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Title:
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Date:
2025-05-01

Citation:
Collin, L. J., Cushing-Haugen, K. L., Terry, K. L., Goode, E. L., Wu, A. H., Harris, H. R., Sasamoto, N., Cramer, D. W., Modugno, F., Elishaev, E., Fu, Z., Moysich, K. B., Fasching, P. A., Pearce, C. L., Menon, U., Gentry-Maharaj, A., Gayther, S. A., Wentzensen, N., Goodman, M. T. ,... Doherty, J. A. (2025). Patterns of Associations with Epidemiologic Factors by High-Grade Serous Ovarian Cancer Gene Expression Subtypes. Cancer Epidemiology Biomarkers and Prevention, 34 (5), pp.762-773. https://doi.org/10.1158/1055-9965.EPI-24-1143.

Persistent Link:
https://hdl.handle.net/11343/359992

# Patterns of Associations with Epidemiologic Factors by High-Grade Serous Ovarian Cancer Gene Expression Subtypes

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## ABSTRACT

**Background:** Ovarian high-grade serous carcinomas (HGSC) comprise four distinct molecular subtypes based on mRNA expression patterns, with differential survival. Understanding risk factor associations is important to elucidate the etiology of HGSC. We investigated associations between different epidemiologic risk factors and HGSC molecular subtypes.

**Methods:** We pooled data from 11 case–control studies with epidemiologic and tumor gene expression data from custom NanoString CodeSets developed through a collaboration within the Ovarian Tumor Tissue Analysis consortium. The PrOTYPE-validated NanoString-based 55-gene classifier was used to assign HGSC gene expression subtypes. We examined associations between epidemiologic factors and HGSC subtypes in 2,070 cases and 16,633 controls using multivariable-adjusted polytomous regression models.

**Results:** Among the 2,070 HGSC cases, 556 (27%) were classified as C1.MES, 340 (16%) as C5.PRO, 538 (26%) as C2.IMM, and 636 (31%) as C4.DIF. The key factors, including oral contraceptive use, parity, breastfeeding, and family history of ovarian cancer, were similarly associated with all subtypes. Heterogeneity was observed for several factors. Former smoking [OR = 1.25; 95% confidence interval (CI) = 1.03, 1.51] and genital powder use (OR = 1.42; 95% CI = 1.08, 1.86) were uniquely associated with C2.IMM. History of endometriosis was associated with C5.PRO (OR = 1.46; 95% CI = 0.98, 2.16) and C4.DIF (OR = 1.27; 95% CI = 0.94, 1.71) only. Family history of breast cancer (OR = 1.44; 95% CI = 1.16, 1.78) and current smoking (OR = 1.40; 95% CI = 1.11, 1.76) were associated with C4.DIF only.

**Conclusions:** This study observed heterogeneous associations of epidemiologic and modifiable factors with HGSC molecular subtypes.

**Impact:** The different patterns of associations may provide key information about the etiology of the four subtypes.

## Introduction

Epithelial ovarian cancer (EOC) represents a heterogeneous disease with distinct histotypes that have different cells of origin, molecular features, epidemiologic risk factors, clinical characteristics, and survival (1). The 2014 and 2020 World Health Organization guidelines (2, 3) classify EOC into five main histotypes: high-grade serous carcinoma (HGSC), low-grade serous tumor, endometrioid tumor, clear-cell tumor, and mucinous tumor. HGSC is the most common histotype, representing approximately 70% of EOC diagnoses (4, 5).

Previous studies have identified associations between epidemiologic factors and EOC, overall and by EOC histotype (6–18). Factors associated with a decreased risk of EOC include oral contraceptive (OC) use, parity, having a full-term pregnancy after 35 years of age, breastfeeding, tubal ligation, and aspirin use (7, 10, 17, 19). Those associated with an increased risk include age, family history of breast and ovarian cancers, polygenic risk score (PRS), lifetime ovulatory years, and estrogen (E)-only hormone replacement therapy (13, 20–24). Several of these factors are associated with all EOC histotypes, including age, parity, and OC use, although the strength of the association varies across histotypes. Other factors have heterogeneous associations (6, 11, 18, 25). For example, endometriosis is primarily associated with a risk of endometrioid and clear-cell tumors (11, 26), and cigarette smoking is associated with an increased risk of mucinous tumor (18). Associations between many epidemiologic factors and HGSC are weaker than the corresponding associations with the other histotypes (6, 27). Some epidemiologic factors have been associated with tumor aggressiveness, specifically. For example, high body mass index (BMI) and current smoking are associated with highly aggressive invasive serous EOC (death within

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1 year of diagnosis), whereas family history of ovarian or breast cancer is positively associated and parity is negatively associated with less aggressive disease (lived five or more years; ref. 28).

Tothill and colleagues (29) first reported molecular subtypes of HGSC in 2008, which were subsequently reproduced and refined across multiple studies (30–35). There are an estimated four gene expression–based subtypes of HGSC labeled using combined terminology from the Tothill and The Cancer Genome Atlas articles (29–36): mesenchymal (C1.MES), proliferative (C5.PRO), immunoreactive (C2.IMM), and differentiated (C4.DIF). These HGSC subtypes demonstