a similar inverse association for the first pregnancy and ovarian cancer risk, regardless of tumor aggressiveness. However, subsequent pregnancies (i.e., beyond the first) were only associated with lower risk of less aggressive disease. When excluding the two studies included in Poole et. al., the results were similar (data not shown). These results are in agreement with a recent study on ovarian cancer survival, reporting lower risk of death following a diagnosis among women with higher parity (19), although this has not been consistently observed in all studies ((20); reviewed in (21)). Recent studies based on the hypothesized dualistic pathway (22) demonstrate a stronger inverse association between parity and “type I” (less aggressive), as compared to “type II” (more aggressive) ovarian cancer (23, 24), in agreement with our findings. Considering biologic mechanisms, the first pregnancy is associated with long-term permanent alterations in hormone regulation, including lower prolactin levels, which have been associated with ovarian cancer risk. This may impact all tumor types similarly. However, it also has been hypothesized that pregnancy can lead to a clearance of malignant cells (i.e., a “wash out” effect) (25), which might impact relatively slowing progressing tumors (i.e., developing over a period of years), rather than rapidly progressing disease that is more likely to have developed in the interval since pregnancy or after child-bearing years are complete. That said, we did not observe

a clear pattern of association for age at last birth across categories of aggressiveness, even when not adjusting for parity (data not shown), although relatively few studies had these data, reducing power. Parity-related reductions in ovulatory cycles (26) are unlikely to explain the observed heterogeneity, given we observed no differences by aggressiveness for oral contraceptive use, or ages at menarche or menopause, all contributors to the number of lifetime ovulatory cycles.

Higher BMI was positively associated with risk of highly aggressive ovarian cancer, but not less aggressive disease. The association between BMI and ovarian cancer did not differ by aggressiveness in the study by Poole et al. (15); however, results on ovarian cancer survival are in line with our findings (21, 27). In a large study within the Ovarian Cancer Cohort Consortium (OCAC), Nagle et al. observed 3% higher risk of death following an ovarian cancer diagnosis for each 5-unit increment in BMI above 18.5 kg/m^2 (HR: 1.03, 95% CI: 1.00–1.07) in a study (27); the authors' reported similar results in analyses restricted to deaths from ovarian cancer. Consistent with our study, results for survival in OCAC were of the same general magnitude for serous and endometrioid disease. The results in our study did not appear to be influenced substantially by reverse causation (i.e., ovarian cancer influencing weight before diagnosis), or concurrence of other morbidities, such as cardiovascular disease.

Obesity may impact disease aggressiveness via its impact on the metabolic milieu, reduced efficacy of treatment, or by providing a permissive local microenvironment for metastases. The associations between BMI and adipokines, insulin resistance and the metabolic syndrome (28), and oxidative stress and chronic low-grade inflammation (29) are well described; in turn, these factors have been hypothesized to be associated with ovarian cancer progression ((30, 31); reviewed in (32-34)). In addition to impacting metabolic markers, adiposity is associated with higher endogenous estrogen concentrations, as a result of an upregulation of aromatase activity

(35), particularly in postmenopausal women (36, 37). However, the trends we observed for exogenous HT use were in the opposite direction of those observed for BMI, providing no support for the hypothesis that endogenous estrogens may be an intermediate mechanism. In terms of treatment-related factors, suboptimal surgical cytoreductive (i.e., debulking) surgery and insufficient chemotherapy dosing, may result in more highly aggressive (i.e., rapidly fatal) disease (38-41)) in obese women. A recent study evaluated the chemotherapeutic "relative dose intensity", considering mg/kg of administered chemotherapeutic agent (38), reporting that women with BMI of 40 or higher received 45% lower dose intensity, relative to normal weight women. In turn, relative dose intensity <85% was associated with worse survival (38). Finally, omental adipose tissue has been identified as a tumor promoting microenvironment (42); thus, this adipose depot proximate to the ovarian tumor may promote tumor progression and metastasis. Interestingly, we also observed that individuals with very low BMI were at increased risk for highly aggressive disease; this should be confirmed in other studies and mechanisms explored to better understand this potential relationship.

We observed an association between current smoking and highly aggressive, but not less aggressive, disease. Smoking is associated with higher risk of death following an ovarian cancer diagnosis ((43); reviewed in (21)) and may drive development of a more aggressive phenotype via its well-described inflammation- and oxidative stress-inducing effects (44). Further, limited data suggest that smoking may impact the effectiveness of neoadjuvant therapy (45), particularly for mucinous tumors. This is agreement with observed differences between smoking and ovarian cancer mortality by histology in OCAC (43), with the strongest associations between smoking and mortality observed for mucinous disease. We observed similar associations in serous and

endometrial/clear cell subgroups in the current study; case numbers precluded evaluating smoking by aggressiveness among mucinous cases.

Family history of ovarian cancer was most strongly associated with less aggressive ovarian cancer, with a similar trend observed for family history of breast cancer. This is consistent with prior investigations suggesting a survival benefit proximate to diagnosis for women carrying an inherited BRCA mutation (46, 47), potentially due to better response to platinum-based chemotherapies and PARP inhibitors (21). This survival benefit is evident in the relative short term after diagnosis (i.e., 3-5 years) (46), as would be captured in our moderately and less aggressive disease categories.

Finally, we observed suggestive heterogeneity in the associations between duration of postmenopausal HT use and tubal ligation and ovarian cancer risk by aggressiveness. The associations between HT use and tubal ligation did not differ by aggressiveness in the prior analysis by Poole et al. (15), nor are they consistently associated with survival (21). In the current study, longer duration of HT use was more strongly associated with increased risk of less aggressive disease. Data on circulating sex steroid hormones suggest heterogeneity by disease subtype, with a study in the OC3 reporting significantly different associations between circulating pre-diagnosis endogenous androgens and ovarian cancer risk by the dualistic pathway (48). Higher androgen concentrations increased risk of type I (less aggressive) ovarian cancer risk, but not type II (more aggressive) disease, providing indirect support for our findings. Androgens are a substrate for estrogen production, and correlated in postmenopausal women (e.g. testosterone and estradiol, postmenopausal women, r=0.23-0.38; (49, 50)). We observed an inverse association between tubal ligation and less aggressive ovarian cancer, and no association with highly aggressive disease. This is supported by one prior study demonstrating a stronger

inverse association between type I vs. type II ovarian cancer (24). Tubal ligation was only associated with lower risk of the endometrioid/clear cell histologic subtype in our prior analysis in the OC3 (10); we observed a stronger inverse association with less aggressive disease in this subgroup in the current study.

We hypothesized that pre-diagnosis exposures may influence whether ovarian cancers develop toward “less” vs. “more” aggressive phenotypes, defined by survival time following an ovarian cancer diagnosis. Overall, results were similar by histologic subgroups, suggesting the observed heterogeneity was not principally driven by tumor histology. Importantly, in clustering analysis, our results suggested that risk factor associations track more clearly by tumor aggressiveness rather than by histology. This suggests that other metrics of tumor heterogeneity should be evaluated to identify potential etiologic mechanisms that relate risk factors to disease development. For example, Kurman and colleagues suggested that ovarian cancer develops along two pathways: type I disease, a less aggressive phenotype including low grade serous and endometrioid, mucinous, clear cell, and malignant Brenner tumors, and; type II disease, more aggressive disease, primarily including high grade serous and endometrioid tumors (22). Prognosis for type I tumors is significantly better than that observed for type II disease (5, 51). An alternative, complementary, approach to that implemented here would be to evaluate risk by the proposed dualistic model (22), classifying tumors using histology and grade. However, grade data were not available for the majority of the cases in this study. A further limitation of this investigation is the lack of post-diagnosis treatment information, including chemotherapy regimen and debulking status. Poole et al. (15) observed minimal impact on the differences between rapidly fatal vs. less aggressive disease before and after adjusting for both chemotherapy regimen and debulking status, suggesting that these factors may not be important

covariates. Finally, despite the relatively large sample size, data availability for the investigated risk factors varied by cohort and was limited for some exposures (e.g., endometriosis, duration of breastfeeding) and analyses by aggressiveness within histologic subgroups were limited; these analyses were restricted to the two major subgroups identified in our earlier investigation (10).

We provide novel data on risk factors for ovarian cancer by aggressiveness, defined by time to death, in this pooled analysis in the OC3, identifying obesity and current smoking as modifiable risk factors predominantly associated with higher risk of highly aggressive (i.e., rapidly fatal) ovarian cancer. Further research is required to more fully describe the mechanistic pathways underlying these associations. However, our study supports a role for maintaining healthy weight and smoking cessation in reducing risk of ovarian cancers with the least favorable outcomes.

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Table 1. Characteristics of cohorts in the Ovarian Cancer Cohort Consortium

Study name	Location	Baseline enrollment period	Baseline cohort size[a]	Median study participant age	Median follow-up (years)	Last year of follow-up	Invasive ovarian cancer cases
Adventist Health Study II	U.S.	2002-2007	39,030	53	8	2015	57
Breast Cancer Detection Demonstration Project Follow-up Study	U.S.	1987-1989	36,209	61	9	1999	145
California Teachers Study	U.S.	1995-1999	43,778	50	15	2010	185
Campaign against Cancer and Stroke	U.S.	1989	12,382	46	22	2012	82
Canadian Study of Diet, Lifestyle, and Health	Canada	1991-1999	2,745[b]	58	16	2010	90
Cancer Prevention Study II Nutrition Cohort	U.S.	1992-1993	65,884	62	15	2009	533
European Prospective Investigation into Cancer and Nutrition Study	Europe	1992-2000	263,796	51	13	2010	671
Iowa Women's Health Study	U.S.	1986	30,537	61	23	2010	263
Melbourne Collaborative Cohort Study	Australia	1990-1994	20,836	55	16	2009	95
Multiethnic/Minority Cohort Study[c]	U.S.	1993-1998	16,462	57	11	2011	63
New York University Women's Health Study	U.S.	1984-1991	12,420	49	24	2012	122
Netherlands Cohort Study on diet and cancer	Netherlands	1986	2,755[b]	62	17	2009	446
NIH-AARP Diet and Health Study	U.S.	1995-1997	153,049	62	11	2006	703
Nurses' Health Study 1980[d]	U.S.	1980-1982	86,627	46	16	1998	351
Nurses' Health Study 1996[d]	U.S.	1996-1998	67,522	62	14	2010	408
Nurses' Health Study II	U.S.	1989-1990	111,800	35	20	2011	214
Prostate, Lung, Colorectal and Ovarian	U.S.	1993-2002	60,191	62	12	2009	358

Cancer Screening Trial							
Singapore Chinese Health Study	Singapore	1993-1999	31,939	56	14	2011	95
Swedish Mammography Cohort Study	Sweden	1997	34,425	60	14	2011	161
VITamins And Lifestyle Cohort	U.S.	2000-2002	28,331	60	10	2011	130
Women's Health Study	U.S.	1993-1996	33,548	53	18	2012	204
Women's Lifestyle and Health	Sweden	1991-1992	49,087	40	21	2012	201

[a]After exclusions for baseline cancers and women with bilateral oophorectomy

[b]These cohorts were included as a case-cohort design, reflecting a total cohort population of 39,618 women for the CSDLH and 62,573 women for the NLCS. Appropriate weights for subcohort selection were applied in all analyses.

[c]Including only Caucasian women.

[d]The Nurses' Health Study was broken into two study periods (1980-June 1996 and July 1996-2010) because the follow-up was nearly twice as long as any other study. We updated the exposures in 1996 for that follow-up period.

Table 2: Associations[a] of ovarian cancer risk factors with invasive epithelial ovarian cancer by tumor aggressiveness in the Ovarian Cancer Cohort Consortium

	Highly aggressive RR (95% CI)	Very Aggressive RR (95% CI)	Moderately aggressive RR (95% CI)	Less aggressive RR (95% CI)	p-het. by aggress.[b]	p-trend across categories of agress.[c]
Parity						
No children	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)		
First child	0.72 (0.58,0.88)	0.80 (0.67,0.94)	0.98 (0.76,1.28)	0.87 (0.74,1.01)	**0.01**	0.13
Subsequent children	0.97 (0.92,1.02)	0.94 (0.90,0.98)	0.95 (0.90,1.01)	0.87 (0.83,0.91)		**0.002**
Age at first birth, per yr	0.99 (0.97,1.00)	1.00 (0.98,1.01)	0.99 (0.97,1.01)	1.01 (1.00,1.02)	0.19	0.08
<20	1.13 (0.85,1.50)	1.07 (0.86,1.33)	1.05 (0.78,1.41)	1.01 (0.83,1.24)		0.54
20-<25	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	0.56	
25-<30	0.98 (0.81,1.17)	0.92 (0.80,1.05)	0.97 (0.79,1.19)	1.05 (0.92,1.19)		0.30
30+	0.85 (0.65,1.10)	1.02 (0.84,1.23)	0.81 (0.60,1.09)	1.10 (0.93,1.31)		0.18
Age at last birth, per yr	1.00 (0.97,1.02)	1.01 (0.99,1.03)	0.98 (0.95,1.00)	1.01 (0.99,1.03)	0.26	0.51
<25	1.31 (0.86,2.01)	0.96 (0.67,1.39)	1.01 (0.64,1.58)	0.89 (0.66,1.19)		0.20
25-<30	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	0.32	
30-<35	1.20 (0.88,1.62)	1.14 (0.90,1.43)	1.04 (0.75,1.43)	1.08 (0.89,1.31)		0.56
35+	1.19 (0.85,1.68)	1.06 (0.82,1.39)	0.59 (0.37,0.92)	1.06 (0.84,1.33)		0.51
Duration of breastfeeding, per yr[d]	0.96 (0.80,1.15)	0.82 (0.68,0.98)	1.00 (0.86,1.18)	0.97 (0.87,1.09)	0.24	0.48
Ever vs never	0.90 (0.58,1.39)	0.67 (0.48,0.93)	0.98 (0.59,1.61)	1.01 (0.77,1.33)	0.27	0.20
Duration of oral contraceptive use, per 5 yr	0.89 (0.81,0.99)	0.82 (0.76,0.89)	0.87 (0.78,0.97)	0.82 (0.77,0.88)	0.48	0.38
Never	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)		
≤1	0.91 (0.68,1.21)	0.90 (0.73,1.10)	1.03 (0.77,1.37)	1.02 (0.86,1.21)		0.31
>1-≤5	0.83 (0.65,1.06)	0.87 (0.73,1.03)	0.98 (0.77,1.24)	0.84 (0.73,0.98)	0.95	0.99
>5-≤10	0.74 (0.56,0.99)	0.66 (0.54,0.82)	0.80 (0.59,1.07)	0.76 (0.64,0.91)		0.52
>10	0.72 (0.52,1.01)	0.59 (0.45,0.77)	0.60 (0.41,0.88)	0.57 (0.46,0.72)		0.37
Age at menarche, per 1 yr	0.99 (0.95,1.04)	0.97 (0.94,1)	1.01 (0.96,1.06)	0.97 (0.94,1.01)	0.64	0.78
≤11	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	0.13	

12	0.88 (0.70,1.10)	0.84 (0.71,0.99)	1.00 (0.78,1.28)	0.95 (0.82,1.10)		0.32
13	0.96 (0.79,1.18)	0.86 (0.74,1.00)	1.14 (0.91,1.43)	0.90 (0.79,1.04)		0.98
14	0.83 (0.65,1.06)	0.81 (0.67,0.98)	0.89 (0.67,1.19)	1.00 (0.85,1.18)		0.10
≥15	0.99 (0.78,1.26)	0.83 (0.69,1.01)	1.11 (0.83,1.48)	0.75 (0.62,0.91)		0.17
Age at menopause, per 5 yr	1.02 (0.94,1.12)	1.04 (0.97,1.11)	0.98 (0.89,1.09)	1.09 (1.02,1.16)	0.38	0.31
≤40	1.13 (0.83,1.54)	1.02 (0.79,1.33)	1.18 (0.81,1.71)	0.71 (0.54,0.95)		0.05
>40-≤45	0.89 (0.67,1.19)	0.71 (0.55,0.90)	1.08 (0.77,1.51)	0.82 (0.65,1.03)		0.87
>45-≤50	1.02 (0.85,1.23)	0.95 (0.82,1.10)	1.04 (0.83,1.31)	0.89 (0.77,1.03)	0.48	0.33
>50-≤55	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)		
>55	1.20 (0.87,1.64)	1.02 (0.78,1.33)	1.14 (0.77,1.69)	0.94 (0.72,1.24)		0.35
Duration of hormone therapy use, per 1 yr[e]	1.03 (1.01,1.04)	1.03 (1.02,1.04)	1.05 (1.03,1.06)	1.04 (1.03,1.05)	0.27	0.12
Never	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)		
≤5 years	0.92 (0.74,1.14)	1.18 (0.99,1.40)	1.29 (1.00,1.66)	1.26 (1.06,1.47)	0.12	**0.05**
>5 years	1.26 (1.01,1.58)	1.52 (1.28,1.80)	1.87 (1.47,2.39)	1.69 (1.43,1.99)		**0.03**
Tubal ligation, ever vs. never	0.94 (0.65,1.36)	0.95 (0.75,1.21)	0.78 (0.55,1.11)	0.66 (0.53,0.82)	0.12	**0.02**
Hysterectomy, ever vs. never[f]	0.88 (0.73,1.06)	0.83 (0.72,0.97)	1.09 (0.89,1.34)	0.92 (0.80,1.06)	0.21	0.36
Endometriosis, ever vs. never	1.41 (0.66,3.00)	1.07 (0.59,1.95)	1.41 (0.75,2.68)	1.58 (1.06,2.33)	0.76	0.46
Family history of breast cancer, yes vs. no	0.88 (0.70,1.11)	1.08 (0.91,1.28)	1.21 (0.95,1.54)	1.20 (1.04,1.41)	0.12	**0.03**
Family history of ovarian cancer, yes vs. no	0.70 (0.38,1.32)	1.45 (1.04,2.04)	1.62 (1.01,2.60)	1.94 (1.47,2.55)	**0.02**	**0.01**
Body mass index in adulthood, per 5kg/m2	1.15 (1.07,1.23)	1.04 (0.98,1.10)	1.03 (0.95,1.12)	0.99 (0.94,1.04)	**0.01**	**0.002**
<20	1.36 (1.04,1.77)	1.02 (0.81,1.27)	0.98 (0.71,1.36)	0.94 (0.78,1.15)		0.06
20-<25	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)		
25-<30	1.15 (0.98,1.35)	0.99 (0.87,1.12)	0.94 (0.78,1.13)	0.95 (0.85,1.07)	**0.04**	0.10
30-<35	1.34 (1.07,1.67)	0.96 (0.80,1.16)	1.10 (0.85,1.42)	0.96 (0.81,1.14)		0.07
≥35	1.93 (1.46,2.56)	1.34 (1.07,1.69)	1.01 (0.70,1.45)	0.98 (0.78,1.24)		**0.0002**
Body mass index at age 18-20, per 5kg/m2	1.11 (0.97,1.28)	1.06 (0.95,1.19)	1.01 (0.86,1.18)	0.97 (0.87,1.08)	0.45	0.10
<18	1.04 (0.76,1.42)	0.84 (0.64,1.11)	0.83 (0.57,1.21)	1.04 (0.83,1.3)		0.71
18-<20	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	0.62	
20-<22	1.09 (0.87,1.36)	1.05 (0.87,1.25)	0.84 (0.65,1.10)	1.06 (0.91,1.24)		0.79

≥22	1.04 (0.83,1.31)	0.99 (0.82,1.19)	1.01 (0.78,1.31)	0.93 (0.79,1.10)		0.46
Height, per 0.05m	1.06 (1.01,1.12)	1.09 (1.04,1.13)	1.04 (0.98,1.10)	1.07 (1.03,1.11)	0.71	0.86
<1.60m	0.81 (0.67,0.98)	0.89 (0.76,1.03)	0.88 (0.70,1.09)	0.94 (0.82,1.07)		0.30
1.60-<1.65m	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	0.70	
1.65-<1.70m	0.90 (0.75,1.08)	1.05 (0.91,1.21)	1.07 (0.87,1.31)	1.10 (0.97,1.26)		0.12
≥1.70m	1.13 (0.93,1.37)	1.21 (1.04,1.41)	1.05 (0.83,1.32)	1.11 (0.97,1.28)		0.63
Smoking						
Never	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)		
Former	0.91 (0.77,1.08)	1.07 (0.95,1.21)	1.02 (0.85,1.22)	0.95 (0.85,1.07)	**0.004**	0.79
Current	1.30 (1.07,1.57)	1.00 (0.85,1.17)	0.78 (0.60,1.01)	088 (0.76,1.02)		**0.002**

[a]Stratified on birth year and cohort, and adjusted for age at study entry, parity, and duration of oral contraceptive use (except when parity or oral contraceptive use was the primary exposure of interest and then we adjusted only for the other risk factor) using pooled analyses of all cohorts combined.

[b]Assessed using a likelihood ratio test comparing a Cox proportional hazards competing risks model allowing the association to vary by subtype to a model forcing the association to be the same across subtypes Highly aggressive: death <1 yr; Very ggressive: death 1-<3 yr; Moderately aggressive: death 3-<5 yr; Less aggressive: alive at 5 yr).

[c]Trend across the ordinal aggressiveness subtypes using meta-regression with a subtype-specific random effect term

[d]Parous women only.

[e]Postmenopausal women only.

[f]Additionally adjusted for duration of hormone therapy use.

Supplemental Table 1. Number of invasive epithelial ovarian cancer cases by tumor aggressiveness and histologic type for each exposure

	Highly aggressive	Very Aggressive	Moderately aggressive	Less aggressive
All invasive cases				
Number of children	817	1342	611	1618
Age at first birth	659	1105	514	1310
Age at last birth	274	444	216	628
Duration of breastfeeding	162	283	140	424
Duration of oral contraceptive use	816	1347	618	1645
Age at menarche	835	1370	631	1677
Age at menopause (postmenopausal only)	636	956	428	964
Duration of hormone therapy use (postmenopausal only)	695	1012	470	1019
Tubal ligation	610	985	460	1226
Hysterectomy	811	1284	599	1531
Endometriosis	169	292	195	457
First degree family history of breast cancer	839	1329	610	1603
First degree family history of ovarian cancer	694	1070	500	1287
Body mass index in adulthood	827	1336	615	1611
Body mass index at age 18-20	543	777	381	1008
Height	845	1355	624	1630
Smoking status	848	1377	631	1674
Invasive serous cases				
Number of children	681	1175	524	1069
Age at first birth	554	974	447	890
Age at last birth	217	380	188	409
Duration of breastfeeding	129	248	123	280
Duration of oral contraceptive use	699	1189	542	1094
Age at menarche	695	1192	541	1093
Age at menopause (postmenopausal only)	540	836	370	653
Duration of hormone therapy use (postmenopausal only)	622	958	429	741
Tubal ligation	511	859	401	796
Hysterectomy	689	1122	517	999
Endometriosis	137	257	170	306
First degree family history of breast cancer	707	1155	526	1044

First degree family history of ovarian cancer	591	942	431	850
Body mass index in adulthood	689	1161	529	1048
Body mass index at age 18-20	459	674	321	657
Height	705	1176	535	1058
Smoking status	707	1195	541	1088
Invasive endometrioid and clear cell cases				
Number of children	315	352	144	617
Age at first birth	239	288	110	463
Age at last birth	75	102	35	227
Duration of breastfeeding	47	66	20	163
Duration of oral contraceptive use	319	356	143	630
Age at menarche	321	362	143	640
Age at menopause (postmenopausal only)	249	249	90	353
Duration of hormone therapy use (postmenopausal only)	277	261	100	357
Tubal ligation	240	271	112	474
Hysterectomy	305	340	138	576
Endometriosis	60	94	51	193
First degree family history of breast cancer	326	356	141	618
First degree family history of ovarian cancer	286	286	123	494
Body mass index in adulthood	326	356	141	618
Body mass index at age 18-20	217	199	89	379
Height	330	365	143	628
Smoking status	329	366	146	644

Supplemental Table 2: Associations[a] of ovarian cancer risk factors with invasive serous epithelial ovarian cancer by tumor aggressiveness in the Ovarian Cancer Cohort Consortium

	Highly aggressive RR (95% CI)	Very Aggressive RR (95% CI)	Moderately aggressive RR (95% CI)	Less aggressive RR (95% CI)	p-het. by aggress.[b]	p-trend across categories of agress.[c]
Parity						
No children	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)		
First child	0.82 (0.64,1.03)	0.87 (0.72,1.04)	1.08 (0.81,1.44)	1.02 (0.84,1.24)	0.24	0.09
Subsequent children	0.95 (0.90,1.01)	0.94 (0.90,0.98)	0.97 (0.92,1.03)	0.91 (0.86,0.95)		0.26
Age at first birth, per yr	0.99 (0.97,1.01)	1.00 (0.98,1.01)	1.00 (0.98,1.02)	1.01 (1.00,1.03)	0.33	0.11
<20	1.05 (0.77,1.43)	1.05 (0.83,1.32)	1.02 (0.74,1.40)	1.09 (0.85,1.39)		0.85
20-<25	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	0.73	
25-<30	0.99 (0.81,1.21)	0.96 (0.82,1.11)	1.00 (0.81,1.24)	1.11 (0.95,1.30)		0.22
30+	0.86 (0.65,1.14)	1.02 (0.83,1.24)	0.89 (0.65,1.22)	1.21 (0.98,1.49)		0.07
Age at last birth, per yr	0.99 (0.96,1.02)	1.01 (0.99,1.03)	0.99 (0.97,1.02)	1.01 (0.99,1.04)	0.46	0.29
<25	1.43 (0.90,2.27)	0.93 (0.62,1.39)	0.90 (0.53,1.50)	1.04 (0.72,1.51)		0.43
25-<30	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	0.48	
30-<35	1.15 (0.81,1.61)	1.16 (0.91,1.49)	1.20 (0.86,1.69)	1.27 (1.00,1.62)		0.57
35+	1.14 (0.78,1.66)	1.03 (0.77,1.38)	0.68 (0.42,1.10)	1.23 (0.92,1.64)		0.75
Duration of breastfeeding, per yr[d]	0.89 (0.71,1.13)	0.81 (0.67,0.98)	1.03 (0.88,1.20)	1.02 (0.90,1.16)	0.11	0.09
Ever vs never	0.87 (0.54,1.40)	0.74 (0.52,1.05)	1.10 (0.65,1.86)	1.14 (0.82,1.60)	0.32	0.13
Duration of oral contraceptive use, per 5 yr	0.84 (0.75,0.96)	0.78 (0.72,0.86)	0.88 (0.79,0.99)	0.81 (0.74,0.88)	0.40	1.00
Never	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)		
≤1	0.92 (0.68,1.25)	0.82 (0.66,1.03)	1.10 (0.81,1.49)	1.02 (0.83,1.26)		0.26
>1-≤5	0.74 (0.56,0.98)	0.85 (0.70,1.02)	0.98 (0.76,1.27)	0.87 (0.72,1.04)	0.87	0.40
>5-≤10	0.68 (0.49,0.95)	0.60 (0.47,0.76)	0.82 (0.60,1.13)	0.70 (0.56,0.87)		0.51
>10	0.65 (0.44,0.96)	0.54 (0.40,0.72)	0.61 (0.40,0.92)	0.56 (0.42,0.75)		0.76
Age at menarche, per 1 yr	0.98 (0.93,1.03)	0.97 (0.93,1.00)	0.99 (0.94,1.04)	0.98 (0.94,1.02)	0.90	0.72
≤11	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)		
12	0.82 (0.64,1.05)	0.83 (0.69,0.99)	0.97 (0.75,1.27)	0.94 (0.78,1.13)	0.41	0.25
13	0.94 (0.76,1.17)	0.89 (0.75,1.04)	1.09 (0.85,1.39)	0.86 (0.72,1.02)		0.74
14	0.81 (0.62,1.06)	0.82 (0.67,1.01)	0.85 (0.62,1.15)	1.03 (0.84,1.27)		0.10

≥15	0.90 (0.69,1.17)	0.80 (0.65,0.99)	1.01 (0.74,1.38)	0.78 (0.62,0.99)		0.64
Age at menopause, per 5 yr	1.02 (0.93,1.11)	1.02 (0.95,1.10)	0.99 (0.89,1.10)	1.05 (0.97,1.13)	0.88	0.67
≤40	1.18 (0.85,1.65)	1.08 (0.82,1.42)	1.17 (0.78,1.76)	0.76 (0.54,1.07)		0.09
>40-≤45	0.88 (0.64,1.19)	0.72 (0.55,0.94)	1.10 (0.77,1.57)	0.91 (0.69,1.20)		0.44
>45-≤50	1.00 (0.82,1.22)	0.97 (0.83,1.14)	1.06 (0.83,1.35)	0.92 (0.77,1.11)	0.70	0.67
>50-≤55	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)		
>55	1.14 (0.81,1.62)	1.00 (0.75,1.32)	1.11 (0.73,1.71)	0.91 (0.64,1.27)		0.70
Duration of hormone therapy use, per 1 yr[e]	1.02 (1.01,1.04)	1.04 (1.02,1.05)	1.05 (1.04,1.07)	1.04 (1.03,1.06)	0.10	**0.03**
Never	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)		
≤5 years	0.92 (0.72,1.16)	1.16 (0.97,1.39)	1.39 (1.07,1.82)	1.19 (0.97,1.46)	0.06	0.10
>5 years	1.25 (0.99,1.59)	1.55 (1.29,1.85)	1.99 (1.53,2.58)	1.88 (1.55,2.28)		**0.01**
Tubal ligation, ever vs. never	1.05 (0.71,1.55)	0.97 (0.74,1.25)	0.74 (0.51,1.07)	0.73 (0.57,0.95)	0.28	0.06
Hysterectomy, ever vs. never[f]	0.88 (0.72,1.07)	0.87 (0.74,1.01)	1.11 (0.90,1.38)	1.00 (0.84,1.17)	0.25	0.15
Endometriosis, ever vs. never	1.03 (0.38,2.79)	1.01 (0.52,1.96)	1.14 (0.53,2.44)	1.21 (0.69,2.12)	0.98	0.68
Family history of breast cancer, yes vs. no	0.90 (0.71,1.16)	1.03 (0.86,1.24)	1.10 (0.84,1.43)	1.23 (1.02,1.48)	0.24	**0.04**
Family history of ovarian cancer, yes vs. no	0.75 (0.38,1.45)	1.34 (0.93,1.94)	1.65 (1.00,2.72)	2.31 (1.70,3.13)	**0.01**	**0.001**
Body mass index in adulthood, per 5kg/m2	1.11 (1.03,1.2)	1.04 (0.98,1.11)	1.02 (0.94,1.12)	0.96 (0.90,1.02)	**0.03**	**0.05**
<20	1.40(1.05,1.87)	1.01 (0.80,1.28)	0.98 (0.69,1.40)	0.92 (0.72,1.18)		**0.05**
20-<25	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)		
25-<30	1.10(0.92,1.31)	0.97 (0.84,1.11)	0.84 (0.69,1.04)	0.93 (0.80,1.07)	0.06	0.14
30-<35	1.32 (1.04,1.68)	0.96 (0.79,1.16)	1.11 (0.85,1.45)	0.87 (0.70,1.08)		**0.04**
≥35	1.70 (1.24,2.35)	1.38 (1.08,1.74)	1.03 (0.71,1.51)	0.89 (0.66,1.20)		**0.002**
Body mass index at age 18-20, per 5kg/m2	1.06 (0.91,1.24)	1.03 (0.91,1.17)	1.00 (0.84,1.19)	0.98 (0.86,1.12)	0.87	0.40
<18	0.97 (0.69,1.35)	0.79 (0.59,1.06)	0.87 (0.58,1.31)	0.98 (0.74,1.30)		0.72
18-<20	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	0.88	
20-<22	1.08 (0.85,1.37)	1.05 (0.86,1.27)	0.89 (0.67,1.18)	1.10(0.90,1.33)		0.99
≥22	0.92 (0.72,1.19)	0.91 (0.75,1.12)	1.00 (0.75,1.33)	0.97 (0.79,1.19)		0.66
Height, per 0.05m	1.06 (1.00,1.12)	1.08 (1.03,1.13)	1.05 (0.99,1.11)	1.07 (1.02,1.12)	0.90	0.97

<1.60m	0.88 (0.71,1.08)	0.90 (0.77,1.06)	0.83 (0.66,1.06)	0.85 (0.71,1.01)		0.66
1.60-<1.65m	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	0.60	
1.65-<1.70m	1.00 (0.82,1.23)	1.05 (0.90,1.23)	1.09 (0.87,1.36)	1.17 (1.00,1.37)		0.21
≥1.70m	1.18 (0.95,1.46)	1.19 (1.01,1.41)	0.96 (0.75,1.24)	1.04 (0.87,1.25)		0.22
Smoking						
Never	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)		
Former	0.90 (0.75,1.08)	1.07 (0.94,1.22)	0.96 (0.79,1.17)	0.97 (0.85,1.11)	**0.05**	0.97
Current	1.26 (1.02,1.55)	0.98 (0.82,1.16)	0.81 (0.61,1.06)	0.90 (0.75,1.08)		**0.02**

[a]Stratified on birth year and cohort, and adjusted for age at study entry, parity, and duration of oral contraceptive use (except when parity or oral contraceptive use was the primary exposure of interest and then we adjusted only for the other risk factor) using pooled analyses of all cohorts combined.

[b]Assessed using a likelihood ratio test comparing a Cox proportional hazards competing risks model allowing the association to vary by subtype to a model forcing the association to be the same across subtypes Highly aggressive: death <1 yr; Very ggressive: death 1-<3 yr; Moderately aggressive: death 3-<5 yr; Less aggressive: alive at 5 yr).

[c]Trend across the ordinal aggressiveness subtypes using meta-regression with a subtype-specific random effect term

[d]Parous women only.

[e]Postmenopausal women only.

[f]Additionally adjusted for duration of hormone therapy use.

Supplemental Table 3: Associations[a] of ovarian cancer risk factors with invasive endometrioid and clear cell epithelial ovarian cancer by tumor aggressiveness in the Ovarian Cancer Cohort Consortium

	Highly aggressive RR (95% CI)	Very Aggressive RR (95% CI)	Moderately aggressive RR (95% CI)	Less aggressive RR (95% CI)	p-het. by aggress.[b]	p-trend acros categories o agress.[c]
Parity						
No children	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)		
First child	0.69 (0.47,1.01)	1.02 (0.70,1.49)	0.97 (0.55,1.69)	0.84 (0.63,1.12)	0.32	0.67
Subsequent children	0.92 (0.85,1.01)	0.87 (0.79,0.95)	0.86 (0.75,0.98)	0.83 (0.76,0.90)		0.09
Age at first birth, per yr	0.98 (0.95,1.02)	0.97 (0.94,1.00)	1.00 (0.96,1.05)	1.01 (0.99,1.03)	0.15	0.05
<20	1.21 (0.77,1.91)	1.39 (0.95,2.03)	0.87 (0.42,1.77)	0.89 (0.62,1.27)		0.13
20-<25	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	0.32	
25-<30	0.91 (0.66,1.26)	0.68 (0.52,0.91)	0.91 (0.57,1.43)	0.93 (0.75,1.15)		0.43
30+	0.84 (0.54,1.29)	0.92 (0.65,1.31)	0.81 (0.44,1.51)	1.09 (0.83,1.44)		0.29
Age at last birth, per yr	0.98 (0.94,1.03)	1.00 (0.96,1.05)	0.93 (0.87,0.99)	1.04 (1.01,1.07)	**0.01**	**0.02**
<25	1.10 (0.49,2.46)	1.33 (0.69,2.57)	0.68 (0.22,2.14)	0.57 (0.33,0.97)		0.06
25-<30	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	**0.04**	
30-<35	1.24 (0.71,2.16)	1.04 (0.64,1.69)	0.40 (0.17,0.95)	0.84 (0.60,1.16)		0.20
35+	0.87 (0.44,1.73)	1.22 (0.71,2.11)	0.24 (0.07,0.88)	1.26 (0.88,1.82)		0.46
Duration of breastfeeding, per yr[d]	0.69 (0.45,1.05)	1.01 (0.74,1.38)	0.94 (0.54,1.66)	1.03 (0.86,1.23)	0.37	0.19
Ever vs never	0.53 (0.24,1.16)	0.64 (0.33,1.25)	0.60 (0.18,2.03)	1.02 (0.67,1.56)	0.38	0.10
Duration of oral contraceptive use, per 5 yr	0.85 (0.71,1.02)	0.87 (0.75,1.01)	0.77 (0.61,0.96)	0.82 (0.74,0.93)	0.81	0.56
Never	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)		
≤1	0.79 (0.48,1.29)	1.07 (0.73,1.57)	1.13 (0.61,2.07)	0.98 (0.74,1.28)		0.71
>1-≤5	0.85 (0.58,1.25)	0.99 (0.71,1.37)	1.16 (0.73,1.84)	0.85 (0.67,1.08)	0.95	0.77
>5-≤10	0.62 (0.38,1.02)	0.85 (0.57,1.27)	0.79 (0.42,1.5)	0.84 (0.64,1.09)		0.45
>10	0.64 (0.36,1.15)	0.64 (0.39,1.07)	0.43 (0.17,1.09)	0.52 (0.36,0.78)		0.47
Age at menarche, per 1 yr	0.97 (0.90,1.04)	0.95 (0.89,1.02)	1.05 (0.94,1.17)	0.95 (0.90,1.00)	0.37	0.79
≤11	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)		
12	0.77 (0.53,1.12)	0.68 (0.49,0.93)	0.95 (0.55,1.66)	0.91 (0.73,1.15)	0.20	0.20
13	1.02 (0.73,1.42)	0.67 (0.50,0.91)	1.32 (0.81,2.14)	0.83 (0.66,1.04)		0.82

14	0.81 (0.54,1.21)	0.71 (0.50,1.01)	1.13 (0.63,2.03)	0.92 (0.70,1.19)		0.35
≥15	0.85 (0.57,1.27)	0.70 (0.48,1.01)	1.37 (0.75,2.52)	0.61 (0.44,0.83)		0.30
Age at menopause, per 5 yr	1.07 (0.92,1.23)	1.07 (0.92,1.23)	0.92 (0.75,1.12)	1.14 (1.02,1.27)	0.39	0.57
≤40	1.18 (0.73,1.88)	0.99 (0.57,1.72)	1.04 (0.40,2.69)	0.64 (0.39,1.06)		0.09
>40-≤45	0.60 (0.37,0.97)	0.75 (0.47,1.20)	1.11 (0.54,2.29)	0.80 (0.55,1.18)		0.34
>45-≤50	0.87 (0.64,1.17)	0.92 (0.69,1.24)	1.35 (0.83,2.19)	0.92 (0.72,1.17)	0.65	0.70
>50-≤55	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)		
>55	1.17 (0.71,1.94)	1.30 (0.81,2.07)	0.82 (0.32,2.15)	1.00 (0.64,1.58)		0.50
Duration of hormone therapy use, per 1 yr[e]	1.02 (1.00,1.05)	1.03 (1.01,1.06)	1.06 (1.03,1.09)	1.03 (1.01,1.05)	0.46	0.71
Never	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)		
≤5 years	0.72 (0.49,1.06)	1.27 (0.91,1.78)	1.15 (0.62,2.14)	1.24 (0.95,1.63)	0.15	0.08
>5 years	1.18 (0.83,1.69)	1.59 (1.12,2.27)	1.97 (1.15,3.38)	1.28 (0.95,1.72)		0.88
Tubal ligation, ever vs. never	0.96 (0.50,1.83)	0.71 (0.42,1.19)	0.85 (0.44,1.64)	0.41 (0.27,0.62)	0.08	**0.02**
Hysterectomy, ever vs. never[f]	0.83 (0.62,1.13)	0.75 (0.55,1.01)	1.01 (0.65,1.56)	0.84 (0.66,1.06)	0.76	0.76
Endometriosis, ever vs. never	1.47 (0.46,4.73)	1.65 (0.73,3.71)	1.9 (0.68,5.34)	1.82 (1.05,3.14)	0.99	0.74
Family history of breast cancer, yes vs. no	0.90 (0.62,1.30)	1.09 (0.79,1.51)	1.04 (0.61,1.77)	1.30 (1.02,1.65)	0.40	0.11
Family history of ovarian cancer, yes vs. no	0.58 (0.19,1.83)	2.06 (1.14,3.71)	1.53 (0.56,4.16)	1.14 (0.64,2.02)	0.18	0.78
Body mass index in adulthood, per 5kg/m2	1.18 (1.06,1.33)	1.14 (1.03,1.27)	1.09 (0.94,1.26)	1.01 (0.93,1.10)	0.11	**0.02**
<20	1.06 (0.67,1.67)	0.81 (0.51,1.29)	0.87 (0.42,1.81)	1.01 (0.75,1.37)		0.88
20-<25	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)		
25-<30	1.19 (0.93,1.54)	1.16 (0.91,1.48)	1.29 (0.88,1.89)	0.91 (0.75,1.11)	0.44	0.08
30-<35	1.41 (0.99,2.01)	1.20 (0.85,1.69)	1.16 (0.67,2.01)	0.93 (0.71,1.23)		0.07
≥35	2.12 (1.35,3.31)	1.58 (1.01,2.48)	0.95 (0.41,2.24)	1.22 (0.86,1.72)		**0.05**
Body mass index at age 18-20, per 5kg/m2	1.08 (0.88,1.34)	1.35 (1.12,1.62)	0.94 (0.69,1.30)	0.97 (0.81,1.16)	0.09	0.18
<18	1.04 (0.64,1.68)	0.92 (0.52,1.62)	1.43 (0.68,3.00)	1.01 (0.70,1.42)		0.992
18-<20	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	0.07	
20-<22	1.01 (0.71,1.44)	1.06 (0.72,1.56)	1.29 (0.73,2.28)	0.98 (0.76,1.26)		0.86
≥22	1.07 (0.75,1.54)	1.67 (1.16,2.39)	1.18 (0.67,2.08)	0.79 (0.60,1.04)		0.13

Height, per 0.05m	1.06 (0.97,1.15)	1.14 (1.05,1.23)	1.02 (0.89,1.18)	1.06 (1.00,1.13)	0.43	0.59
<1.60m	0.68 (0.50,0.92)	0.82 (0.61,1.11)	1.13 (0.71,1.78)	1.09 (0.87,1.35)	0.10	**0.01**
1.60-<1.65m	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)		
1.65-<1.70m	0.69 (0.51,0.92)	0.93 (0.70,1.24)	1.00 (0.63,1.58)	1.15 (0.92,1.43)		**0.01**
≥1.70m	0.98 (0.73,1.33)	1.40 (1.06,1.86)	1.16 (0.71,1.89)	1.23 (0.98,1.55)		0.49
Smoking						
Never	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	**0.01**	
Former	0.86 (0.66,1.12)	1.28 (1.01,1.63)	0.89 (0.61,1.30)	0.94 (0.78,1.12)		0.82
Current	1.19 (0.87,1.63)	1.23 (0.91,1.65)	0.64 (0.37,1.10)	0.71 (0.55,0.92)		**0.002**

[a]Stratified on birth year and cohort, and adjusted for age at study entry, parity, and duration of oral contraceptive use (except when parity or oral contraceptive use was the primary exposure of interest and then we adjusted only for the other risk factor) using pooled analyses of all cohorts combined.

[b]Assessed using a likelihood ratio test comparing a Cox proportional hazards competing risks model allowing the association to vary by subtype to a model forcing the association to be the same across subtypes Highly aggressive: death <1 yr; Very ggressive: death 1-<3 yr; Moderately aggressive death 3-<5 yr; Less aggressive: alive at 5 yr).

[c]Trend across the ordinal aggressiveness subtypes using meta-regression with a subtype-specific random effect term

[d]Parous women only.

[e]Postmenopausal women only.

[f]Additionally adjusted for duration of hormone therapy use.

Supplemental Table 4. Sensitivity analyses exploring the relationship of BMI with risk of ovarian cancer by tumor aggressiveness

BMI categories in kg/m2	Highly aggressive RR (95% CI)	Very Aggressive RR (95% CI)	Moderately aggressive RR (95% CI)	Less aggressive RR (95% CI)	p-het. by aggress.[b]	p-trend across categories of agress.[c]
All women (n, cases)	827	1336	615	1611		
<20	1.36 (1.04,1.77)	1.02(0.81,1.27)	0.98 (0.71,1.36)	0.94 (0.78,1.15)		0.06
20-<25	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)		
25-<30	1.15 (0.98,1.35)	0.99 (0.87,1.12)	0.94 (0.78,1.13)	0.95 (0.85,1.07)	**0.04**	0.10
30-<35	1.34 (1.07,1.67)	0.96 (0.80,1.16)	1.10 (0.85,1.42)	0.96 (0.81,1.13)		0.07
≥35	1.93 (1.46,2.56)	1.34 (1.07,1.69)	1.01 (0.70,1.45)	0.98 (0.77,1.24)		**<0.001**
Excluding cases diagnosed within 2 yr of baseline	770	1193	538	1381		
<20	1.30 (0.98,1.72)	1.03 (0.81,1.29)	1.05 (0.75,1.49)	0.93 (0.76,1.15)		0.09
20-<25	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)		
25-<30	1.13 (0.96,1.34)	0.98 (0.86,1.12)	0.97 (0.79,1.19)	0.95 (0.84,1.08)	**0.05**	0.16
30-<35	1.31 (1.04,1.64)	0.95 (0.78,1.16)	1.17 (0.89,1.53)	0.94 (0.78,1.13)		0.10
≥35	1.94 (1.45,2.58)	1.32 (1.04,1.69)	1.09 (0.74,1.60)	0.90 (0.69,1.18)		**<0.001**
Excluding women with CVD or diabetes at baseline	533	945	421	1117		
<20	1.52 (1.11,2.08)	0.82 (0.61,1.09)	1.07 (0.73,1.58)	0.92 (0.73,1.17)		0.17
20-<25	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)		
25-<30	1.12 (0.92,1.38)	0.93 (0.80,1.08)	0.93 (0.74,1.17)	0.95 (0.83,1.09)	0.18	0.35
30-<35	1.35 (1.02,1.78)	1.05 (0.85,1.30)	1.15 (0.84,1.56)	1.07 (0.88,1.30)		0.36

≥35	1.79 (1.22,2.62)	1.43 (1.08,1.88)	1.06 (0.68,1.67)	1.00 (0.75,1.34)		**0.01**
Only considering stage 1 and 2 cases	148	235	114	701		
<20	1.11 (0.57,2.17)	1.00 (0.57,1.74)	0.79 (0.34,1.84)	1.01 (0.76,1.35)		0.90
20-<25	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)		
25-<30	1.15 (0.80,1.65)	1.09 (0.81,1.47)	0.97 (0.64,1.48)	0.94 (0.79,1.12)	0.86	0.23
30-<35	0.75 (0.39,1.46)	1.13 (0.72,1.76)	0.68 (0.33,1.37)	0.91 (0.70,1.19)		0.95
≥35	1.27 (0.50,3.22)	1.78 (0.99,3.19)	0.61 (0.19,2.00)	1.00 (0.70,1.44)		0.20
Only considering stage 3 or 4 cases	457	766	356	499		
<20	1.30 (0.90,1.87)	1.04 (0.78,1.39)	1.15 (0.77,1.73)	0.81 (0.55,1.18)		0.12
20-<25	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)		
25-<30	1.09 (0.88,1.36)	0.95 (0.80,1.12)	0.92 (0.71,1.18)	0.89 (0.73,1.10)	0.15	0.21
30-<35	1.41 (1.06,1.86)	0.77 (0.60,1.00)	1.30 (0.95,1.77)	0.95 (0.72,1.27)		0.55
≥35	1.41 (0.95,2.11)	1.26 (0.95,1.68)	1.17 (0.75,1.82)	0.98 (0.66,1.44)		0.18
Premenopausal at baseline	79	197	110	435		
<20	1.25 (0.62,2.51)	0.95 (0.58,1.56)	1.31 (0.72,2.38)	0.73 (0.51,1.05)		0.17
20-<25	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)		
25-<30	0.65 (0.34,1.24)	1.14 (0.81,1.61)	0.83 (0.5,1.38)	1.07 (0.85,1.35)	0.26	0.50
30-<35	1.86 (0.95,3.65)	0.79 (0.44,1.41)	1.55 (0.85,2.8)	1.36 (0.98,1.88)		0.90
≥35	1.80	1.21 (0.63,2.32)	1.20 (0.51,2.82)	1.31 (0.85,2.01)		0.76

	(0.71,4.55)					
Postmenopausal at baseline	734	1107	496	1114		
<20	1.33 (0.99,1.78)	1.02 (0.79,1.31)	0.81 (0.54,1.23)	1.03 (0.8,1.31)		0.22
20-<25	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)		
25-<30	1.20 (1.01,1.43)	0.99 (0.86,1.13)	0.95 (0.77,1.16)	0.93 (0.81,1.06)	**0.02**	**0.03**
30-<35	1.29 (1.02,1.64)	1.01 (0.83,1.23)	1.00 (0.76,1.33)	0.84 (0.68,1.03)		**0.01**
≥35	1.99 (1.48,2.68)	1.38 (1.07,1.76)	0.98 (0.65,1.47)	0.89 (0.67,1.19)		**<0.001**

[a]Stratified on birth year and cohort, and adjusted for age at study entry, parity, and duration of oral contraceptive use using pooled analyses of all cohorts combined. HT=Hormone therapy

[b]Assessed using a likelihood ratio test comparing a Cox proportional hazards competing risks model allowing the association to vary by subtype to a model forcing the association to be the same across subtypes Highly aggressive: death <1 yr; Very ggressive: death 1-<3 yr; Moderately aggressive: death 3-<5 yr; Less aggressive: alive at 5 yr).

[c]Trend across the ordinal aggressiveness subtypes using meta-regression with a subtype-specific random effect term

Supplemental Table 5. Sensitivity analyses exploring the relationship of smoking with risk of ovarian cancer by tumor aggressiveness

Smoking categories	**Highly aggressive RR (95% CI)**	**Very Aggressive RR (95% CI)**	**Moderately aggressive RR (95% CI)**	**Less aggressive RR (95% CI)**	**p-het. by aggress.[b]**	**p-trend acros categories o agress.[c]**
All women (n, cases)	848	1376	631	1674		
Never	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)		
Former	0.91 (0.77,1.08)	1.07 (0.95,1.21)	1.02 (0.85,1.22)	0.95 (0.85,1.07)	**0.004**	0.79
Current	1.30 (1.07,1.57)	1.00 (0.85,1.17)	0.78 (0.60,1.01)	088 (0.76,1.02)		**0.002**
Excluding cases diagnosed within 2 yr of baseline	791	1226	551	1434		
Never	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)		
Former	0.92 (0.77,1.09)	1.05 (0.92,1.20)	1.06 (0.88,1.28)	0.94 (0.83,1.06)	**0.01**	0.85
Current	1.29 (1.05,1.57)	0.97 (0.82,1.15)	0.81 (0.62,1.07)	0.84 (0.73,0.99)		**0.004**

Excluding women with CVD or diabetes at baseline	554	986	433	1181		
Never	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)		
Former	0.85 (0.69,1.05)	1.05 (0.90,1.21)	1.03 (0.83,1.28)	0.93 (0.82,1.07)	**0.01**	0.99
Current	1.31 (1.05,1.65)	1.04 (0.87,1.24)	0.73 (0.54,0.99)	0.89 (0.75,1.05)		**0.005**
Only considering stage 1 and 2 cases	152	247	117	735		
Never	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)		
Former	0.75 (0.50,1.13)	1.09 (0.81,1.46)	1.50 (1.00,2.24)	0.90 (0.76,1.07)	0.26	0.79
Current	0.98 (0.62,1.53)	0.87 (0.59,1.28)	1.07 (0.62,1.84)	0.93 (0.75,1.15)		0.97
Only considering stage 3 or 4 cases	474	789	367	519		
Never	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)		
Former	0.96 (0.77,1.19)	1.06 (0.91,1.24)	0.84 (0.66,1.06)	0.92 (0.75,1.11)	**0.001**	0.37
Current	1.52 (1.19,1.94)	0.99 (0.80,1.23)	0.65 (0.45,0.92)	0.79 (0.60,1.04)		**<0.001**

[a]Stratified on birth year and cohort, and adjusted for age at study entry, parity, and duration of oral contraceptive use using pooled analyses of all cohorts combined.

[b]Assessed using a likelihood ratio test comparing a Cox proportional hazards competing risks model allowing the association to vary by subtype to a model forcing the association to be the same across subtypes Highly aggressive: death <1 yr; Very ggressive: death 1-<3 yr; Moderately aggressive: death 3-<5 yr; Less aggressive: alive at 5 yr).

[c]Trend across the ordinal aggressiveness subtypes using meta-regression with a subtype-specific random effect term

Appendix 3: Draft manuscript "Ovarian cancer risk factor associations by tumor dominance: the Ovarian Cancer Cohort Consortium"

Reproductive and hormonal factors and risk of ovarian cancer by tumor dominance: Results from the Ovarian Cancer Cohort Consortium (OC3)

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Abstract

Background: Laterality of epithelial ovarian tumors may reflect the underlying carcinogenic pathways and origins of tumor cells. Predominantly unilateral ovarian cancers (i.e., dominant) are more likely to originate from the ovarian surface epithelium, whereas bilateral ovarian cancers (i.e., non-dominant) are more like to have a fallopian tube origin. Elucidating the associations with ovarian cancer risk factors by tumor dominance may help understand the mechanisms through which these factors influence ovarian cancer risk.

Methods: We pooled data from 10 prospective studies participating in the Ovarian Cancer Cohort Consortium. Information on measures of tumor size or tumor dominance was extracted from surgical pathology reports or obtained through cancer registries. We defined dominant tumors as those restricted to one ovary or where the dimension of one ovary at least twice as large as the other, and non-dominant tumors as those with similar dimensions across the two ovaries. Competing risks Cox model was used to examine whether associations with reproductive and hormonal risk factors differed by ovarian tumor dominance.

Results: Of 1,065 ovarian cancer cases with tumor dominance information, 403 were left-dominant, 443 were right-dominant, and 219 were non-dominant. Parity was more strongly inversely associated with risk of dominant than non-dominant ovarian cancer (p-heterogeneity=0.007). Older age at last birth was associated with lower risk of dominant tumors but higher risk of non-dominant tumors (p-heterogeneity=0.08). Similarly, longer years since last birth had a positive association with dominant tumors but an inverse association with non-dominant tumors (p-heterogeneity=0.06). Although the heterogeneity was not statistically significant, an increased risk associated with endometriosis was observed for dominant tumors, but not for non-dominant tumors.

Conclusions: These data suggest that reproductive risk factors appear to have a stronger impact on dominant tumors, which may have an ovarian origin.

Introduction

Ovarian cancer, the most deadly gynecologic malignancy in the US women, is a highly heterogeneous disease. For example, each histotype of ovarian cancer likely originates through a different etiologic pathway, displaying a high level of heterogeneity in clinical behavior and disease progression; importantly each histotype displays a distinct risk factor profile. Further, recent evidence suggests that different types of ovarian tumors may have distinct cellular origins, potentially representing two major carcinogenic pathways. Type 1 ovarian tumors are more likely to arise from the ovarian surface epithelium, be histologically classified as low-grade serous, endometrioid, mucinous, or clear cell subtypes, and harbor mutations in the genes of KRAS, BRAF, β-catenin and pTEN. By contrast, Type 2 tumors are more likely to be high-grade serous carcinomas with a distal fallopian tube origin and p53 mutations. Prior work suggests that tumors originating from the ovarian surface tend to present with a dominant tumor mass (i.e., tumor growth primarily confined to one ovary), whereas tumors of fallopian tube origin tend to be non-dominant (i.e., bilateral tumors with a similar extent of growth or peritoneal tumors). Thus, tumor dominance can be considered as an indicator for ovarian cancer cell of origin, and as such, elucidating the associations with ovarian cancer risk factors by tumor dominance may provide insights into the mechanisms through which these factors influence ovarian cancer development. We conducted the current analysis in the Ovarian Cancer Cohort Consortium (OC3), a large-scale collaborative effort to understand etiologic heterogeneity in ovarian cancer, to examine whether the associations of ovarian cancer risk with reproductive, hormonal, anthropometric and lifestyle factors differed by ovarian tumor dominance.

Methods

Study populations

Ten prospective cohort studies (out of a total of 23 contributing studies) in the OC3 with available data on tumor dominance were included in this analysis (Table 1). All OC3 participating studies had a prospective design with regular follow-up of ovarian cancer

diagnoses and death, and collected key ovarian cancer risk factors (e.g., age, oral contraceptive [OC] use, parity) at baseline. Individual studies were approved by the respective institutional review board following the institution's requirement. The approaches for data pooling, harmonization and analysis, developed by OC3 Data Coordinating Center, were approved by the institutional review board of the Brigham and Women's Hospital.

Exposure assessment

Exposure information at baseline was obtained and harmonized centrally for either the full cohort (9 studies) or a case-cohort sample with weights for subcohort members (1 study). We examined multiple putative and known ovarian cancer risk factors, including parity (nulliparous, 1 child, 2 children, 3 children, ≥4 children; per 1 child), age at first birth (<20, 20-<25, 25-<30, 30+ years; per 1 year), age at last birth (<25, 25-<30, 30-<35, 35+ years; per 1 year), years since last birth (per 1 year), duration of OC use (ever, never; never, ≤1, >1-≤5, >5-≤10, >10 years; per 5 years of use), duration of breastfeeding (per 1 year among parous women), age at menarche (≤11, 12, 13, 14, ≥15 years; per 1 year), age at natural menopause (among postmenopausal women: ≤40, >40-≤45, >45-≤50, >50-≤55, >55 years; per 5 years), duration of postmenopausal hormone therapy (HT) use (among postmenopausal women: ever, never; never, ≤5, >5 years; per 1 year), tubal ligation (yes, no), hysterectomy (yes, no), endometriosis (yes, no), first degree family history of breast cancer (yes, no), first degree family history of ovarian cancer (yes, no), BMI at baseline (<20, 20-<25, 25-<30, 30-<35, ≥35 kg/m^2; per 5 kg/m^2), BMI at age 18-20 years (<18, 18-<20, 20-<22, ≥22 kg/m^2; per 5 kg/m^2), height (<1.60, 1.60-<1.65, 1.65-1.70, ≥1.70 m; per 0.05m), and smoking at baseline (never, ever).

Ovarian cancer ascertainment and tumor dominance definition

Incident cases of epithelial ovarian cancer or peritoneal cancer were identified by self-report or through linkage with cancer registry. Diagnoses were confirmed, and tumor characteristics, including histology, stage, grade, and tumor size, were obtained, by review of

medical or surgical pathology report or linkage with cancer registry data. For cases with surgical pathology report available, we abstracted dimensions, area, or volume recorded for ovarian tumors identified on each side of the peritoneal cavity (left and right). For cases classified through cancer registry, we collected information regarding the extent of tumor growth on each ovary, further extracting data on tumor size on the left and right when available. We considered an ovarian cancer case as having dominant tumor mass if (1) the growth of tumor was limited to one ovary, (2) a tumor mass was found on one ovary, with only tumor foci on the other ovary, or (3) the tumor dimensions, area, or volume on one side was at least twice that of the other side. A case was considered non-dominant if (1) the tumor was classified as primary peritoneal cancer, (2) only tumor foci were found on both ovaries, (3) no ovaries could be identified on either side of the peritoneal cavity, or (4) the tumor dimensions, area, or volume on one side was within two times that of the other side. Cases without appropriate information to classify tumor dominance were censored at time of diagnosis. Of all invasive ovarian cancer cases in each cohort (Table 1), the proportion of cases with tumor dominance data ranged from 28% (Women's Lifestyle and Health) to 69% (Sister Study).

Statistical analysis

Women with a history of cancer (other than non-melanoma skin cancer), with bilateral oophorectomy prior to study entry, or missing age at baseline were excluded. We calculated hazard ratios (HR) and 95% confidence intervals (95% CI) using competing risks Cox proportional hazards regression to evaluate associations between exposures and ovarian cancer based on aggressiveness. Follow-up time was time between study entry and date of i) invasive ovarian cancer diagnosis, ii) death, or iii) end of follow-up, whichever occurred first. Given the relatively small number of available cases, we pooled data from all cohorts, and stratified on year of birth and cohort to account for potential differences in baseline hazards by these factors. Statistical heterogeneity of associations across tumor aggressiveness categories

was assessed via a likelihood ratio test comparing a model allowing the association for the risk factor of interest to vary by dominance (right dominant, left dominant, non-dominant) versus one not allowing the association to vary. All models were adjusted for age at entry, number of children, and duration of OC use, unless the exposure of interest was collinear with one of these factors. Hysterectomy analyses were additionally adjusted for HT use. For missing data in covariates, we included a missing indicator in the model. SAS 9.4 was used to conduct the analyses. A p-value of <0.05 was considered statistically significant. As a sensitivity analysis, we corrected for multiple comparisons for the test of heterogeneity using an adjusted alpha of 0.003 (0.05/18 exposures).

Results

Compared to women who did not develop ovarian cancer during follow-up, those later diagnosed with ovarian cancer were older, had shorter duration of OC use, were more likely to be postmenopausal, and were less likely to be parous, have ever used OC, or have tubal ligation at baseline (Table 2). Compared to ovarian cancer patients with non-dominant tumors, those with dominant tumor mass were older at baseline, had fewer children, were older age at last birth, and were more likely to be postmenopausal, but were less likely to be parous, have ever smoked, have ever used OC, have tubal ligation or hysterectomy, or have a family history of breast cancer or ovarian cancer. Further, compared with women with right-dominant tumors, women with left-dominant tumors were more likely to be parous or postmenopausal, and have ever smoked or used OC.

Of 1,065 incident ovarian cancer cases identified during follow-up with tumor dominance information, 846 (79.4%) were classified as dominant tumors, with 403 (47.6%) having dominant tumor mass on the left and 443 (52.4%) on the right (Table 3). There were higher proportions of serous or stage 3 tumors among non-dominant cases, whereas non-serous and stage 1/2 tumors were more common in dominant cases. When comparing tumor characteristics by

laterality of tumor dominance, there were more serous tumors in left-dominant tumors and more clear cell subtype in right-dominant tumors; other tumor characteristics were similar.

When evaluating reproductive factors with ovarian cancer risk by tumor dominance, parity, tubal ligation, and endometriosis appeared more strongly associated with risk of dominant versus non-dominant ovarian cancer (Table 4). The HR (95% CI) for each additional child was 0.92 (0.86, 0.98) for left-dominant tumors and 0.81 (0.76, 0.87) for right-dominant tumors, compared to 0.98 (0.91, 1.07) for non-dominant tumors (p-heterogeneity=0.007). In addition, age at last birth and years since last birth were differentially associated with ovarian cancer risk by tumor dominance. Older age at last birth tended to be associated with lower risk of dominant tumors but higher risk of non-dominant tumors (p-heterogeneity=0.08). By contrast, longer years since last birth had weak positive associations with dominant tumors but an inverse association with non-dominant tumors (p-heterogeneity=0.06). Although the difference was not statistically significant (p-heterogeneity=0.25), tubal ligation was associated with significantly lower risk of left-dominant tumors (HR: 0.66; 95% CI: 0.44, 0.99) and suggestively lower risk of right-dominant tumors (HR: 0.85; 95%: 0.59, 1.20), but was not associated with non-dominant tumors (HR: 1.06; 95% CI: 0.71, 1.59). Similarly, despite a lack of statistically significant heterogeneity, there was a suggestion of positive associations of endometriosis with dominant tumors but no association with non-dominant tumors.

Overall, we did not observe clear differences by tumor dominance in the associations with hormonal factors, family history, anthropometric factors, and smoking (Table 5). Postmenopausal HT, family history of ovarian cancer, and height were positively associated with ovarian cancer risk regardless of tumor dominance (p-heterogeneity>0.14). However, the associations with OC use and current BMI differed significantly between left- and right-dominant ovarian tumors. The reduced ovarian cancer risk for every 5 years of OC use was significantly lower for right dominant tumors (HR: 0.72; 95% CI: 0.62, 0.85) than for left dominant tumors (HR: 0.92; 95% CI: 0.80, 1.06; p-heterogeneity=0.03). Current BMI was associated with

significantly increased risk of right-dominant ovarian cancer (HR for every 5-unit increase in BMI: 1.11, 95% CI: 1.01, 1.22), although no associations were observed for left-dominant or non-dominant tumors (p-heterogeneity=0.03). After testing for multiple comparisons, no results remained statistically significant.

Discussion

In this pooled analysis of 10 prospective cohort studies, we observed that several reproductive factors, including parity, age at last birth, years since last birth, tubal ligation, and endometriosis, were differentially associated with ovarian cancer risk by tumor dominance, with suggestively stronger relationships with dominant versus non-dominant ovarian tumors. However, the associations with other reproductive factors, hormonal factors, anthropometric measures, family history and smoking did not vary substantially between dominant and non-dominant tumors. Intriguingly, OC use and current BMI showed a different association with left-dominant and right-dominant ovarian tumors.

Our results were consistent with a prior study in NHS, NHSII, and New England Case-Control Study, which reported stronger associations of parity, tubal ligation and endometriosis with dominant tumors than with non-dominant tumors. Taken together, these findings suggest that parity, tubal ligation and endometriosis are more likely to influence ovarian tumors originating from ovarian surface epithelial cells. Indeed, higher parity leads to a lower number of ovulatory cycles, which reduces the possibility of neoplastic progression on the ovarian surface epithelium resulting from ovulation-induced wounds. On the other hand, the elevated progesterone levels during pregnancy may confer potential protection against ovarian carcinogenesis by suppressing proliferation and inducing apoptosis of ovarian epithelial cells. Also, it is hypothesized that the mechanism through which endometriosis increases ovarian cancer risk is possibly due to the reflux and implantation of endometrial fragments onto ovarian surface during menstruation, which leads to inflammation and malignant transformation. Similarly, tubal ligation may be protective for ovarian cancer by blocking the retrograde of

endometrial tissues through fallopian tube and preventing subsequent potential carcinogenesis on ovarian surface.

The observed differences by tumor dominance for age at last birth and years since last birth, which were not examined in the prior study, may also be related to the protection of pregnancy-related progesterone surge on development of dominant ovarian tumors. The trend towards an inverse association between age at last birth and risk of dominant tumors may be explained by the prolonged protection by progesterone that extends to later years of life, whereas the increased risk associated with years since last birth may result from the fact that the potential anti-cancer effects by progesterone are likely to wane over time after last birth. Our results on age at last birth and years since last birth as well as on parity all point to the notion that progesterone may be etiologically relevant for ovarian tumors arising from ovarian surface epithelium. However, further study is needed to understand why the associations for non-dominant tumors appear to go in the opposite direction for age at last birth and time since last birth compared to dominant tumors.

Interestingly, endometriosis and ovarian endometrioma have been shown to have left lateral predisposition. This is consistent with our observation that the association between endometriosis and risk of dominant ovarian cancer was suggestively stronger for left- versus right-dominant tumors. However, it is unclear why the associations with OC use and current BMI also differed by laterality of dominant ovarian tumors. There is some evidence suggesting that BMI had a stronger positive association with distal colon cancer than proximal colon cancer, suggesting that the hormonal impact of adiposity may have different impact across tissue types. Future investigation should replicate these analyses in independent data sets and evaluate potential underlying mechanisms.

This study is strengthened by the relatively large sample size including data from 10 prospective studies, each with abstracted data on tumor size and laterality using a standardized

abstraction procedure. Further, the use of harmonized exposure data reduced the potential for misclassification. However, this study was still limited by a relatively low number of cases, in part because tumor data was not available on a large number of cases, usually because a pathology report was not available or size information about the tumor was not listed in the report. This also precluded an analysis examining associations by tumor dominance within histotypes. Given that we previously showed associations of reproductive factors, in particular, varied by histotypes, we cannot fully separate if the observed differences in association were due to dominance or histotype.

In summary, we found that reproductive factors were more strongly associated with dominant tumors, suggesting that progesterone exposure may be particularly relevant for tumors of ovarian origin. Further, the intriguing, albeit suggestive, differences in association between dominant tumors on the left versus right side for endometrioisis, OC use, and BMI, should be explored in future studies. Additional research should also examine other ways to leverage pathology report data to assess key metrics of tumor heterogeneity to better elucidate etiologic mechanisms underlying ovarian cancer development.

Table 1. Characteristics of included cohorts participating in the Ovarian Cancer Cohort Consortium

Study	Location	Baseline	Cohort size	Median age (yrs)	Median follow-up (yrs)	All invasive cases	Cases with for tumor dominance data
Melbourne Collaborative Cohort Study	Australia	1990-1994	20,836	55	16	95	62
Nurses' Health Study 1980[1]	US	1980-1982	86,608	46	16	351	296
Nurses' Health Study 1996[1]	US	1996-1998	67,530	62	14	408	112
Nurses' Health Study II	US	1989-1990	111,800	35	20	214	143
Netherlands Cohort Study on diet and cancer[2]	Netherlands	1986	2,757	62	17	446	228
New York University Women's Health Study	US	1987-1991	12,427	49	24	122	52
Sister Study	US	2003-2009	39,195	55	5	39	27
Swedish Mammography Cohort Study	Sweden	1997	34,427	60	14	95	29
VITamins And Lifestyle Cohort	US	2000-2002	28,331	60	10	130	40
Women's Health Study	US	1993-1996	33,548	53	18	204	70
Women's Lifestyle and Health	Sweden	1991-1992	49,087	40	21	201	56

[1]The Nurses' Health Study was broken into two study periods (1980-June 1996 and July 1996-2010) because the follow-up was nearly twice as long as any other study. We updated the exposures in 1996 for that follow-up period.

[2]This cohort was included as a case-cohort design, reflecting a total cohort population of 62,573 women. Appropriate weights for subcohort selection were applied in all analyses.

Table 2. Reproductive and hormonal risk factors at baseline by ovarian cancer status and tumor dominance in OC3

	Non-cases	Ovarian cancer cases			
		Non-dominant tumor mass	Dominant tumor mass		
			All	Left-dominant	Right-dominant
N	483,494	219	846	403	443
Age	48.9 (12.5)	51.2 (10.6)	54.1 (11.0)	54.0 (10.7)	54.2 (11.2)
Height (meters)	1.6 (0.1)	1.6 (0.1)	1.7 (0.1)	1.7 (0.1)	1.7 (0.1)
BMI at age 18 (kg/m^2)	21.1 (3.1)	21.1 (2.9)	21.3 (2.8)	21.5 (2.8)	21.2 (2.8)
Current BMI (kg/m^2)	25.2 (5.1)	24.9 (5.4)	25.3 (4.5)	24.9 (4.2)	25.7 (4.8)
Ever smoker, %	47.1	54.3	45.3	47.3	43.5
Age at menarche (yrs)	12.6 (1.5)	12.6 (1.3)	12.9 (1.6)	12.8 (1.6)	13.0 (1.6)
Ever OC use, %	67.4	53.2	42.3	47.5	37.6
Duration of OC use (yrs)[1]	3.4 (4.6)	2.3 (3.8)	1.9 (3.6)	2.2 (3.9)	1.6 (3.3)
Parous, %	85.1	86.2	77.8	81.3	74.5
Parity[2]	2.3 (1.5)	2.8 (1.7)	2.2 (1.8)	2.4 (1.8)	2.1 (1.8)
Age at first birth[2]	22.4 (8.8)	21.8 (9.2)	21.6 (10.2)	22.2 (9.1)	21.0 (11)
Age at last birth[2]	26.2 (11.3)	27.1 (11.9)	25.4 (12.1)	26.5 (10.9)	24.5 (13)
Breastfeeding (months)[2]	10.5 (12.9)	9.0 (12.4)	9.4 (12.5)	10.3 (13.1)	8.6 (11.9)
Postmenopausal status, %	44.2	47.8	64.2	65.7	62.8
Age at menopause[3]	50.2 (4.2)	50.0 (3.4)	49.9 (3.9)	49.9 (3.8)	49.9 (4)
Duration HT use (years)[3]	1.7 (4.3)	2.1 (4.9)	1.3 (3.4)	1.4 (3.5)	1.2 (3.3)
Hysterectomy, %	11.4	14.6	11.8	13.1	10.6
Unilateral oophorectomy, %	3.6	2.7	3.0	2.2	3.6
Tubal ligation, %	16.9	15.6	7.9	7.3	8.6
Family history of breast cancer, %	17.6	15.2	12.9	13.2	12.7
Family history of ovarian cancer, %	2.4	4.3	2.7	2.7	2.7

[1]Among ever users

[2]Among parous women

[3]Among postmenopausal women

Table 3. Ovarian tumor dominance by tumor characteristics in OC3

	Non-dominant tumor mass	Dominant tumor mass		
		All	Left-dominant	Right-dominant
N	219	846	403	443
Histology				
Serous, %	44.3	40.0	42.7	37.5
Endometrioid, %	2.3	13.2	12.9	13.5
Mucinous, %	2.3	8.0	8.4	7.7
Clear cell, %	1.4	7.9	5.5	10.2
Poorly differentiated, %	4.1	2.7	2.7	2.7
Stage				
1 (Localized), %	1.8	24.2	22.3	26.0
2 (Regional), %	9.1	20.2	20.8	19.6
3 (Distant), %	41.1	23.5	24.6	22.6
Unknown, %	4.6	6.7	6.7	6.8
Grade				
Well-differentiated, %	2.7	9.7	9.7	9.7
Moderately differentiated, %	11.4	15.7	14.4	16.9
Poorly differentiated, %	33.3	38.8	40.0	37.7
Undifferentiated, %	2.3	2.3	2.5	2.0
Unknown, %	9.6	15.4	15.1	15.6

Table 4. Associations of ovarian cancer risk factors with reproductive factors by tumor dominance

Risk factors	Left dominant	Right dominant	Non-dominant	P-het
		Hazard ratio (95% CI)		
Parity				
Nulliparous	1.00 (ref)	1.00 (ref)	1.00 (ref)	
1 child	0.80 (0.54, 1.18)	0.81 (0.59, 1.11)	0.60 (0.32, 1.11)	
2 children	0.83 (0.62, 1.13)	0.47 (0.35, 0.62)	0.52 (0.32, 0.84)	
3 children	0.65 (0.47, 0.91)	0.47 (0.35, 0.62)	0.75 (0.47, 1.20)	
≥4 children	0.62 (0.45, 0.85)	0.35 (0.26, 0.48)	0.72 (0.45, 1.16)	
Per 1 child	0.92 (0.86, 0.98)	0.81 (0.76, 0.87)	0.98 (0.91, 1.07)	0.007
Tubal ligation				
No	1.00 (ref)	1.00 (ref)	1.00 (ref)	
Yes	0.66 (0.44, 0.99)	0.85 (0.59, 1.20)	1.06 (0.71, 1.59)	0.25
Hysterectomy				
No	1.00 (ref)	1.00 (ref)	1.00 (ref)	
Yes	0.85 (0.62, 1.15)	0.70 (0.51, 0.96)	0.83 (0.56, 1.23)	0.65
Endometriosis				
No	1.00 (ref)	1.00 (ref)	1.00 (ref)	
Yes	1.97 (0.90, 4.29)	1.55 (0.71, 3.35)	0.92 (0.22, 3.94)	0.82
Age at menarche				
≤11 yrs	1.00 (ref)	1.00 (ref)	1.00 (ref)	
12 yrs	0.81 (0.60, 1.09)	0.95 (0.70, 1.30)	1.12 (0.76, 1.64)	
13 yrs	0.81 (0.61, 1.08)	1.26 (0.95, 1.67)	1.06 (0.72, 1.55)	
14 yrs	1.07 (0.78, 1.46)	1.10 (0.78, 1.53)	1.15 (0.72, 1.82)	
≥15 yrs	0.77 (0.54, 1.11)	1.15 (0.82, 1.62)	0.98 (0.56, 1.70)	
Per 1-year increase	0.98 (0.92, 1.05)	1.03 (0.97, 1.09)	1.01 (0.93, 1.10)	0.56
Age at menopause				
≤40 yrs	0.33 (0.11, 0.98)	1.12 (0.57, 2.19)	0.37 (0.05, 2.84)	
40-45 yrs	1.03 (0.65, 1.64)	0.68 (0.42, 1.11)	0.59 (0.25, 1.44)	
45-50 yrs	1.00 (0.72, 1.37)	1.03 (0.77, 1.39)	0.95 (0.59, 1.51)	
50-55 yrs	1.00 (ref)	1.00 (ref)	1.00 (ref)	
>55 yrs	1.40 (0.76, 2.58)	1.23 (0.70, 2.18)	0.30 (0.04, 2.06)	
Per 1-year increase	1.17 (0.99, 1.38)	1.12 (0.95, 1.32)	1.14 (0.87, 1.51)	0.93
Age at first birth				
<20 yrs	0.99 (0.58, 1.66)	1.02 (0.56, 1.85)	0.45 (0.14, 1.48)	
20-25 yrs	1.00 (ref)	1.00 (ref)	1.00 (ref)	

25-30 yrs	0.91 (0.72, 1.16)	1.46 (1.13, 1.90)	0.88 (0.65, 1.20)	
≥30 yrs	0.68 (0.47, 0.98)	1.42 (1.03, 1.97)	0.78 (0.48, 1.27)	
Per 1-year increase	0.98 (0.96, 1.01)	1.03 (1.00, 1.05)	1.00 (0.96, 1.03)	0.09
Age at last birth				
<25 yrs	0.83 (0.45, 1.53)	0.78 (0.41, 1.48)	0.78 (0.37, 1.64)	
25-30 yrs	1.00 (ref)	1.00 (ref)	1.00 (ref)	
30-35 yrs	1.02 (0.72, 1.43)	0.98 (0.70, 1.39)	1.06 (0.72, 1.56)	
≥35 yrs	0.75 (0.47, 1.20)	0.90 (0.60, 1.37)	1.38 (0.90, 2.11)	
Per 1-year increase	0.98 (0.95, 1.01)	0.99 (0.96, 1.02)	1.03 (1.00, 1.07)	0.08
Years since last birth				
Per 1-year increase	1.02 (0.99, 1.06)	1.01 (0.98, 1.04)	0.97 (0.93, 1.00)	0.06
Breastfeeding				
Per 1-year increase	1.01 (0.83, 1.24)	0.92 (0.73, 1.15)	1.06 (0.85, 1.33)	0.63

*Stratified by cohort and adjusted for age, parity and duration of OC use

Table 5. Associations of hormonal factors, family history, anthropometric factors, and smoking with ovarian cancer risk by tumor dominance

Risk factors	Left dominant	Right dominant	Non-dominant	P-het
	Hazard ratio (95% CI)			
OC use				
Never	1.00 (ref)	1.00 (ref)	1.00 (ref)	
Ever	0.97 (0.77, 1.21)	0.56 (0.45, 0.70)	1.05 (0.77, 1.44)	0.0005
Duration of OC use				
Never	1.00 (ref)	1.00 (ref)	1.00 (ref)	
<1	1.27 (0.92, 1.75)	0.56 (0.39, 0.81)	1.28 (0.84, 1.94)	
1-5	0.87 (0.63, 1.19)	0.56 (0.41, 0.77)	1.23 (0.82, 1.84)	
5-10	0.91 (0.64, 1.28)	0.56 (0.40, 0.78)	0.86 (0.53, 1.41)	
≥10	0.90 (0.58, 1.41)	0.33 (0.18, 0.58)	0.98 (0.54, 1.78)	
Per 5 years of use	0.92 (0.80, 1.06)	0.72 (0.62, 0.85)	0.93 (0.77, 1.13)	0.03
PMH use				
Never	1.00 (ref)	1.00 (ref)	1.00 (ref)	
Ever	1.47 (1.11, 1.96)	1.20 (0.86, 1.67)	1.30 (0.85, 1.98)	0.66
Duration of PMH use				
Never	1.00 (ref)	1.00 (ref)	1.00 (ref)	
<5	1.18 (0.83, 1.69)	1.19 (0.82, 1.73)	1.19 (0.72, 1.94)	
≥5	1.64 (1.12, 2.39)	1.01 (0.65, 1.59)	1.79 (1.10, 2.92)	
Per 1 year of use	1.03 (1.00, 1.06)	1.00 (0.97, 1.04)	1.06 (1.02, 1.09)	0.14
Family history: breast cancer				
No	1.00 (ref)	1.00 (ref)	1.00 (ref)	
Yes	1.20 (0.86, 1.68)	1.11 (0.80, 1.55)	1.42 (0.93, 2.19)	0.67
Family history: ovarian cancer				
No	1.00 (ref)	1.00 (ref)	1.00 (ref)	
Yes	1.60 (0.82, 3.14)	1.59 (0.85, 2.99)	1.62 (0.83, 3.15)	0.99
BMI				
<20	0.85 (0.57, 1.28)	1.06 (0.72, 1.57)	1.52 (0.99, 2.33)	
20-25	1.00 (ref)	1.00 (ref)	1.00 (ref)	
25-30	0.94 (0.75, 1.19)	1.41 (1.14, 1.75)	0.81 (0.57, 1.14)	
30-35	0.79 (0.54, 1.15)	1.37 (0.99, 1.88)	1.10 (0.70, 1.73)	
≥35	0.59 (0.31, 1.12)	1.41 (0.89, 2.25)	1.19 (0.66, 2.16)	
Per 5 units	0.92 (0.82, 1.03)	1.11 (1.01, 1.22)	0.94 (0.80, 1.11)	0.03
BMI at 18				

<18	0.76 (0.46, 1.25)	1.05 (0.69, 1.61)	1.45 (0.90, 2.36)	
18-20	1.00 (ref)	1.00 (ref)	1.00 (ref)	
20-22	1.05 (0.77, 1.43)	1.00 (0.74, 1.35)	0.91 (0.62, 1.33)	
≥22	1.12 (0.82, 1.52)	1.02 (0.75, 1.37)	0.97 (0.67, 1.42)	
Per 5 units	1.12 (0.95, 1.33)	0.95 (0.79, 1.14)	0.88 (0.68, 1.13)	0.25
Height				
<1.60	1.06 (0.80, 1.42)	0.77 (0.58, 1.02)	0.74 (0.49, 1.11)	
1.60-1.65	1.00 (ref)	1.00 (ref)	1.00 (ref)	
1.65-1.70	1.04 (0.80, 1.37)	0.97 (0.75, 1.24)	1.35 (0.96, 1.90)	
≥1.70	1.31 (0.99, 1.73)	1.02 (0.79, 1.33)	1.04 (0.70, 1.53)	
Per 5 units	1.09 (1.00, 1.18)	1.09 (1.01, 1.18)	1.08 (0.98, 1.19)	0.99
Smoking				
Never	1.00 (ref)	1.00 (ref)	1.00 (ref)	
Ever	1.08 (0.88, 1.31)	0.93 (0.76, 1.12)	1.16 (0.88, 1.52)	0.35
Pack-years				
Never	1.00 (ref)	1.00 (ref)	1.00 (ref)	
<10	0.96 (0.73, 1.27)	1.05 (0.82, 1.35)	1.43 (1.02, 1.99)	
10-20	1.25 (0.91, 1.74)	0.73 (0.50, 1.07)	0.93 (0.57, 1.53)	
20-35	1.18 (0.85, 1.65)	0.67 (0.45, 0.99)	0.95 (0.60, 1.49)	
≥35	0.94 (0.61, 1.44)	0.95 (0.64, 1.40)	1.16 (0.72, 1.86)	
Per 20 units	1.04 (0.90, 1.19)	0.89 (0.76, 1.04)	0.97 (0.82, 1.14)	0.36

*Stratified by cohort and adjusted for age, parity and duration of OC use

Appendix 4: Published manuscript "Androgens are differentially associated with ovarian cancer subtypes in the Ovarian Cancer Cohort Consortium"

Androgens are differentially associated with ovarian cancer subtypes in the Ovarian Cancer Cohort Consortium

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Abstract

Invasive epithelial ovarian cancer (EOC) is the most lethal gynecologic malignancy. The etiology of EOC remains elusive; however, experimental and epidemiologic data suggest a role for hormone-related exposures in ovarian carcinogenesis and risk factor differences by histologic phenotypes and developmental pathways. Research on pre-diagnosis androgen concentrations and EOC risk has yielded inconclusive results, and analyses incorporating EOC subtypes are sparse. We conducted a pooled analysis of 7 nested case-control studies in the Ovarian Cancer Cohort Consortium to investigate the association between pre-diagnosis circulating androgens (testosterone, free testosterone, androstenedione, dehydroepiandrosterone sulfate (DHEAS)), sex hormone binding globulin (SHBG), and EOC risk by tumor characteristics (i.e. histology, grade, and stage). The final study population included 1,331 EOC cases and 3,017 matched controls. Multivariable conditional logistic regression was used to assess risk associations in pooled individual data. Testosterone was positively associated with EOC risk (all subtypes combined, Odds Ratio $(OR)_{log2}$=1.12 [95% Confidence Interval (CI) 1.02-1.24]); other endogenous androgens and SHBG were not associated with overall risk. Higher concentrations of testosterone and androstenedione associated with an increased risk in endometrioid and mucinous tumors (e.g., testosterone, endometrioid tumors, OR_{log2}=1.40 [1.03-1.91]), but not serous or clear cell. An inverse association was observed between androstenedione and high grade serous tumors (OR_{log2}=0.76 [0.60-0.96]). Our analyses provide further evidence for a role of hormone-related pathways in EOC risk, with differences in associations between androgens and histologic subtypes of EOC.

Introduction

Reproductive history influences risk of ovarian cancer and it has been hypothesized that these associations are mediated by exposure to endogenous hormones, including androgens (1). Data from experimental studies link androgen-related signalling to ovarian cancer through increased cellular proliferation and reduced apoptotic rates (2-4). The relationship between androgens and epithelial ovarian cancer (EOC) risk has been examined in 7 nested case-control studies with the numbers of cases in these studies ranging from 31 to 1,052 (5-10); these studies predominantly investigated EOC as a composite outcome. Emerging data show heterogeneity in risk factors by histologic subtypes (e.g., serous, endometrioid, mucinous, clear cell) and by the hypothesized "dualistic pathway" of ovarian carcinogenesis (defined by differences in the genetic make-up and the morphological architecture of histologic phenotypes) (11-18). The relationship between androgens and EOC risk by disease subtype has been minimally explored. Analyses to date suggest heterogeneity by subtype (9, 10); however, individual studies evaluating EOC by subtype were either limited by small case numbers in subtype analyses (9), or restricted to women pregnant at the time of serum sampling (10).

We pooled and harmonized available data from 6 nested case-control studies within the Ovarian Cancer Cohort Consortium (OC3), plus the Finnish Maternity Cohort (FMC), to investigate the relationship of pre-diagnosis concentrations of androgens (e.g., testosterone, free testosterone, androstenedione, dehydroepiandrosterone-sulfate (DHEAS)) and sex-hormone binding globulin (SHBG) with EOC risk, overall and by subtype. Subtype analyses included analyses by histology, grade and stage, and by the hypothesized dualistic model of EOC development, i.e., type I vs. type II (19). Our study represents the largest investigation to date including individual-level data from 1,331 EOC cases and 3,017 matched controls, with 61 (clear cell) to 667 (serous) cases represented in the major histologic subtypes.

Methods

Study Population: Ovarian Cancer Cohort Consortium (OC3)

The OC3 has been described previously (12). For this investigation, eligible cohorts were required to have data on a defined set of *a priori* selected covariates (e.g., menopausal status at blood donation, oral contraceptive use at blood donation, parity) and pre-diagnosis measurements of testosterone, free

testosterone, androstenedione or DHEAS. In addition to the OC3 cohorts, the FMC, a cohort of women pregnant at blood collection, contributed data to this investigation (for contributing cohorts see Supplementary Table S1). Available biomarker and questionnaire data from each cohort were centrally collated and harmonized at the Data Coordinating Center at the Brigham and Women's Hospital.

Case characteristics

Eligible cases included women diagnosed with invasive EOC (International Classification of Disease Codes (ICD): ICD9 codes 183 and 158; ICD10 code C56) ascertained by self-report with medical record confirmation and/or linkage to cancer registries. Cases were individually matched to two or three controls (free of cancer and alive at the time of diagnosis of the index case) on age, date, menopausal status and day or phase of menstrual cycle at blood collection in premenopausal women, with the exception of the FMC (matched on age and date at blood collection, parity at blood collection and at diagnosis/index date). Histomorphological data was complete, and the majority of cases had data on stage (82%); grade was available for 36% of the cases. We used histology and grade to classify tumors into type I ((48%, n=291); low-grade serous and endometrioid, all mucinous and clear cell) and type II ((52%, n=314); high-grade serous, high-grade endometrioid) (19). Serous and endometrioid cases missing grade data were excluded from these analyses; mucinous and clear cell tumors were included regardless of grade data availability, as these tumors are classified as type I independent of grade. In a sensitivity analysis, all mucinous and clear cell cases missing grade were excluded from the type I subgroup (after exclusion, case n=77). The proportion of type I tumors was higher than expected; however, we observed the expected distribution (type I: 28% vs. type II: 72%) after excluding women from the FMC (all missing grade; younger at diagnosis and more frequently diagnosed with mucinous tumors than cases from the other cohorts).

Laboratory methods

In all studies, case-control sets were measured in the same batch and technicians performing the assays were blinded to case-control status and quality control samples. Information on sample type, laboratory assays, and intra- and inter-batch coefficients of variations for each cohort is summarized in Supplemental Table S2. Free testosterone was calculated based on measured concentrations of

testosterone and SHBG, with albumin assumed to be a constant 40g/L, according to the mass law of action (20).

Statistical analyses

Hormone measurements were standardized across studies based on the cohort-specific mean concentrations in controls (see supplemental methods; Supplemental Table S3). Conditional logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CI). ORs were estimated using log2-transformed biomarker concentrations and study-specific tertiles based on the distribution in controls. A continuous probit score, generating a rank for each participant in each cohort by hormone concentration, was used to test for trend across tertiles. We additionally evaluated associations in quintiles for EOC overall and the serous subtype. Multivariable models included: parity [never, ever, missing (2.8%)] and OC use [never, ever, missing (47%); excluding FMC 2.3% missing]. Additional adjustment for body mass index (BMI; kg/m^2) among women with data available (n=747 cases), did not change the ORs (data not shown).

Statistical analyses were conducted using a two-stage approach: First, ORs were calculated within each cohort and pooled using DerSimonian and Laird random effects meta-analysis models to assess between-study heterogeneity (21). Second, ORs were calculated based on pooled individual participant data (22). ORs estimated from meta-analysis and the data pooling method were similar, and we observed no significant between-study heterogeneity. Therefore, presented results are based on the pooled analysis. The assumption of linearity was tested using restricted cubic splines; no significant deviations from linearity were observed. Statistical heterogeneity of associations across subtypes was assessed via a likelihood ratio test comparing a model allowing the association for the risk factor of interest to vary by subtype versus one assuming the same association across subtype using polytomous conditional logistic regression (23).

We evaluated associations after stratification by menopausal status at blood collection (premenopausal vs. postmenopausal) and age at diagnosis (<55 vs. ≥55 years). Androgen concentrations are relatively stable in pregnancy (24), however, we excluded FMC members in sensitivity analyses given that all women were pregnant at the time they provided a blood sample. Finally, we conducted a sensitivity

analysis after exclusion of women diagnosed within two years after blood donation. A more detailed description of statistical procedures is available in the supplemental methods.

SAS Statistical Software, version 9.3 (SAS Institute, Cary NC, USA) was used for statistical analyses. P-values<0.05 were considered as statistically significant; all statistical tests and p-values were two-sided.

Results

In total 1,331 cases and 3,017 matched controls from 7 cohorts were included in this investigation (Table 1). Average age at blood collection ranged from 32 (FMC) to 61 years (CLUE II), and the majority of women were parous (89% cases, 94% controls) (Table 1). Average age at diagnosis ranged from 45 (FMC) to 67 (CLUE II) (Supplemental Table S4).

Androgens and overall EOC risk

A doubling of testosterone (i.e., 1-unit increase in $\log_2$-transformed testosterone) was associated with a 12% increase in overall EOC risk (OR_{log2}=1.12; 95% Confidence Interval (CI) [1.02-1.24)], and a 25% increase in risk comparing top to bottom tertile (OR_{T3-T1}=1.25 [1.06-1.48]; p_{trend}=0.03), Table 2). Free testosterone, androstenedione, DHEAS and SHBG were not associated with overall risk of EOC. Results from analyses evaluating quintiles of androgen and SHBG concentrations were similar to those from models using tertiles (Supplemental Table S5); however, the OR comparing highest vs. lowest quintile of testosterone was not statistically significant (OR_{Q5-Q1}=1.22 [0.99-1.52]).

Histologic subtypes

The association between testosterone and EOC risk differed by histologic subtype (p_{het}=0.06). Higher concentrations of circulating testosterone were associated with increased risk of endometrioid and mucinous tumors (e.g., endometrioid tumors: OR_{log2}=1.40 [1.03-1.91]), but not with serous or clear cell tumors (e.g., serous tumors: OR_{log2}=0.96 [0.84-1.11]). Free testosterone and androstenedione were associated with increased risk of mucinous tumors (e.g., androstenedione: OR_{log2}=1.33 [1.03-1.72], Table 2), but not with any of the other histologic subtypes (e.g., androstenedione and endometrioid

tumors: OR_{log2}=1.04 [0.76-1.43]). DHEAS and SHBG were not associated with any of the examined histologic subtypes.

Tumor grade and developmental pathways

We observed significant heterogeneity in the association between androstenedione and low grade EOC and high grade serous disease; androstenedione was significantly inversely associated with high grade serous EOC (p_{het}=0.02; all low grade cases: OR_{log2}=1.41 [0.86-2.31]; high grade serous OR_{log2}=0.76 [0.60-0.96]) (Table 3). The association between SHBG and EOC risk differed significantly by grade (p_{het}=0.02); however, the individual effect estimates were not statistically significant.

The association between androgens and EOC risk differed by developmental pathway (type I vs. type II tumors, p_{het}, testosterone: 0.02; free testosterone: 0.01; androstenedione: <0.01; DHEAS: <0.01) (Figure 1). Overall, higher concentrations of androgens were associated with increased risk of type I tumors, and reduced risk of type II tumors (e.g., androstenedione: type I: OR_{log2}=1.29 [1.05-1.60]; cases n=287; type II: OR_{log2}=0.74 [0.59-0.92], cases n=307; p_{het}<0.01). Significant heterogeneity for androstenedione (p<0.01) and DHEAS (p=0.03) remained after exclusion of mucinous and clear cell cases missing data on grade from the type I subgroup (before exclusion, n=291 case-control sets; after exclusion, n=77 case-control sets). However, while of the same general magnitude, the effect estimates were no longer statistically significant (Supplemental Figure S1).

Sensitivity and Subgroup Analyses

We observed some evidence of heterogeneity for the androgens and SHBG and overall EOC by menopausal status at blood collection (androstenedione, p_{het}=0.05; SHBG, p_{het}=0.02) and age at diagnosis (<55 years vs ≥55 years: androstenedione, p_{het}=0.02; DHEAS, p_{het}=0.05; SHBG, p_{het}=0.05). Both androstenedione and SHBG were positively associated with risk only among women premenopausal at blood collection (androstenedione: premenopausal women, OR_{log2}=1.18 [1.03-1.35], postmenopausal women OR_{log2}=0.95 [0.82-1.12]; SHBG: premenopausal women, OR_{log2}=1.18 [1.00-1.39], postmenopausal women OR_{log2}=0.89 [0.76-1.04]). No further significant heterogeneity was

observed by menopausal status at blood collection. Androstenedione was associated with increased risk of EOC among women diagnosed before age 55 years, but not among women diagnosed at age 55 or older (<55 at diagnosis, OR_{log2}=1.21 [1.05-1.40], ≥55 years at diagnosis, OR_{log2}=0.95 [0.82-1.10]). While the association between DHEAS and SHBG and EOC differed by age at diagnosis, the ORs were not statistically significant in either age at diagnosis subgroup (e.g., SHBG, <55 at diagnosis, OR_{log2}=1.16 [0.98-1.38], ≥55 years at diagnosis, OR_{log2}=0.92 [0.79-1.07]).

We observed no heterogeneity in analyses by stage at diagnosis. We observed an attenuation of the association between testosterone and EOC after excluding the FMC (n=576 cases, 43% of sample; after exclusion: OR_{log2}=1.06 [0.93 - 1.21]). Overall, ORs were similar for the histologic subtypes after this exclusion, however, no longer statistically significant (e.g., testosterone and endometrioid tumors, before exclusion: n=164, OR_{log2}=1.40 [1.03 - 1.91]; after exclusion: n=73, OR_{log2}=1.39 [0.81 - 2.36]. The most substantial attenuation was for the association between androstenedione and mucinous tumors (before exclusion: n=191 cases, OR_{log2}=1.33 [1.03 - 1.72]; after exclusion: n=49 cases, OR_{log2}=1.19 [0.74 - 1.92]). Excluding women diagnosed within two years after blood donation did not meaningfully impact the results (data not shown).

Discussion

We investigated pre-diagnosis circulating concentrations of androgens and risk of EOC overall (n=1,331 cases) and by subtype (case range, n=61 clear cell to 667 serous), in a collaborative re-analysis of 7 nested case-control studies. The association between testosterone and risk of EOC differed by histologic subtype: endogenous androgens were predominantly associated with increased risk of endometrioid and mucinous tumors, while no significant associations were observed for serous or clear cell tumors, although some androgens were inversely associated with high-grade serous and endometrioid (Type II) disease.

Ovarian cancer is comprised of four predominant histologic subtypes: serous, mucinous, endometrioid and clear cell. These histologic subtypes differ substantially by molecular alterations at diagnosis and presumed tissue of origin. The majority of serous tumors are high-grade neoplasms; this subtype represents the majority of invasive EOCs. Separate etiologic pathways are hypothesized for low- and

high-grade serous EOC. It is hypothesized that a proportion of low-grade serous carcinomas develop from distal epithelium of the fallopian tube that implants on the ovarian surface epithelium (~ 80%), while high-grade serous tumors may arise from serous tubal intraepithelial carcinomas (STIC) within the fimbriated end of the fallopian tube (25, 26). Mucinous carcinomas are hypothesized to develop from the gastrointestinal mucosa or from transitional-type epithelium located at the tubal-peritoneal junction; borderline mucinous ovarian tumors are established precursors for this subtype (19). Both endometrioid and clear cell tumors have been proposed to arise from endometrial tissue, and have been associated with endometriosis and retrograde menstruation (19, 27).

Beyond histologic subgroups, two hypothesized developmental pathways of tumorigenesis (type I and type II) have been defined using tumor molecular genetic characteristics (19, 25); in the absence of data on the tumor molecular profile, EOC is classified as type I or type II based on data on histology and grade. Type I tumors include low-grade serous, low-grade endometrioid, mucinous and malignant Brenner tumors (commonly present with *KRAS, BRAF, PTEN, PIK3CA, CTNNB1, and ERBB2* mutations)—subtypes that have been hypothesized to develop in a step-wise manner from borderline tumors or endometriosis within or on the surface of the ovary, and are typically diagnosed at earlier disease stage (27). Type II tumors include high-grade serous, high-grade endometrioid, malignant mixed and undifferentiated tumors (typically present with *TP53* mutations, but none of the mutations observed in type I disease) (19). These latter tumors comprise the majority of EOCs, are aggressive, and typically present at an advanced stage.

Prior epidemiologic data suggest risk factor differences by EOC subtype defined by histology (e.g. (12, 15-18)) and developmental pathway (11, 14). Consistent differences by histologic subtype of invasive EOC are observed for hormone-related risk factors including duration of OC use (lower risk of all histologic subtypes but mucinous; (12, 15)), older age at menopause (higher risk of all but mucinous; (12)), smoking (higher risk of mucinous, lower risk of clear cell; (12, 17)), parity (more strongly protective in non-serous subtypes; (12)), postmenopausal hormone therapy (HT) use (higher risk of serous and endometrioid subtypes only; (12, 18)), and adiposity (among non-HT users; higher risk of serous and endometrioid subtypes only); (16)). Data by the type I/II classification are sparse, but

consistently show stronger associations between parity and type I, relative to type II, disease (11, 14). Three prospective studies evaluated circulating estrogens (10, 28) and/or androgens (9, 10) and invasive EOC risk by subtype. Higher concentrations of both estrogens and androgens were associated with increased risk of non-serous EOC subtypes (9, 10, 28), whereas higher concentrations of androstenedione had opposing effects on risk of type I (higher risk) and type II (lower risk) EOC (9).

In women, androgens are produced in the ovary, adrenal glands, and via peripheral conversion of androgen precursors (e.g., DHEA); in turn, androgens are the substrate for estrogen production by aromatase. DHEAS is a pre-androgen synthesized in the adrenal gland, and subsequently metabolized toward androstenedione and testosterone, or estradiol (29). Androstenedione, an intermediate between DHEA and DHEAS and testosterone, is produced in both the ovary (premenopausal women: 40%; postmenopausal women: 20-30%) and the adrenal gland. In premenopausal women, approximately 25% of circulating testosterone originates in the ovary, 25% in the adrenal glands, and 50% is metabolized from precursors such as androstenedione in peripheral tissues (e.g., liver, adipose tissue) (29, 30); the proportion of testosterone of ovarian origin is higher in postmenopausal women (~50%) (29). These androgens are correlated with each other (e.g., r=0.54 between DHEAS and androstenedione to r=0.69 between DHEAS and testosterone; adjusted for menopausal status (6)) and weakly correlated with estradiol (e.g., estradiol and testosterone: premenopausal women: r=0.08 (31); postmenopausal women, r=0.23-0.38; (32, 33)) and body mass index (r=0.07-0.13; (31-33)).

Androgens may (1) directly influence ovarian carcinogenesis through androgen receptor (AR) signaling, or (2) impact risk through their role as estrogen precursors; associations with estrogens may be most evident in the context of progesterone insufficiency as observed in polycystic ovarian syndrome (PCOS). ARs and estrogen (ER) receptors are expressed in the normal ovary, including ovarian surface epithelial cells and cortical inclusion cysts, and the fallopian tube (34-36). *In vivo* data show that ovarian cancer preferentially develops in a hormonal milieu enriched with androgens (e.g., testosterone induces epithelial neoplasms in guinea pigs (37)) or estrogens (e.g., estrogen-induced tumor growth in high-grade serous ovarian cancers) (38, 39). The hyperandrogenic PCOS is characterized by functional ovarian hyperandrogenism, with an excess of testosterone produced in the ovarian thecal cells (40); up

to 45% of cases additionally present with adrenal hyperandrogenism (41). Estimates of PCOS prevalence range from 5 to 15% (30); the syndrome has highest prevalence among reproductive-age women. PCOS-related androgen excess is observed in both pre- and postmenopausal women (42). Progesterone deficiency is a hallmark of PCOS, resulting in a higher ratio of estrogens to progesterone. PCOS (43, 44) and relatively high levels of estrogens unopposed by progesterone are associated with increased endometrial cancer risk (i.e., estrogen-alone HT (45), relatively high endogenous estrogens in postmenopausal women (33, 46)). These associations with endometrial cancer may be most relevant to the endometrioid or clear cell EOC, given endometrial tissue is a proposed tissue of origin for these subtypes. PCOS itself has not consistently been associated with ovarian cancer (43, 44, 47), though data by subtype are limited. Data to date suggest both estrogen-alone and estrogen plus progesterone HT are associated with increased risk of endometrioid EOC (18).

In the current study, we evaluated three members of the androgen synthesis pathway—DHEAS, androstenedione, testosterone—and EOC risk by histology (i.e., accounting for hypothesized differences in cell of origin) and developmental pathway (i.e., "less" relative to "more" aggressive disease). We observed a significant positive association between testosterone and risk of endometrioid ovarian cancer. There is limited *in vitro* evidence to support a role of androgens in the etiology of endometrioid EOC (34, 48). However, given the possible common tissue of origin, it is plausible that androgens impact risk similarly in both endometrial cancer and endometrioid EOC. With respect to endometrial cancer, recent *in vivo* data have demonstrated that androgens induce epithelial proliferation in the mouse uterus (49), and epidemiologic data provide some support for an association between androgens and endometrial cancer risk (50). Together, this data on endometrial cancer provides indirect evidence supporting an association between androgens and endometrioid EOC. Androgens are an intermediate on the estrogen-synthesis pathway, and estrogen exposure unopposed by progesterone may be the underlying biological mechanism linking androgens to endometrioid EOC, particularly if in the context progesterone deficiency, as in PCOS and in postmenopausal women. Prior research has linked higher early pregnancy estradiol concentrations to a 2.5-fold increase in risk of endometrioid EOC (10), and postmenopausal HT use (12, 18) and adiposity (16) are associated with increased risk of this subtype. We adjusted for BMI in a sensitivity analysis, given (1) the association between PCOS and obesity and

(2) adipose tissue is a key site of metabolism of androgens to estrogens in postmenopausal women. Adjustment for BMI did not impact the results. Data on history of PCOS were not available.

Higher concentrations of all investigated androgens, except DHEAS, were significantly associated with increased risk of mucinous tumors. Emerging data suggest the ovarian stroma proximal to mucinous EOC has higher concentrations of sex-steroid producing enzymes than distant stroma, providing support for a role for sex steroids in the development of mucinous disease (35). Androgens (directly, or after conversion to estrogens) may contribute to growth promotion in the early stages of mucinous disease; however, to our knowledge, the androgen responsiveness of mucinous tumors is not well characterized, and data on ER expression are limited (51, 52). The precise biological mechanisms underlying the observed associations between androgens and mucinous tumors remain an open question.

In line with two prior prospective studies (9, 10), both included in this analysis, we observed no association with pre-diagnosis androgen concentrations and increased risk of serous carcinomas. Recent data on estrogens and ovarian cancer are in line with our results on androgens, with no association observed between estrogens and risk of invasive serous tumors in the FMC (first-trimester estrogens) (10) or among postmenopausal women in the Women's Health Initiative (28). We observed no associations with clear cell disease. However, sample size for this subtype was limited.

We observed significant heterogeneity in the strength of associations between androgens and risk of type I vs. type II tumors; higher androgen concentrations were associated with higher risk of type I, but lower risk of type II (predominantly high grade serous), tumors. These results are in agreement with the single prior study on endogenous androgens and EOC risk using the dualistic model classification (9); these data from the European Prospective Investigation into Cancer and Nutrition (EPIC) were included in the current analysis. There is indirect evidence for differences in hormone dependency in type I and type II tumors, based on the variation of ER expression between low-grade (ER expression: 58%) and high-grade serous carcinomas (ER expression: 27%) (53). However, the mechanisms linking androgen concentrations to lower risk of type II tumors in our study are unclear. While chance and residual confounding may explain the results, future work should explicitly examine the impact of androgens on type II tumors.

Given the large sample size, our study was powered to investigate risk associations for less common tumors (e.g., mucinous tumors) and by developmental pathway (type I/type II). A general weakness of pooled analyses is the difference in data availability of covariates and differences in laboratory methods. In this investigation, data from each cohort were centrally compiled and harmonized and we addressed differences in absolute biomarker concentrations (I) using study-specific tertiles and (II) standardizing hormone measurements using study-specific mean concentrations. Results were robust regardless of whether we calculated ORs from the pooling of individual data or from meta-analysis. For some of the investigated hormones the number of sets that could be used was reduced for subgroup analyses, which resulted in reduced power. In our primary analyses using the developmental pathway classification, we included all mucinous and clear cell tumors in the "type I" classification, as their classification is independent of grade. If there were systematic differences in the observed associations with type I disease in cases with and without grade data, this may result in a biased interpretation of the differences between type I and type II EOC. However, the associations observed in our primary analysis and in a sensitivity analysis restricted to women with complete data on grade were of similar magnitude. Many statistical tests are reported; therefore some significant observations may be due to chance. However, all statistical analyses were hypothesis driven. In line with the majority of other epidemiological studies, a single measurement of biomarkers was used to assess risk associations. This single measurement may not reflect long-term average concentrations and the storage time and conditions may impact the true value of the biochemical indicators. However, the stability of androgen measurements over time has been shown previously for a period over at least 2-3 years: (1) premenopausal women [ICC ranged from 0.58 (androstenedione) up to 0.81 (DHEAS), (54) and (2) postmenopausal women [ICC ranged from 0.66 (androstenedione) up to 0.92 (SHBG) (55).

The testosterone synthesis pathway (e.g., DHEAS, androstenedione, testosterone) may play an important role in the onset and progression of a subset of epithelial invasive ovarian carcinomas. Androgens may either have a direct impact on ovarian carcinogenesis, or act through increased synthesis of other steroid hormones (e.g., estrogens); this is an area for future epidemiologic research. While androgens were associated with increased risk of non-serous tumors, we observed an inverse association between androstenedione and high grade serous tumors. In addition to providing novel

findings on hormone-related pathways in ovarian carcinogenesis, this study supports emerging data on the heterogeneity of epithelial invasive ovarian cancer and underscores the importance of examining etiologic differences for subtypes.

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Table 1. Case and control characteristics in pooled analysis of prospective data on circulating androgens, SHBG and EOC risk: the Ovarian Cancer Cohort Consortium (OC3)

Cohort	Reference		No	Mean age at blood donation years (SD)	Nulliparous %[1]	Ever OC use, %[1]	Postmenopausal, %[2]	Mean BMI (SD)
Clue II	≠	Case	46	60.8 (13.0)	19%	20%	85%	26.3 (5.8)
	≠	Control	91	61.0 (12.9)	13%	13%	86%	25.4 (4.6)
EPIC	Ose et al. 2014	Case	451	55.9 (8.5)	18%	37%	77%	26.8 (4.9)
		Control	867	55.9 (8.6)	12%	45%	77%	26.3 (4.7)
FMC	Schock et al. 2014	Case	576	32.5 (4.8)	0%	##	0%	##
		Control	1,433	32.5 (4.7)	0%	##	0%	##
NHS	Tworoger et al. 2007	Case	117	57.7 (6.5)	8%	41%	79%	24.8 (4.8)
		Control	348	57.7 (6.5)	5%	47%	79%	24.7 (4.1)
NHS II	Tworoger et al. 2007	Case	15	46.1 (4.4)	20%	93%	20%	29.6 (9.8)
		Control	44	45.8 (4.3)	23%	86%	18%	25.9 (5.8)
NYUWHS	Lukanova et al. 2002	Case	63	52.6 (8.6)	47%	29%	56%	24.5 (3.8)
		Control	112	52.0 (8.5)	38%	36%	54%	25.9 (4.3)
WHS	Tworoger et al. 2007	Case	63	55.7 (7.2)	25%	65%	75%	24.5 (3.9)
		Control	122	55.5 (7.0)	15%	71%	70%	25.1 (4.4)
Total		Case	1,331	45.8 (13.7)	11%	40%	42%	26.2 (5.1)
		Control	3,017	44.8 (13.7)	6%	47%	39%	25.8 (4.6)

[1]Among women with data: parity 2.8% missing; OC use 47% missing (excluding FMC: 2.3% missing)

[2]At blood collection

BMI = body mass index; OC = oral contraceptive; OC3 = Ovarian Cancer Cohort Consortium; SHBG = sex hormone binding globulin; SD = standard deviation; CLUE = Washington County, MD Study 'Give us a clue to cancer and heart disease'; EPIC = European Prospective Investigation into Cancer and Nutrition; FMC = Finnish Maternity Cohort; NHS = Nurses' Health Study; NYUWHS = New York University Women's Health Study; WHS = Women's Health Study.

≠ Data from Clue II have not been published.

Information on BMI and OC use was not collected in the FMC

Table 2: Odds ratios (95% CI) for EOC overall and by histologic subtypes in tertiles and for doubling of androgen concentrations: the Ovarian Cancer Cohort Consortium (OC3)[1]

	Invasive EOC			Serous			Endometrioid			Mucinous			Clear Cell		
	Sets	**OR (95%CI)**	**p_{trend}**	**Sets**	**OR (95%CI)**	**p_{trend}**	**Sets**	**OR (95%CI)**	**p_{trend}**	**Sets**	**OR (95%CI)**	**p_{trend}**	**Sets**	**OR (95%CI)**	**p_{trend}**
Testosterone															
T1	398	ref		222	ref		35	ref		45	ref		15	ref	
T2	443	**1.20 (1.02 - 1.41)**		229	1.16 (0.92 - 1.46)		60	1.46 (0.88 - 2.42)		61	1.34 (0.86 - 2.08)		27	1.65 (0.73 - 3.73)	
T3[2]	460	**1.25 (1.06 - 1.48)**	**0.03**	204	0.97 (0.76 - 1.24)	0.56	69	**1.80 (1.08 - 3.01)**	0.06	84	**1.94 (1.25 - 3.02)**	0.05	17	0.82 (0.34 - 2.00)	0.68
Doubling[3]	1,301	**1.12 (1.02 - 1.24)**	**0.02**	655	0.96 (0.84 - 1.11)	0.61	164	**1.40 (1.03 - 1.91)**	**0.03**	190	**1.29 (1.01 - 1.66)**	**0.04**	59	1.12 (0.69 - 1.80)	0.65
p_{het}[4]															0.06
Free Testosterone															
T1	286	ref		155	ref		25	ref		35	ref		11	ref	
T2	287	1.05 (0.85 - 1.28)		151	1.04 (0.79 - 1.38)		32	0.94 (0.48 - 1.86)		48	1.46 (0.85 - 2.52)		10	0.82 (0.27 - 2.48)	
T3[2]	292	1.06 (0.87 - 1.31)	**0.04**	129	0.83 (0.62 - 1.10)	0.63	36	1.03 (0.53 - 1.99)	0.45	50	1.50 (0.88 - 2.54)	**0.03**	20	2.02 (0.67 - 6.12)	0.26
Doubling[3]	865	1.10 (1.00 - 1.21)	0.05	435	0.97 (0.84 - 1.11)	0.61	93	1.11 (0.80 - 1.53)	0.53	133	**1.33 (1.04 - 1.72)**	**0.03**	41	1.32 (0.82 - 2.11)	0.26
p_{het}[4]															0.12
Androstenedione															
T1	450	ref		235	ref		46	ref		56	ref		21	ref	
T2	387	0.86 (0.73 - 1.02)		204	0.88 (0.70 - 1.12)		45	0.67 (0.4 - 1.13)		51	0.90 (0.57 - 1.42)		15	0.93 (0.41 - 2.10)	
T3[2]	470	1.07 (0.90 - 1.28)	0.13	217	0.99 (0.77 - 1.28)	0.79	73	0.99 (0.59 - 1.67)	0.82	84	**1.57 (1.01 - 2.42)**	**0.03**	24	0.81 (0.37 - 1.77)	0.62
Doubling[3]	1,307	1.08 (0.97 - 1.19)	0.16	656	0.97 (0.84 - 1.12)	0.69	164	1.04 (0.76 - 1.43)	0.82	191	**1.33 (1.03 - 1.72)**	**0.03**	60	1.07 (0.69 - 1.66)	0.77
p_{het}[4]															0.17
DHEAS															
T1	227	ref		128	ref		18	ref		8	ref		7	ref	
T2	245	1.08 (0.86 - 1.36)		127	1.06 (0.77 - 1.45)		23	0.81 (0.36 - 1.82)		16	1.04 (0.35 - 3.08)		12	3.36 (0.88 - 12.8)	
T3[2]	219	0.95 (0.74 - 1.23)	0.87	111	0.83 (0.59 - 1.18)	0.45	28	1.02 (0.42 - 2.47)	0.85	20	1.53 (0.50 - 4.71)	0.29	14	3.50 (1.03 - 11.9)	0.10
Doubling[3]	691	0.99 (0.89 - 1.10)	0.82	366	0.93 (0.81 - 1.08)	0.36	69	1.05 (0.72 - 1.53)	0.78	44	1.34 (0.81 - 2.23)	0.26	33	1.52 (0.87 - 2.65)	0.14
p_{het}[4]															0.22
SHBG															
T1	311	ref		147	ref		30	ref		56	ref		19	ref	
T2	250	0.82 (0.67 - 1.00)		141	0.89 (0.67 - 1.18)		27	0.98 (0.49 - 1.93)		28	0.61 (0.36 - 1.05)		12	0.75 (0.30 - 1.91)	
T3[2]	325	1.09 (0.89 - 1.33)	0.56	157	1.14 (0.86 - 1.52)	0.39	37	1.49 (0.78 - 2.85)	0.44	51	0.96 (0.58 - 1.57)	0.79	12	0.75 (0.29 - 1.97)	0.50
Doubling[3]	886	1.02 (0.91 - 1.14)	0.76	445	1.06 (0.91 - 1.25)	0.43	94	1.16 (0.80 - 1.67)	0.43	135	0.93 (0.68 - 1.28)	0.65	43	0.77 (0.46 - 1.30)	0.33
p_{het}[4]															0.43

[1]Results were derived from conditional logistic regression models, additionally adjusted for OC use (never/ever/missing) and parity (never/ever/missing); [2]The p value for trend across tertiles is based on a continuous probit score (generating a rank for each person in each cohort by hormone level); [3]Linear trends for doubling of hormone concentrations were estimated on $\log_2$ scale; [4] Pair-wise heterogeneity tests were performed, using the likelihood ratio test comparing models assuming (1) the same association between exposure and outcomes compared to (2) a model assuming different associations for each subtype.

DHEAS=dehydroepiandrosterone sulfate; SHBG=sex hormone binding globulin

Table 3: Odds ratios (95% CI) for EOC for doubling of androgen concentrations and stratified by grade at diagnosis overall and for serous tumors: the Ovarian Cancer Cohort Consortium (OC3) [1]

	Sets	OR (95%CI)
Testosterone		
Low grade	55	1.28 (0.80 – 2.07)
High grade		
All	407	0.94 (0.79 - 1.12)
Serous	260	0.84 (0.67 - 1.04)
	p_{het}[2]	0.25
	p_{het}[3]	0.12
Free Testosterone		
Low grade	38	1.34 (0.79 - 2.27)
High grade		
All	277	0.95 (0.80 - 1.13)
Serous	180	0.92 (0.74 - 1.13)
	p_{het}[2]	0.24
	p_{het}[3]	0.19
Androstenedione		
Low grade	55	1.41 (0.86 - 2.31)
High grade		
All	406	0.84 (0.69 - 1.01)
Serous	259	**0.76 (0.60 - 0.96)**
	p_{het}[2]	0.05
	p_{het}[3]	**0.02**
DHEAS		
Low grade	49	1.32 (0.89 - 1.97)
High grade		
All	374	0.93 (0.81 - 1.07)
Serous	234	0.91 (0.76 - 1.08)
	p_{het}[2]	0.07
	p_{het}[3]	0.06
SHBG		
Low grade	38	0.59 (0.33 - 1.03)
High grade		
All	286	1.12 (0.93 - 1.36)
Serous	185	1.17 (0.92 - 1.49)
	p_{het}[2]	**0.02**
	p_{het}[3]	**0.02**

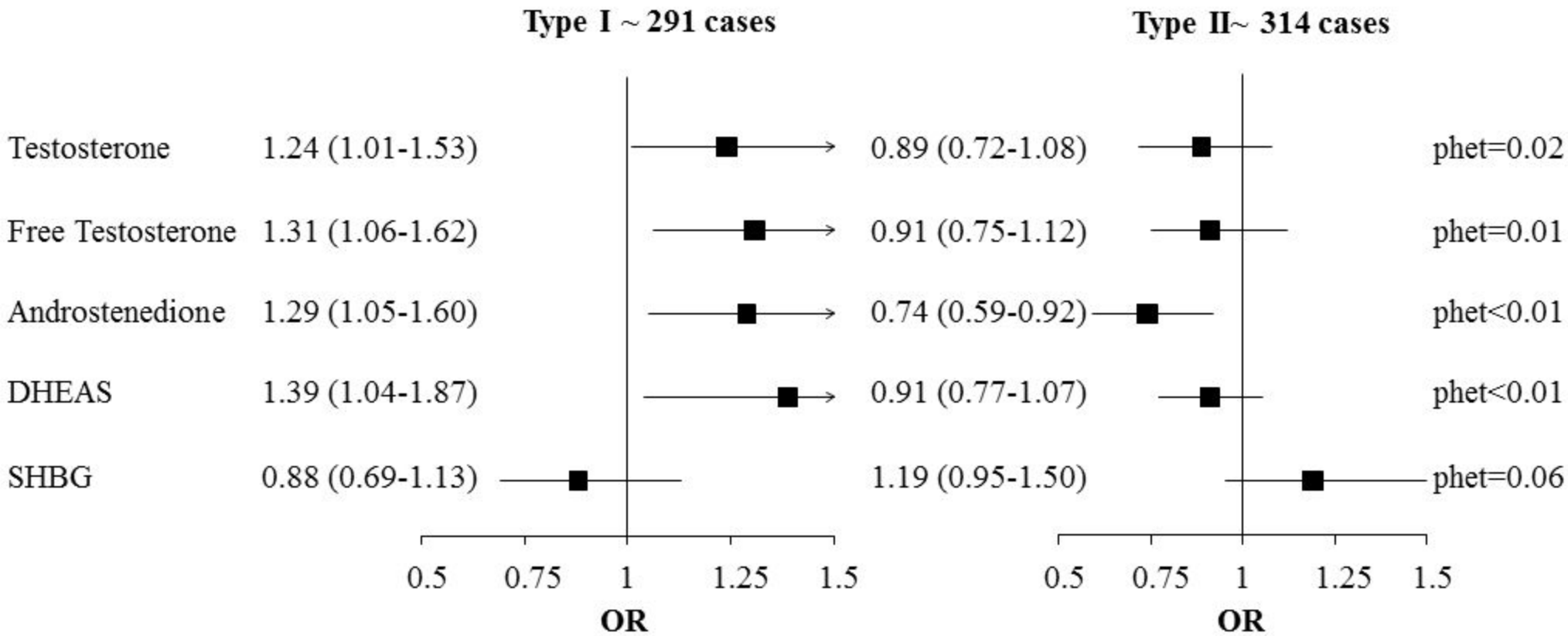
[1]Results were derived from conditional logistic regression models, additionally adjusted for OC use (never/ever/missing) and parity (never/ever/missing). Pair-wise heterogeneity tests were performed, using the likelihood ratio test comparing models assuming (1) the same association between exposure and outcomes compared to (2) a model assuming different associations for each subtype. [2]Comparing all high grade subtypes to low grade. [3]Comparing high grade serous to all low grade. DHEAS=dehydroepiandrosterone sulfate; SHBG=sex hormone binding globulin

Figure 1.

Title: Odds ratios (95% CI) for EOC for doubling of androgen concentrations and EOC risk by the Type I and Type II classification: the Ovarian Cancer Cohort Consortium (OC3).

Label: Results were derived from conditional logistic regression models, additionally adjusted for OC use (never/ever/missing) and parity (never/ever/missing). Pair-wise heterogeneity tests were performed, using the likelihood ratio test comparing models assuming (1) the same association between exposure and outcomes compared to (2) a model assuming different associations for each subtype. DHEAS=dehydroepiandrosterone sulfate; SHBG=sex hormone binding globulin

Figure 1.



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Androgens are differentially associated with ovarian cancer subtypes in the Ovarian Cancer Cohort Consortium

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Appendix 5: Published manuscript “Pre-diagnosis insulin-like growth factor-I and risk of epithelial invasive ovarian cancer by histological subtypes: A collaborative re-analysis from the Ovarian Cancer Cohort Consortium”

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ORIGINAL PAPER

Pre-diagnosis insulin-like growth factor-I and risk of epithelial invasive ovarian cancer by histological subtypes: A collaborative re-analysis from the Ovarian Cancer Cohort Consortium

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Abstract

Purpose Biologic evidence suggests that the Insulin-like growth factor (IGF)-family may be involved in the etiology of epithelial invasive ovarian cancer (EOC). However, prospective studies investigating the role of IGF-I in ovarian carcinogenesis have yielded conflicting results.

Methods We pooled and harmonized data from 6 case–control studies nested within the Ovarian Cancer Cohort Consortium to investigate the association between pre-diagnosis IGF-I concentrations and subsequent risk of EOC. We evaluated IGF-I concentrations and risk of EOC overall and by tumor subtype (defined by histology, grade, stage) in 1,270 cases and 2,907 matched controls. Multivariable conditional logistic regression models were used to calculate odds ratios (OR) and 95% confidence intervals (CI).

Results Doubling of IGF-I concentration was associated with significantly lower risk of overall EOC [$OR_{log2} = 0.82$; CI 0.72–0.93]. We observed no heterogeneity by tumor characteristics (e.g., histology, $p_{het} = 0.62$), menopausal status at blood collection ($p_{het} = 0.79$), or age at diagnosis ($p_{het} = 0.60$).

Conclusions These results suggest that IGF-I concentrations are inversely associated with EOC risk, independent of histological phenotype. Future prospective research should consider potential mechanisms for this association,

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including, considering other members of the IGF-family to better characterize the role of IGF-signaling in the etiology of EOC.

Keywords Epithelial ovarian cancer · IGF-I · Histological subtypes · Developmental pathways

Introduction

Insulin-like growth factor (IGF) signaling has been implicated in the development of various epithelial cancers (e.g., breast and prostate), supported by evidence from in vitro and in vivo studies (as reviewed in: [1]). Data from mechanistic studies demonstrate a role for IGF-I in cellular proliferation, invasion, and angiogenesis of ovarian cancer cells [2, 3]. Thus, a role for IGF-I in the development of epithelial invasive ovarian cancer (EOC) has been hypothesized.

Prospective studies evaluating circulating concentrations of IGF-I and EOC risk have yielded inconclusive results [4–8]. Emerging data support different etiologies for the main EOC histologic subtypes (e.g., serous, endometrioid, mucinous and clear cell tumors) [9], which can be categorized using the hypothesized dualistic model of ovarian carcinogenesis (i.e., type I, predominantly low grade serous and endometrioid histologies, as well as mucinous and clear cell tumors; and type II, predominantly high grade serous and endometrioid tumors) [10]. However, in prior research evaluating circulating IGF-I and risk, EOC was predominantly investigated as a composite outcome due to limited power. To date, two studies evaluated differences in IGF-I associations across histologies and by developmental pathways with no clear heterogeneity [7, 8].

In the present study, we pooled available data from six prospective cohort studies within the Ovarian Cancer Cohort Consortium (OC3) to investigate the association between pre-diagnosis IGF-I and EOC risk among 1270 invasive EOC cases and 2907 matched controls. We investigated overall EOC risk, as well as heterogeneity by EOC subtypes (e.g., histology, grade, stage) and developmental pathways (i.e., type I vs. type II).

Materials and methods

Study populations

The OC3 has been described previously [9]. For this investigation, eligible cohorts were required to have data on a defined set of a priori selected covariates (e.g., menopausal status at blood donation, oral contraceptive use at blood donation, parity) and pre-diagnosis measurements of circulating IGF-I. Data from the following OC3 studies were included in the current study: "give us a clue to cancer and heart disease" (CLUE II), the European Prospective Investigation into Cancer and Nutrition (EPIC) [7], the Harvard Women's Health Study (WHS) [5], and the Nurses' Health Studies (NHS and NHSII) [5]. In addition to the OC3 cohorts, the Finnish Maternity Cohort (FMC) [8], a cohort of women pregnant at blood collection, contributed data to this investigation (for additional information on contributing cohorts, see Table S1). Available biomarker and questionnaire data from each cohort were centrally collated and harmonized.

Ascertainment of cases

Eligible cases included women diagnosed with incident epithelial invasive ovarian cancer [International Classification of Disease (ICD) codes: ICD9 codes 183 and 158; ICD10 codes C56] ascertained by self-report with medical record confirmation and/or linkage to cancer registries. Cases were individually matched to two or three controls on age, date, menopausal status and day or phase of menstrual cycle in premenopausal women, with exception of the FMC, which was restricted to currently pregnant women. Cases and controls in the FMC were matched on age and date at blood collection and parity at blood collection and diagnosis (or reference date for controls) (Table S1). Histologic classification was as follows: 50% of tumors were of serous histology ($n=630$), 13% endometrioid ($n=163$), 15% mucinous ($n=186$), 4% clear cell ($n=57$) and 18% other (including

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"not otherwise specified" (NOS), malignant neoplasms, carcinoma, mixed Mullerian, mixed mesodermal or malignant Brenner tumors; $n=234$). The majority of cases had data on stage at diagnosis ($n=1044$; 82%). Information on grade was available for 34% of the cases (missing for all FMC cases). Well-differentiated tumors (i.e., grade 1) were classified as "low grade", whereas moderately, poorly, and undifferentiated tumors (i.e., grades 2–4) were classified as "high grade"; well-differentiated tumors had a district risk factor profile relative to moderately and poorly differentiated tumors in a previous study in the OC3, whereas moderately and poorly differentiated tumors clustered together [9]. Data on histology and grade were used to classify tumors into developmental pathways. Low-grade serous and endometrioid, and all mucinous and clear cell cases were classified as Type I (49%, $n=277$); high-grade serous and endometrioid were classified as type II (51%, $n=284$) [10]. Mucinous and clear cell cases from the FMC were characterized as Type I; however, all serous and endometrioid tumors from the FMC were excluded from Type I/Type II analyses given no data on grade were available. After excluding participants from FMC, we observed a type I/type II distribution as expected from the literature (type I 28% vs. type II 72%) [10].

Laboratory methods

Case–control sets from all cohorts were measured in the same batch and technicians performing the assays were blinded to case–control status and quality control samples. With the exception of EPIC and the FMC, which used serum, IGF-I was measured in plasma samples (Table S2). All studies, with exception of the FMC, used an enzyme-linked immunosorbent assay (ELISA); the FMC used a chemiluminescent immunoradiometric assay. Coefficients of variation ranged from 2% (NHS, NHSII, WHS) to 14.6% (FMC). To account for differences in study-specific mean concentrations and a different case–control ratio between studies (1:2 vs. 1:3), IGF-I concentrations were standardized based on the cohort-specific mean concentrations in controls (i.e., for each cohort, standardized concentration = original concentration − mean concentration in controls).

Statistical analyses

Conditional logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CI). The association between IGF-I concentrations and EOC risk was evaluated on the log2-transformed continuous scale and in tertiles; quintiles were evaluated in a secondary analysis. Quantiles were defined based on the distribution in controls. Results from models considering study-specific quantiles vs. across-study quantiles based on the standardized IGF-I concentrations were similar. Therefore, only results from across-study quantiles are presented. To account for potential differences in assay distribution between cohorts, a continuous probit score was used to test for trend across tertiles (generating a rank for each participant in each cohort by hormone concentration). Multivariable models were adjusted for parity (never, ever) and OC use [(never, ever, missing (48%); missing excluding FMC (0.3%)]. We evaluated the impact of adjustment for body mass index (BMI; kg/m^2) among the subset of the study population with this data available (686 cases and 1,442 controls).

Statistical analyses were conducted using a two-stage approach. First, the log2 relative risks were calculated within each cohort and pooled using DerSimonian and Laird random effects meta-analysis models [11]. Heterogeneity between cohort-specific effect estimates was tested by DerSimonian and Lairds Q statistic [11]. Second, effect estimates based on pooled individual participant data were calculated. We observed no significant between-study heterogeneity in the meta-analysis, therefore, we present results based on the pooled participant data.

The assumption of linearity was tested using restricted cubic splines; no significant deviations from linearity were observed (data not shown). Statistical heterogeneity of associations across subtypes was assessed via a likelihood ratio test comparing a model allowing the association for the risk factor of interest to vary by subtype vs. one assuming the same association across subtypes using polytomous conditional logistic regression [12]. We evaluated heterogeneity by menopausal status at blood collection and age at diagnosis by including a multiplicative interaction term in the models and evaluating the Wald p value. The FMC (pregnant at blood collection) was excluded in a sensitivity analysis. Given the potential influence of IGF-I in early phases of carcinogenesis, we evaluated risk associations excluding women diagnosed within two years after blood donation.

SAS Statistical Software, version 9.3 (SAS Institute, Cary NC, USA) was used for all statistical analyses. p values <0.05 were considered as statistically significant; all statistical tests and p values were two-sided.

Results

In total, 1,270 cases and 2,907 matched controls were included; the number of cases and controls contributed from each of the participating studies ranged from 15 cases/44 controls (NHS II) up to 575 cases/1,427 controls (FMC) (Table 1). Women who were postmenopausal at blood collection accounted for 42% of the cases and 39% of

Table 1 Case and control characteristics by cohort in pooled analysis of prospective data on circulating IGF-I and EOC risk: the Ovarian Cancer Cohort Consortium (OC3)

Cohort	References	No	Mean age, at blood donation years (SD)	Nulliparous (%[a])	Ever OC use (%[b])	Postmenopausal (%)	Mean BMI (SD)
Clue II	≠						
	Case	46	60.8 (13.0)	19	20	85	26.3 (5.8)
	Control	90	60.9 (12.9)	13	13	86	25.3 (4.7)
EPIC	Ose et al. [7]						
	Case	450	55.9 (8.5)	17	37	77	26.8 (4.9)
	Control	864	55.9 (8.6)	12	45	77	26.3 (4.8)
FMC	Schock et al. [8]						
	Case	575	32.5 (4.8)	0	≠≠	0	≠≠
	Control	1427	32.5 (4.7)	0	≠≠	0	≠≠
NHS	Tworoger et al. [5]						
	Case	121	57.9 (6.5)	8	41	80	24.8 (4.8)
	Control	360	57.8 (6.5)	4	47	80	24.7 (4.0)
NHS II	Tworoger et al. [5]						
	Case	15	46.1 (4.4)	20	93	20	29.6 (9.8)
	Control	44	45.8 (4.2)	23	86	18	25.9 (5.9)
WHS	Tworoger et al. [5]						
	Case	63	55.7 (7.2)	25	65	75	24.5 (3.9)
	Control	122	55.5 (7.0)	15	71	70	25.1 (4.4)
Total							
	Case	1270	45.5 (13.9)	9	40	42	26.3 (5.1)
	Control	2907	44.6 (13.8)	5	47	39	25.8 (4.6)

BMI body mass index, *CLUE* Washington County; MD Study 'Give us a clue to cancer and heart disease', *EPIC* European Prospective Investigation into Cancer and Nutrition, *FMC* Finnish Maternity Cohort, *NHS* Nurses' Health Study, *WHS* Women's Health Study

≠Data from Clue II have not been published

≠≠Information on BMI and OC use was not collected in the FMC

[a]Among women with data: parity 2.2% missing; OC use 48% missing (excluding FMC: 0.3% missing)

[b]Percentages for total presented for women with data: $n = 2168$

the controls, and the majority of women (91% cases, 95% controls) were parous. The median duration of follow-up was 9.1 (SD 6.1) years among incident cancer cases, ranging from 2.7 (SD 1.9) years for NHS II to 12.3 (SD 6.8) years for the FMC. Overall, mean age at diagnosis was 54.6 (SD 12.5) years with youngest cases in FMC [mean: 44.7 (SD 8.1) years] and oldest cases in CLUE II [mean: 67.4 (SD 13.0) years] (Table S3).

Higher IGF-I concentrations were associated with lower EOC risk (all cases: $OR_{log2} = 0.82$; [0.72–0.93]; Table 2). The ORs from analyses considering extreme tertiles vs. quintiles were similar (Tertile 3 vs. 1, OR = 0.75 [0.62–0.90]; Quintile 5 vs. 1, OR = 0.74 [0.59–0.93]). We observed no between-study heterogeneity ($p_{het} = 0.81$; Fig. 1), and results from the meta-analysis were comparable to those of the pooled analysis ($OR_{log2} = 0.82$ [0.73–0.94]). The association between IGF and EOC did not differ significantly across histological subtypes ($p_{het} = 0.62$) or for Type I vs. Type II disease ($p_{het} = 0.67$). We observed no significant heterogeneity by disease stage at diagnosis (local disease: OR_{log2} 0.79 [0.59–1.06]; regional/metastatic disease, OR_{log2} 0.84 [0.71–0.98]; p_{het} 0.79) or tumor grade (low grade: OR_{log2} 1.25 [0.52–3.03]; high grade: OR_{log2} 0.82 [0.63–1.07]; p_{het} 0.43); however, the number of low grade tumors was limited ($n = 49$).

Additional adjustment for BMI did not impact the associations (e.g., overall EOC among women with data on BMI: without adjusting for BMI: OR_{log2}: 0.89; [0.78–1.03] vs. adjusting for BMI OR_{log2}: 0.91; [0.79–1.04]). Results were similar by menopausal status at blood collection ($p_{het} = 0.79$) and age at diagnosis ($p_{het} = 0.60$). Exclusion of women from the FMC (after exclusion, OR_{log2} 0.81 [0.67–0.98]) or women diagnosed within two years after blood donation (after exclusion, OR_{log2} 0.86 [0.75–0.98]) did not impact the results.

Table 2 Odds ratios (95% CI) for tertiles and doubling of IGF-I and EOC risk overall and IGF-I doubling and EOC risk by tumor characteristics, menopausal status at blood donation and age at diagnosis: the Ovarian Cancer Cohort Consortium (OC3)

	Sets	OR (95% CI)	p_{trend}[a]
Overall EOC			
Tertile 1	460	Ref	
Tertile 2	441	0.93 (0.78–1.09)	
Tertile 3	369	0.75 (0.62–0.90)	<0.01
Doubling	1,270	0.82 (0.72–0.93)	<0.01
ORs for doubling histology			
Serous	630	0.89 (0.74–1.06)	0.19
Endometriod	163	0.82 (0.56–1.20)	0.32
Mucinous	186	0.81 (0.58–1.12)	0.21
Clear cell	57	0.50 (0.26–0.99)	0.04
p_{het}[b]			0.62
Grade			
Low grade	49	1.25 (0.52–3.03)	0.62
High grade	377	0.82 (0.63–1.07)	0.15
p_{het}[b]			0.43
Dualistic pathway[c]			
Type I	277	0.78 (0.59–1.03)	0.08
Type II	284	0.87 (0.64–1.18)	0.35
p_{het}[b]			0.67
Disease stage			
Local	246	0.79 (0.59–1.06)	0.12
Regional/metastatic	802	0.84 (0.71–0.98)	0.03
p_{het}[b]			0.79
Menopausal status at blood collection			
Premenopausal	738	0.84 (0.71–0.98)	0.03
Postmenopausal	532	0.80 (0.65–0.99)	0.04
p_{het}[b]			0.79
Age at diagnosis			
Age <55	665	0.80 (0.67–0.94)	0.01
Age ≥55	605	0.86 (0.71–1.04)	0.12
p_{het}[b]			0.60

ORs from conditional logistic regression models adjusted for OC use (never/ever/missing) and parity (never/ever/missing). Tertile cutpoints based on all study controls using IGF-I concentrations standardized to mean = 0 ng/mL: T1 ≤−0.20, T2 >−0.20 to 0.26, T3 >0.26

[a]The *p* value for trend across tertiles is based on a continuous probit score (generating a rank for each person in each cohort by hormone level); p_{trend} for doubling of hormone concentrations was estimated on $\log_2$ scale

[b]*p* for heterogeneity from likelihood ratio test comparing a model allowing the association to vary by subtype vs. one assuming the same association across subtype using polytomous conditional logistic regression; for age at diagnosis, Wald *p* value from interaction term

[c]Type I: Low-grade serous and endometrioid, and all mucinous and clear cell cases; Type II: high-grade serous and endometrioid cases

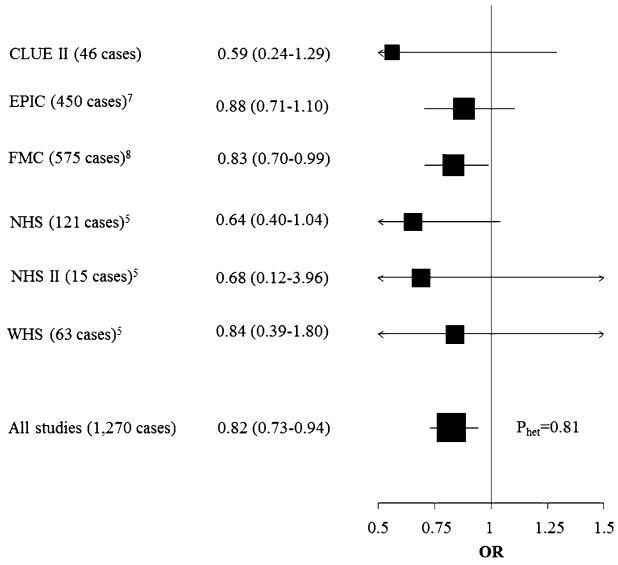

Fig. 1 OR (95% CI) for the association between circulating IGF-I and overall EOC risk for each of the cohorts included in the pooled re-analysis, and results from meta-analysis: the Ovarian Cancer Cohort Consortium (OC3)

Discussion

We present the largest and most comprehensive study to date on the relationship between pre-diagnosis IGF-I and risk of EOC, including 1,270 cases and 2,907 matched controls. In this collaborative re-analysis of six nested case–control studies, we observed an 18% risk reduction for overall EOC risk with a doubling of IGF-I concentration. We observed no heterogeneity between histological subtypes or by other tumor characteristics (e.g., stage, grade, type I/II).

To date, five published prospective studies (*n* cases, range 132–1,052), all of which are included in this pooled analysis, have addressed the association between IGF-I and EOC risk [4–8]. Two of these investigations reported inverse associations overall [7, 8], whereas the others observed significant associations only in subgroups defined by age at diagnosis [4–8]. In the current study, we observed an inverse association between IGF-I and EOC risk overall, with no heterogeneity by age at diagnosis. To date, data on the role of IGF-I in the development of different EOC subtypes are sparse and generally did not support a heterogeneous association [7, 8]. Consistent with those findings, we observed no heterogeneity by EOC subtype in this pooled re-analysis.

IGF-I has well established mitogenic and anti-apoptotic properties (as reviewed in [1]), which are believed to underlie its association with a number of epithelial cancers. We, therefore, hypothesized a positive association between IGF-I and EOC risk. The observed inverse association is

not in line with this hypothesis. The biological pathways underlying the inverse association observed in this study remains to be fully elucidated. One potential explanation for the observed inverse association may be the anti-inflammatory effects of IGF-I [13]. Serum IGF-I is inversely correlated with C-reactive protein (CRP [14]). Recent nested case–control studies have shown a consistent positive association between high CRP concentrations (CRP > 10 mg/L) and subsequent risk of EOC [15–19], although we were unable to adjust for CRP levels in this analysis. Clearly, additional research is needed to understand the potential biological mechanisms underlying the apparent inverse association between IGF-I and EOC risk.

Given the large sample size, our study was powered to investigate risk associations overall, as well as for less common tumors (e.g., mucinous) and by the dualistic model of ovarian carcinogenesis. However, our study also has limitations. Data on tumor characteristics (e.g., missing data on grade: 66%, type I/type II: 56%) and potential confounders (e.g., BMI 49%) was incomplete for some subgroup and sensitivity analyses. A general limitation of pooled analyses is between-cohort differences in data on covariates, biospecimen collection, and laboratory methods. Data from each cohort were centrally compiled and harmonized, and differences in absolute biomarker concentrations were addressed through (I) standardizing hormone measurement using study-specific mean concentrations and (II) using study-specific tertiles. IGF-I standardization was carried out under the assumption that between-study differences in IGF-I concentrations were due to differences in collection and/or laboratory methods, and not due to true underlying differences in concentrations between cohorts. Results were similar in analyses using meta-analysis and calculating OR from the pooling of individual data and we did not observe between-study heterogeneity. Limited covariate data were available for statistical adjustment. However, data from previous studies included in our pooled analysis do not suggest strong confounding of the association between IGF-I and EOC by lifestyle or reproductive factors [5, 7]. Further, we included a cohort of women pregnant at blood collection (FMC) in this study. IGF-I concentrations decrease in early pregnancy, relative to pre-conception concentrations, with a subsequent increase in concentrations in mid-to-late pregnancy until delivery [20]. FMC blood samples were collected at a mean 10.4 (controls)—10.7 (cases) weeks gestation. Pre- and early pregnancy concentrations are modestly correlated (8 weeks gestation: $r = 0.32$; 16 weeks gestation: $r = 0.15$) [20].We excluded the FMC in sensitivity analyses, and observed similar results. An additional limitation is the quantification of circulation IGF-I in a single blood sample. However, the stability of IGF-I measurements over a five year period and its utility as epidemiologic biomarker has been shown previously [intra-class coefficient of variation: 0.74 (95% CI 0.55–0.93)] [21]. Finally, IGF signaling is exceptionally complex as the distinct members of the IGF-family activate different downstream signaling pathways. This investigation only evaluated one member of the IGF-I family and EOC risk. Finally, it is unclear if circulating measures of IGF-I are related to exposure in the peritoneal cavity.

In conclusion, our investigation does not support the hypothesis that elevated IGF-I concentrations increase risk of EOC overall or for specific disease subtypes. In contrast, in this large, pooled analysis, we observed a significant inverse association and no heterogeneity by subtype. To more fully characterize the function of the IGF-pathway in ovarian carcinogenesis future investigations should consider other growth factors and binding proteins (e.g., IGF-II or Insulin-like factor III, IGFBP2, IGFBP3).

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Compliance with ethical standards

Conflict of interest No potential conflicts of interest were disclosed.

Disclaimer The authors assume full responsibility for analyses and interpretation of these data.

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Appendix 6: Draft manuscript "Frequent Aspirin, Nonsteroidal Anti-inflammatory Drug, and Acetaminophen Use and Risk for Ovarian Cancer: An Analysis in the Ovarian Cancer Cohort Consortium (OC3)"

Title: Frequent Aspirin, Nonsteroidal Anti-inflammatory Drug, and Acetaminophen Use and Risk for Ovarian Cancer: An Analysis in the Ovarian Cancer Cohort Consortium (OC3)

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Formatted for Annals of Internal Medicine (US Preventive Services Task Force – total assessment of aspirin and health – contributes to further understanding relationship between aspirin and ovarian cancer risk/prevention)

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ABSTRACT

BACKGROUND: Aspirin use is associated with reduced risk of several cancers. A recent analysis of individual-level data from 12 case-control studies including over 7,500 cases showed a modest 10% decreased ovarian cancer risk with regular aspirin use, which was stronger for daily and low dose users. High dose non-aspirin nonsteroidal anti-inflammatory drug (NSAID) use was also associated with lower risk, but acetaminophen was not associated ovarian cancer risk.

OBJECTIVE: To prospectively investigate associations of aspirin, non-aspirin NSAID, and acetaminophen use with incident invasive ovarian cancer among population-based cohort studies.

SETTING: Ovarian Cancer Cohort Consortium (OC3)

PARTICIPANTS: 836,084 women who provided data about aspirin, non-aspirin NSAID, or acetaminophen use at baseline study enrollment.

MEASUREMENTS: Incident invasive ovarian cancer (outcome) and aspirin, non-aspirin NSAID, and acetaminophen use (exposure). Associations between frequency, duration, and dose of analgesic use and ovarian cancer risk were evaluated.

RESULTS: Frequent (at least ~4-5 days per week or more vs. infrequent/non-regular aspirin use was not associated with ovarian cancer risk [hazard rate ratio (95% confidence interval): 0.95 (0.88-1.03)]; while daily or almost daily (~7 days per week/28+ days per month) aspirin use was marginally associated with a 10% reduction in ovarian cancer risk [0.90 (0.81-1.00)]. Frequent use of non-aspirin NSAIDs or acetaminophen

was not associated with ovarian cancer [non-aspirin NSAIDS: 1.00 (0.90-1.11); acetaminophen 1.05 (0.88-1.25)]. Very frequent (daily or almost daily) use of acetaminophen [1.29 (1.00-1.67)], but not non-aspirin NSAIDs [0.98 (0.85-1.12)], was associated with elevated ovarian cancer risk. Risk estimates for frequent, longer duration (10+ years) use of aspirin or non-aspirin NSAID were modestly elevated [aspirin: 1.15 (0.98-1.34); NSAID: 1.20 (0.86-1.69)].

LIMITATIONS: Self-reported medication use, limited information on low dose aspirin use or indication for use.

CONCLUSIONS: In this large, prospective analysis of pooled cohort study data daily aspirin use was associated with a modest (10%) reduced risk of ovarian cancer. This finding is consistent with a recent pooled analysis of individual-level case-control study data, which demonstrated a 20% reduced ovarian cancer risk with daily aspirin use. We observed an elevated risk of ovarian cancer with daily acetaminophen use and elevated, albeit not statistically significant, risk estimates with very long duration (10+ years) use of aspirin or NSAIDs, suggesting that indications for long-term use may be related to ovarian cancer development.

PRIMARY FUNDING SOURCE: Department of Defense Ovarian Cancer Research Program and National Cancer Institute Intramural Research Program

INTRODUCTION

Ovarian cancer continues to be the most fatal gynecologic cancer, largely due to delayed presentation of symptoms, lack of successful early detection strategies, and poor overall survival. Chemopreventive strategies have not been widely studied but may present approaches to reduce ovarian cancer burden. Chronic inflammation is proposed to play a key role in ovarian carcinogenesis.[1] Factors associated with ovarian epithelial disruption through ovulation [2, 3], inflammation-related exposures such as endometriosis and pelvic inflammatory disease [4, 5], and circulating inflammation-related markers [6, 7] have been associated with ovarian cancer risk.

Inhibition of COX-1 and COX-2 enzymes in the synthesis of prostaglandins is thought to be the major mechanism responsible for the anti-inflammatory and anti-neoplastic effects of nonsteroidal anti-inflammatory drugs (NSAIDs).[8, 9] In addition, NSAIDs may suppress ovulation and affect cell proliferation, angiogenesis, and apoptosis of the epithelium.[10] Acetaminophen, another commonly used analgesic and antipyretic drug, has weak anti-inflammatory activity and may have an anti-gonadotropic effect.[11] It has also been proposed that acetaminophen inhibits ovarian carcinogenesis through the depletion of glutathione leading to necrosis.[12] Therefore, aspirin, non-aspirin NSAIDs, or acetaminophen may be potential agents for the chemoprevention of ovarian cancer. Because of the widespread use of these drugs, any association with an increased or decreased cancer risk may have important public health implications.

Intervention trials have shown that daily aspirin use is associated with reduced risk and mortality of several malignancies.[13] However, individual trials were not powered for

rare cancer endpoints, and meta-analyses of trial data have not provided sufficient ovarian cancer endpoints for evaluation.[14] Rothwell et al. reported fewer female cancers (uterus/ovary/breast combined) with at least 3 years of 300mg+ use daily; for ovarian cancer, this included 6 cases in combined intervention arm data and 12 cases in combined control/placebo arms.[13]

Few prospective observational studies have evaluated the association between aspirin or other NSAIDs use and the risk of ovarian cancer, with inconsistent results.[15-18] However, a recent pooled analysis of 12 ovarian cancer case-control studies participating in the Ovarian Cancer Association Consortium (OCAC) reported a reduced risk of ovarian cancer with aspirin use, especially among daily users of low dose aspirin.[19] Case-control studies, though well powered, are limited by retrospective ascertainment of exposure. Evaluating the association between aspirin use and epithelial ovarian cancer in larger prospective datasets is crucial to better understanding the biology behind tumor development as well as improving potential prevention recommendations for women who may be at increased risk of ovarian cancer. We evaluated the association between aspirin, non-aspirin NSAID, and acetaminophen use with ovarian cancer using prospective individual-level data from the Ovarian Cancer Cohort Consortium (OC3). Given prior findings that frequent use was most relevant for ovarian cancer risk reduction, we focused the current evaluation on frequent analgesic use to the extent possible using cohort study data.

METHODS

Study population

The study population for this analysis included women participating in 16 prospective cohort studies from North America and Europe (Supplemental Table 1). Eligible studies were a cohort study or clinical trial with prospective follow-up that included women, with determination of ovarian cancer endpoints either through questionnaire/medical record follow-up or confirmation by cancer registries, as well as follow-up for death. For the current analysis, eligible studies were limited to those that collected information on aspirin, non-aspirin NSAID or acetaminophen (paracetamol) use. All studies obtained institutional approval at their respective institution(s) for cohort maintenance and participation in the OC3. The OC3 Data Coordinating Center and analytic approaches were approved by the institutional review board of the Brigham and Women's Hospital (BWH).

Exposure definitions

Data for medication use was self-reported in most studies (14 of 16, Supplemental Table 1); the other studies used data from prescription databases (NLCS, WLHS). Of the studies utilizing questionnaires to ascertain medication usage, 10 of 14 studies asked about 'regular use' of medications over either a specified period (BGS, MEC, VITAL, NHS00, NHSII01; one month to 2 years) and/or at a defined frequency of use (BGS, CTS, MEC, SS, VITAL, WHI, SMC, NHS80). The definition for frequency of "regular" use also varied by study ranging from once per week to daily; most studies (six out of seven) specified once or twice per week as the minimum frequency of "regular" use. The time frame for ascertainment ranged from ever (over entire lifetime – BGS, CTS, IWHS, MEC, SMC) to current (at time of questionnaire – CLUE, Sisters, NHS80)

with intermediate categories including use during the past two weeks (WHI), past year (PLCO, CPS2, AARP), past two years (NHS00, NHSII), or past 10 years (VITAL).

Medication usage was initially harmonized as 'regular', at least once per week, use of aspirin, non-aspirin NSAIDs, and acetaminophen and the reference group was non-regular users, less than once a week for each category. However, given mechanistic rationale for assessment of frequent use, we focused on frequent use (used at least 4-5 days per week) to the extent possible. Frequency of medication use was available in eleven of 16 studies. Frequent medication use was defined as use at least 4-5 times per week (depending on the categories used in the questionnaire) for at least 6 months duration, less frequent use or non-regular use (described above) were combined to form the reference group. We also evaluated very frequent (almost daily) use, based on categories of 6-7 days per week, 7 days per week, or ≥28 days per month (available in eleven studies). Variables for frequent medication use were further divided by duration (≥0.5-5, >5-10, >10 years, 9 studies) of use and dose (<100 (or "baby aspirin") and ≥100 mg) for aspirin to differentiate between use of low- and regular/high-dose formulations (4 studies NHS, NHS2, VITAL, WHI)), based on available data from the individual studies.

Potential confounding variables were available from all studies as part of a core dataset and were harmonized by the coordinating center (Brigham and Women's Hospital). Except for age, continuous variables were categorized in all analyses to reduce the effect of outliers. Variables that were selected *a priori* as adjustment factors included: baseline age (continuous), body mass index (<20, 20-24.9, 25-29.9, 30-34.9, ≥35 kg/m^2), number of births (none, one, two, three, four or more full-term births), duration of

oral contraceptive (OC) use (never, ≤1, >1-5, >5-10, >10 years), duration of menopausal hormone therapy (MHT) use (premenopausal, never, ≤5, >5-10, >10 years).

Outcome definitions

We included borderline and invasive epithelial ovarian or peritoneal tumors, which were identified either through cancer registries or medical record review (ICD9 codes 183 and 158; ICD10 codes C56). We first evaluated associations of risk factors with all tumors combined (n=4,275). Second, we evaluated associations for invasive epithelial ovarian cancers (n=3,897) and further evaluated associations for the four most common histologic types: serous (n=1,827, including tumors coded as poorly differentiated), endometrioid (n=297), mucinous (n=183), and clear cell (n=136). The remaining 1,832 cases had another histology (e.g., mixed) or were missing specific histology information and were censored at diagnosis date in histology-specific analyses.

Statistical methods

Women were excluded from primary analyses if they had a history of cancer (other than non-melanoma skin cancer) or bilateral oophorectomy prior to study entry, or were missing age at study entry. We calculated hazard ratios (HR) and 95% confidence intervals (95% CI) using competing risks Cox proportional hazards regression to evaluate the association between the analgesic medications and risk of ovarian cancer overall and by histologic type (17). Follow-up time was defined as time between study entry and the first occurrence of: 1) ovarian cancer diagnosis, 2) death, or 3) end of follow-up. In our primary analyses, we pooled data from all cohorts, and stratified on

cohort to account for potential differences in baseline hazards. Statistical heterogeneity of associations across subtypes was assessed via a likelihood ratio test comparing a model allowing the association for the analgesic use category of interest to vary by histology versus one not allowing the association to vary (15). Secondarily, we used meta-analysis to combine cohort-specific estimates and to assess between-study heterogeneity.

Effect modification by factors that influence inflammation (history of chronic disease, smoking, BMI) as well as other ovarian cancer risk factors, age, parity, MHT use, OC use, menopausal status were evaluated using multiplicative interaction terms.

The following sensitivity analyses were performed: 1) use of a common reference group analysis, coding “non-frequent users” as women who reported no or infrequent use of aspirin and non-aspirin NSAIDs and acetaminophen, 2) excluding women who reported a history of chronic disease (cardiovascular disease, diabetes, auto-immune disease [if available]) at baseline, and 3) exploring the potential for reverse causation by evaluating associations of frequent analgesic use with ovarian cancer cases that occurred less than 5 years, 5 to less than 10 years, and 10 or more years after baseline. All statistical tests were 2-sided, and p-values less than 0.05 were considered statistically significant; analyses were performed using SAS 9.1.

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RESULTS

Aspirin

Frequent aspirin use (use of medication at least 4-5 times per week vs. infrequent/non-regular use) was not associated with ovarian cancer risk [n=853 exposed cases, HR (95% CI): 0.95 (0.88-1.03)] (Table 2). In analyses evaluating daily or almost daily use (at least 6 days per week or more), there was a 10% reduction in ovarian cancer risk [n=449 cases, 0.90 (0.81-1.00)], albeit of borderline statistical significance. This association was specific to the pattern of use that included daily or almost daily use for 0.5-<5 years in duration [0.85 (0.75-0.96), n=300 cases], however, the risk estimate was comparable among daily users for 5-10 years at baseline [0.89 (0.66-1.20), n=50 cases], albeit with wide confidence intervals. No associations were observed when analyzing dose or other patterns of duration of aspirin use and ovarian cancer risk (Table 2). In analyses of frequent, long duration use (10 or more years at baseline), risk estimates were suggestively elevated [vs. infrequent/non-regular use: 1.15 (0.98-1.34), n=213 cases]. Associations with aspirin use strengthened for serous ovarian cancers in analyses by histologic subtype (Table 3), daily aspirin use was associated with a 15% reduction in risk [95% CI: 0.71-1.00)] whereas an elevated risk [1.27 (0.99-1.62)] was

suggested with 10 or more years of frequent aspirin use. A similar pattern was observed for clear cell tumors; however, risk estimates were imprecise due to limited numbers.

Non-aspirin NSAID

Frequent non-aspirin NSAID use was not associated with ovarian cancer risk [1.00 (0.90-1.11)] (Table 2). No associations were observed when analyzing duration or daily frequency of non-aspirin NSAID use and ovarian cancer risk (Table 2). In analyses by histologic subtype there was an increased risk of serous ovarian cancer with frequent, long duration non-aspirin NSAID use [2.06 (1.14-3.74), p-het 0.03, n=11 cases] (Table 3). The risk estimate for ovarian cancer with frequent, long duration non-aspirin NSAID use also was elevated [1.20 (0.86-1.96), n=37)], albeit non-signficant.

Acetaminophen

Frequent acetaminophen use was not associated with ovarian cancer risk [0.99 (0.85-1.15)] (Table 2). However, there was a suggestion of an elevated risk with daily acetaminophen use [1.29 (1.00-1.67), n=67 exposed cases], which seemed to be consistently elevated regardless of duration of daily use. The association with daily acetaminophen use strengthened for serous ovarian cancer in analyses by histologic subtype [1.70 (1.14-2.55), p-het 0.09] (Table 3).

Additional analyses

Results were similar in analyses utilizing meta-analysis of individual study-specific estimates (results not reported). There was very little study-related heterogeneity across

associations and exclusion of individual studies did not substantially change the summary risk estimates (results not reported).

Risk estimates were generally similar across age strata (Supplemental Table 2). Reduced risk with daily aspirin use was apparent among women less than 50 [HR=0.87], 50-59 [HR=0.93], and 60-69 [HR=0.88] years old at baseline, whereas the risk estimate was null for women 70 years old or older at baseline [HR=1.04, p-value for interaction = 0.05]. Increased risk with daily acetaminophen use was only apparent among women 70 years old or older at baseline [1.77 (1.15-2.72), p-interaction 0.33]. Results were similar across strata of other ovarian cancer risk factors (results not shown).

The pattern of results, reduced risk for daily aspirin use and elevated risk with daily acetaminophen use, were similar in secondary analyses restricting to invasive ovarian cancers only, utilizing a common reference group, excluding women with a history of chronic disease at baseline (results not shown). Elevated risk estimates with frequent longer duration use of aspirin or non-aspirin NSAIDs were attenuated in analyses excluding women with a history of chronic disease at baseline [aspirin: 1.11 (0.94-1.33); NSAIDs: 1.07 (0.70-1.62), results not tabled].

CONCLUSIONS

We observed a 10% reduced ovarian cancer risk for daily aspirin use, although this was only for women who used for short to moderate durations at baseline (<10 years). While we did not observe reduced risk with daily use of non-aspirin NSAIDs, we did see an increased ovarian cancer risk with very frequent (daily/almost daily for at least six

months) acetaminophen use. This provides an important contrast to the findings for daily aspirin use and these results are supported by mechanistic differences in the drugs' anti-inflammatory effects and other mechanisms of action. Importantly, the increased risk with daily acetaminophen use is based on a very limited number of exposed cases and should be interpreted with caution; additional analyses suggested that the increased risk was only apparent among women 70 years of age or older at baseline, or during the first 5 years of follow-up.

Taken together with the case-control study data [19] on daily aspirin use and ovarian cancer risk, this summary of the available cohort data suggests that daily or almost daily use of aspirin is likely needed to observe any reduction in ovarian cancer risk, although this reduction was relatively modest (10-20% depending on study design). The weaker association in the prospective studies versus case-control studies is similar to results for breast cancer risk.[14] Although recall bias and reverse causation may lead to a stronger association in case-control studies, our use of baseline analgesic use only may lead to misclassification that could attenuate the results. The collection of updated exposure information during follow-up is needed across many prospective cohorts to reduce the impact of such misclassification.

Importantly, in this analysis, we were able evaluate patterns of duration to characterize a dose-response association; however, unlike colorectal cancer, in which longer duration of use is associated with further risk reductions, the lower risk of ovarian cancer with frequent aspirin use was most apparent with short to moderate duration (the largest exposure strata) and appeared null or slightly elevated with longer duration use (10+ years). Data availability was limited with respect to very long durations of use. A

better understanding of the relationship between frequency and duration of use leveraging updated exposure data is needed to assess potential causality of the daily aspirin-ovarian cancer relationship, including ascertainment of use during critical time periods (e.g. premenopausal versus postmenopausal use). Critically, consideration of daily aspirin use and its timing/duration for the potential primary prevention of ovarian cancer must be weighed against overall cancer chemoprevention as well as risk related adverse events (e.g. upper gastrointestinal bleeding, peptic ulcer, etc). Specifically, pooled analyses of clinical trial data demonstrate that daily aspirin use is most relevant for chemoprevention of colorectal cancer and cancer risk overall [20], as alternate dosing trials did not show clear benefits.[21]

Long durations of frequent aspirin and non-aspirin NSAID use were associated, at least suggestively, with elevated risk. This could suggest confounding by indication, which we could not directly address in this analysis since indication for use was not collected in the studies. We attempted to address this limitation in sensitivity analyses by excluding women who reported a history of chronic disease at baseline and observed some attenuation in risk estimates. We also noted that elevated risks were most apparent for cases diagnosed within 5 years of baseline (potential reverse causation). That said, the consistency of the positive associations for frequent, long duration aspirin and non-aspirin NSAID suggests that further assessment to identify potential residual confounding by age or unmeasured factors is needed.

Of note is that the inverse association for daily aspirin and the suggestive increased risk with long duration of aspirin and NSAIDs was stronger in serous tumors. Some data suggest that serous tumors may be more strongly related to inflammatory factors. For

example, pre-diagnostic circulating inflammatory marker, c-reactive protein, has been associated more strongly with the serous subtype.[6, 22] Further, more aggressive high grade serous tumors have been associated with inducible nitric oxide synthase and other inflammatory markers than low grade tumors.[23] Lifetime ovulations also were more strongly associated with tumors expressing p53 [24], a hallmark of serous disease.[25] The stronger positive relationship for frequent long duration use of aspirin or NSAIDs with serous disease, may suggest that long-term users likely have other factors that increase inflammation and thus risk of this subtype. However, further research is needed using updated exposure information to assess this carefully in prospective studies as well as reanalysis of the case-control studies.

Frequent acetaminophen use was generally not associated with ovarian cancer incidence in this analysis, however daily use did seem to be associated with elevated risk, a finding that requires further evaluation given the limited number of exposed cases. Acetaminophen use was not associated with ovarian cancer risk in the pooled case-control study data[19], based on more than 400 exposed cases (odds ratio for daily acetaminophen use vs. non-regular use: 0.95 (0.74-1.23). Acetaminophen and non-aspirin NSAIDs are commonly used interchangeably; however acetaminophen has only weak anti-inflammatory properties, and may have gonadotrophic effects[11]. The differential associations between daily use of aspirin and acetaminophen further support findings for both medications that are supported by their respective biologic mechanisms.

The prospective design of the pooled studies precludes recall bias. Additional strengths of the study include the ability to identify deaths as well as capture loss to follow-up and

the ability to account for many known and suspected risk factors for ovarian cancer. Limitations included the use of primarily self-reported exposure data, limited information on low dose aspirin use, and limited data on indication for analgesic use, which could independently affect risk. The combination of long-term follow-up and ascertainment of exposure at baseline (in most studies) or an early questionnaire meant that individuals could have started or stopped use during follow-up, which would contribute to measurement error. Further, most studies did not capture lifetime use.

Our findings have more mechanistic than clinical implications, because the absolute incidence of ovarian cancer is low, our findings are unlikely to alter the balance of more common and clinically significant risks and benefits associated with daily aspirin use. However, the associations stratified by age at baseline provide information relevant to current USPSTF recommendations regarding aspirin use for cardio-prevention, as decreased ovarian cancer risk estimates associated with daily aspirin use were observed among women <70 years old. The association was null for women 70 or older at baseline. The USPSTF does not recommend frequent aspirin use in this age group given increased risks for adverse events. While daily aspirin use may provide a very modest reduced risk with respect to incident ovarian cancer, the potential increased risk associated with frequent aspirin use for more than 10 years duration requires further study.

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Table 1. Distribution of frequent analgesic use by demographic and health characteristics in the Ovarian Cancer Cohort Consortium, n=836,084.

	Aspirin				non-aspirin NSAID				Acetaminophen			
	Infrequent/ non-regular use		Frequent* use		Infrequent/ non-regular use		Frequent* use		Infrequent/ non-regular use		Frequent* use	
	n	%	n	%	n	%	n	%	n	%	n	%
Age mean (SD)	54.7	(11.4)	59.4	(10.1)	59.1	(9.5)	59.6	(8.5)	57.7	(10.6)	60.9	(10.0)
Age strata												
<50	171,049	31.0	28,462	17.7	68,208	15.8	10,496	12.9	69,762	22.9	3,973	14.4
50-59	182,326	33.0	48,432	30.1	144,873	33.6	29,425	36.1	101,553	33.4	8,351	30.3
60+	198,689	36.0	84,044	52.2	218,295	50.6	41,609	51.0	132,697	43.6	15,244	55.3
BMI (kg/m^2)												
<20	38,712	7.0	9,460	5.9	28,981	6.7	3,239	4.0	20,937	6.9	1,513	5.5
20-24.9	246,476	44.6	63,791	39.6	183,064	42.4	25,614	31.4	127,806	42.0	9,216	33.4
25-29.9	157,968	28.6	49,716	30.9	130,232	30.2	25,969	31.9	89,960	29.6	8,560	31.1
30-34.9	61,441	11.1	21,816	13.6	51,919	12.0	14,072	17.3	36,797	12.1	4,342	15.8
35+	33,201	6.0	12,620	7.8	26,604	6.2	10,813	13.3	21,015	6.9	3,068	11.1
Missing	14,266	2.6	3,535	2.2	10,576	2.5	1,823	2.2	7,497	2.5	869	3.2
Age at menarche												
≤11	129,521	23.5	39,029	24.3	104,278	24.2	22,428	27.5	58,358	19.2	5,549	20.1
12	132,550	24.0	43,314	26.9	107,177	24.8	22,151	27.2	82,000	27.0	8,085	29.3
13	155,896	28.2	42,510	26.4	122,489	28.4	19,967	24.5	87,684	28.8	6,628	24.0
14	71,928	13.0	21,378	13.3	55,615	12.9	10,314	12.7	44,990	14.8	4,640	16.8
≥15	48,479	8.8	13,304	8.3	38,367	8.9	6,361	7.8	27,904	9.2	2,428	8.8
Missing	13,690	2.5	1,403	0.9	3,450	0.8	309	0.4	3,076	1.0	238	0.9
Duration oral contraceptive use												
Never	210,399	38.1	79,036	49.1	193,635	44.9	32,992	40.5	112,760	37.1	11,756	42.6
>0-1 year	43,208	7.8	14,589	9.1	32,672	7.6	7,606	9.3	27,743	9.1	2,557	9.3
>1-5 years	97,165	17.6	24,065	15.0	67,121	15.6	13,458	16.5	47,757	15.7	3,612	13.1
>5-10 years	78,116	14.1	16,254	10.1	48,201	11.2	9,520	11.7	36,471	12.0	2,323	8.4
>10 years	104,143	18.9	24,316	15.1	76,349	17.7	16,530	20.3	65,839	21.7	6,257	22.7
Missing	19,033	3.4	2,678	1.7	13,398	3.1	1,424	1.7	13,442	4.4	1,063	3.9
Number pregnancies												
0	85,920	15.6	16,579	10.3	56,916	13.2	9,977	12.2	42,630	14.0	2,899	10.5
1	60,572	11.0	14,426	9.0	45,993	10.7	8,030	9.8	35,178	11.6	2,988	10.8
2	177,064	32.1	44,857	27.9	128,389	29.8	23,169	28.4	97,780	32.2	7,997	29.0
3	131,053	23.7	42,162	26.2	110,188	25.5	21,291	26.1	67,767	22.3	6,372	23.1
4+	93,130	16.9	41,287	25.7	85,208	19.8	17,992	22.1	55,969	18.4	6,706	24.3
Missing	4,325	0.8	1,627	1.0	4,682	1.1	1,071	1.3	4,688	1.5	606	2.2
Menopausal status												
Premenopausal	188,738	34.2	31,168	19.4	83,184	19.3	12,792	15.7	82,248	27.1	3,986	14.5
Postmenopausal	348,494	63.1	125,619	78.1	342,938	79.5	67,335	82.6	216,731	71.3	22,957	83.3
Missing	14,832	2.7	4,151	2.6	5,254	1.2	1,403	1.7	5,033	1.7	625	2.3
Age at menopause												
39-45	45,905	8.3	15,523	9.6	45,476	10.5	8,341	10.2	33,314	11.0	3,162	11.5
46-50	89,057	16.1	32,661	20.3	86,398	20.0	15,875	19.5	60,363	19.9	6,024	21.9
51-55	123,290	22.3	43,577	27.1	125,242	29.0	22,357	27.4	77,772	25.6	7,313	26.5
>55	24,452	4.4	9,294	5.8	25,889	6.0	5,503	6.7	14,587	4.8	1,600	5.8
Premenopausal	188,738	34.2	31,168	19.4	83,184	19.3	12,792	15.7	82,248	27.1	3,986	14.5
Missing	80,622	14.6	28,715	17.8	65,187	15.1	16,662	20.4	35,728	11.8	5,483	19.9

Table 2. Associations between analgesic use and ovarian cancer risk in the Ovarian Cancer Cohort Consortium, n=836,084.

Duration menopausal hormone use												
Never	273,921	49.6	73,279	45.5	165,228	38.3	26,744	32.8	112,911	37.1	9,282	33.7
>0-5 year	78,836	14.3	29,980	18.6	73,431	17.0	16,284	20.0	54,914	18.1	6,446	23.4
>5-10 years	43,492	7.9	16,040	10.0	41,755	9.7	9,652	11.8	30,399	10.0	3,512	12.7
>10 years	42,380	7.7	20,700	12.9	44,658	10.4	13,673	16.8	28,174	9.3	4,487	16.3
Missing	113,435	20.5	20,939	13.0	106,304	24.6	15,177	18.6	77,614	25.5	3,841	13.9
Any cardiovascular disease												
No	19,146	3.5	11,630	7.2	22,121	5.1	8,655	10.6	26,078	8.6	4,698	17.0
Yes	1,763	0.3	1,545	1.0	2,500	0.6	808	1.0	2,859	0.9	449	1.6
Missing	531,155	96.2	147,763	91.8	406,755	94.3	72,067	88.4	275,075	90.5	22,421	81.3
Diabetes												
No	440,316	85.2	113,913	82.0	308,678	79.5	55,152	81.8	200,184	72.8	14,468	64.5
Yes	15,142	2.9	9,472	6.8	16,115	4.2	4,131	6.1	9,268	3.4	1,500	6.7
Missing	61,381	11.9	15,541	11.2	63,500	16.4	8,161	12.1	65,623	23.9	6,453	28.8
Autoimmune disease												
No	86,690	18.2	35,539	25.5	104,565	28.7	20,401	31.8	115,614	49.5	9,414	49.4
Yes	6,192	1.3	4,179	3.0	7,292	2.0	3,159	4.9	9,630	4.1	1,855	9.7
Missing	383,645	80.5	99,626	71.5	252,748	69.3	40,667	63.3	108,156	46.3	7,787	40.9

*at least ~4-5 days/week for 6 months or longer

	Pooled cohort analysis				
Aspirin	N cases	Person-years	HR	95% CI	p
Infrequent/non-regular *(reference)*	2,404	4,946,925	1.00	ref	
Frequent use*	853	1,411,155	0.95	(0.88-1.03)	0.25
Frequent use by duration vs. infrequent/non-regular use					
frequent/0.5-<5 yr dur	547	925,611	0.91	(0.83-1.00)	0.06
frequent/5-10 yr dur	93	176,357	0.89	(0.71-1.10)	0.27
frequent/10+ yr dur	213	309,188	1.15	(0.98-1.34)	0.08
Categories of frequent use vs. infrequent/non-regular use					
<daily use	404	865,656	1.02	(0.91-1.15)	0.74
daily use**	449	545,499	0.90	(0.81-1.00)	0.05
Categories of frequent use by duration vs. infrequent/non-regular use					
<daily/0.5-<5 yr dur	247	488,823	1.00	(0.87-1.15)	0.96
<daily/5-10 yr dur	43	113,129	0.89	(0.66-1.22)	0.48
<daily/10+ yr dur	114	263,704	1.13	(0.93-1.37)	0.23
daily/0.5-<5 yr dur	300	436,788	0.85	(0.75-0.96)	0.01
daily/5-10 yr dur	50	63,227	0.89	(0.66-1.20)	0.43
daily/10+ yr dur	99	45,484	1.20	(0.94-1.52)	0.14
Frequent use by dose vs. infrequent/non-regular use					
frequent low dose	115	72,719	0.99	(0.79-1.23)	0.91
frequent normal dose	144	130,684	0.94	(0.77-1.15)	0.55
Non-aspirin NSAID					
Infrequent/non-regular *(reference)*	2,305	3,799,022	1.00	ref	
Frequent use*	427	616,272	1.00	(0.90-1.11)	0.98
Frequent use by duration vs. infrequent/non-regular use					
frequent/0.5-<5 yr dur	326	509,342	0.97	(0.86-1.09)	0.56
frequent/5-10 yr dur	64	76,022	1.09	(0.84-1.41)	0.51
frequent/10+ yr dur	37	30,908	1.20	(0.86-1.69)	0.29
<daily use	188	293,384	1.03	(0.88-1.20)	0.74
daily use**	239	322,887	0.98	(0.85-1.12)	0.74
<daily/0.5-<5 yr dur	133	230,290	0.96	(0.80-1.14)	0.63
<daily/5-10 yr dur	39	44,255	1.30	(0.94-1.79)	0.11
<daily/10+ yr dur	16	18,840	1.16	(0.71-1.91)	0.56
daily/0.5-<5 yr dur	193	279,052	0.97	(0.84-1.13)	0.73
daily/5-10 yr dur	25	31,767	0.86	(0.57-1.30)	0.47
daily/10+ yr dur	21	12,068	1.24	(0.78-1.96)	0.36
Acetaminophen					
Infrequent/non-regular *(reference)*	1,425	2,604,565	1.00	ref	
Frequent use*	148	197,009	1.05	(0.88-1.25)	0.61
Frequent use by duration vs. infrequent/non-regular use					
frequent/0.5-<5 yr dur	61	95,060	0.99	(0.76-1.29)	0.94
frequent/5-10 yr dur	50	50,683	1.16	(0.87-1.54)	0.32
frequent/10+ yr dur	37	51,266	1.01	(0.73-1.41)	0.95
Categories of frequent use vs. infrequent/non-regular use					
<daily use	80	145,185	0.91	(0.72-1.14)	0.40

daily use**	68	51,824	1.29	(1.00-1.67)	0.05
Categories of frequent use by duration vs. infrequent/non-regular use					
<daily/0.5-<5 yr dur	33	69,923	0.87	(0.62-1.22)	0.42
<daily/5-10 yr dur	25	35,311	0.98	(0.66-1.47)	0.94
<daily/10+ yr dur	22	39,950	0.89	(0.58-1.36)	0.59
daily/0.5-<5 yr dur	28	25,137	1.21	(0.81-1.81)	0.34
daily/5-10 yr dur	25	15,372	1.42	(0.95-2.14)	0.09
daily/10+ yr dur	15	11,315	1.25	(0.75-2.08)	0.40

*at least ~4-5 days/week for 6 months or longer

Table 3. Associations between analgesic use and ovarian carcinoma histologic subtypes, Ovarian Cancer Cohort Consortium.

		Serous (n=1,827)		Endometrioid (n=297)		Mucinous (n=183)		Clear Cell (n=136)	
Aspirin	p-het	HR	(95% CI)	HR	(95% CI)	HR	(95% CI)	HR	(95% CI)
Infrequent/non-regular *(reference)*	0.26	1.00	ref	1.00	ref	1.00	ref	1.00	ref
Frequent use*		0.93	(0.81-1.05)	0.90	(0.64-1.27)	1.13	(0.73-1.75)	1.11	(0.71-1.74)
Frequent use by duration vs. infrequent/non-regular use									
frequent/0.5-<5 yr dur	0.03	0.85	(0.73-0.99)	0.93	(0.62-1.40)	1.03	(0.6-1.74)	0.75	(0.40-1.42)
frequent/5-10 yr dur		0.89	(0.64-1.24)	1.28	(0.62-2.66)	0.67	(0.16-2.87)	1.46	(0.52-4.12)
frequent/10+ yr dur		1.27	(0.99-1.62)	0.64	(0.31-1.31)	1.69	(0.83-3.42)	1.97	(0.98-3.97)
Categories of frequent use vs. infrequent/non-regular use									
<daily use	0.13	1.04	(0.86-1.25)	0.86	(0.55-1.34)	0.93	(0.53-1.63)	1.35	(0.75-2.41)
daily use**		0.85	(0.71-1.00)	0.95	(0.59-1.54)	1.40	(0.77-2.56)	0.87	(0.44-1.73)
Non-aspirin NSAID									
Infrequent/non-regular *(reference)*	0.06	1.00	ref	1.00	ref	1.00	ref	1.00	ref
Frequent use*		1.09	(0.92-1.30)	1.03	(0.61-1.73)	0.86	(0.41-1.77)	0.53	(0.23-1.22)
Frequent use by duration vs. infrequent/non-regular use									
frequent/0.5-<5 yr dur	0.03	1.01	(0.83-1.23)	1.09	(0.63-1.89)	0.84	(0.38-1.86)	0.54	(0.22-1.34)
frequent/5-10 yr dur		1.39	(0.87-2.22)	1.04	(0.25-4.31)	1.51	(0.2-11.63)	0.71	(0.10-4.95)
frequent/10+ yr dur		2.06	(1.14-3.74)	--	--	--	--	--	--
Categories of frequent use vs. infrequent/non-regular use									
<daily use	0.04	1.15	(0.87-1.53)	1.36	(0.61-3.00)	1.65	(0.65-4.20)	0.45	(0.11-1.83)
daily use**		1.06	(0.86-1.31)	0.87	(0.45-1.67)	0.49	(0.15-1.58)	0.58	(0.21-1.59)
Acetaminophen									
Infrequent/non-regular *(reference)*	0.21	1.00	ref	1.00	ref	1.00	ref	1.00	ref
Frequent use*		1.29	(0.94-1.77)	1.77	(0.96-3.29)	0.70	(0.16-2.99)	1.49	(0.43-5.17)
Frequent use by duration vs. infrequent/non-regular use									
frequent/0.5-<5 yr dur	0.01	1.36	(0.87-2.12)	0.72	(0.20-2.64)	--	--	2.42	(0.57-10.35)
frequent/5-10 yr dur		1.44	(0.85-2.43)	3.66	(1.54-8.69)	1.68	(0.23-12.17)	1.48	(0.18-11.91)
frequent/10+ yr dur		0.97	(0.48-1.96)	1.92	(0.58-6.32)	1.30	(0.16-10.48)	--	--
Categories of frequent use vs. infrequent/non-regular use									
<daily use	0.09	0.95	(0.60-1.51)	1.70	(0.78-3.69)	1.15	(0.22-6.03)	1.69	(0.33-8.59)
daily use**		1.70	(1.14-2.55)	1.85	(0.75-4.57)	--	--	1.15	(0.17-8.01)

*at least ~4-5 days/week for 6 months or longer

**at least ~6-7 days/week or ≥28 days per month for 6 months or longer

Competing risk histology analyses based on fixed covariate effects, variable covariate effect results were practically identical.

Appendix 7: Results for other on-going projects in the OC3

1. Title: C-reactive protein and ovarian cancer risk: Preliminary results from the Ovarian Cancer Cohort Consortium (OC3)
First and Last Authors: Elizabeth M. Poole, Shelley S. Tworoger
Abstract: C-reactive protein (CRP), a general marker of inflammation has been consistently associated with increased ovarian cancer risk. However, few studies have had the ability to evaluate differences in association by ovarian tumor subtype. We pooled existing data on CRP and ovarian cancer risk among nested case-control studies conducted within 6 OC3 participating studies: CLUEII, EPIC, NHS, NHSII, NYUWHS, and PLCO. Cohort-specific tertiles and quartiles were analyzed using conditional logistic regression, as were common cutpoints at <1mg/L, 1-<10mg/L and ≥10mg/L and Odds Ratios (OR) and 95% confidence intervals (CI) were calculated using random-effects meta-analysis. We also evaluated differences in the association between serous and non-serous cancers. There was no significant association between the top vs. bottom tertile (OR: 1.10; 95% CI: 0.91-1.32) or the top vs. bottom quartile (OR: 1.10; 95% CI: 0.89-1.36). However, there was a strong, positive association between very high CRP (≥10mg/L vs. <1 mg/L: OR: 1.96; 95% CI: 1.36-2.84). Associations of very high (≥10 mg/L vs. < 1 mg/L) did not differ by histologic type: serous OR: 1.63; 95% CI: 1.11-2.43; endometrioid OR: 1.78; 95% CI: 0.63-5.00; mucinous OR: 10.43; 95% CI: 1.27-85.86; clear cell OR: 2.30; 95% CI: 0.39-13.34). Our results confirm the consistent observation of a positive association between very high CRP levels and ovarian cancer risk. Pooled analyses accounting for potential confounders are ongoing.

2. Title: Reproductive and lifestyle factors and risk of ovary, fallopian tube, and peritoneum cancer in the OC3
First and Last Authors: Megan S. Rice, Leo Schouten
Abstract: Multiple cancers can be diagnosed in the peritoneal cavity based on presumed primary cell of origin, including ovarian cancers, primary peritoneal tumors (only small nodules on the ovaries), and fallopian tube cancers (central tumors in the tube). We examined whether risk factor associations differed across these three types in 19 studies that provided information on at least 2 of the three tumor types with 3,927 ovarian, 339 peritoneal, and 183 fallopian tube tumors. BMI at age 18 was positively associated with primary peritoneal cancer (HR, per 5 kg/m^2=1.28, 95%CI: 1.06-1.55), but was not associated with ovarian (comparable HR=0.99) or fallopian tube (HR=0.84) cancers (p-heterogeneity=0.03). Further, ever vs. never parity was only associated with ovarian tumors (HR=0.68; 95%CI: 0.62-0.74), but not peritoneal (HR=0.96) or fallopian tube (HR=1.00) tumors (p-heterogeneity=0.04). Tubal ligation was associated only with ovarian and fallopian tube tumors (p-heterogeneity=0.05). Other risk factor associations did not significantly differ by anatomic location, although power was somewhat limited particularly for fallopian tube tumors.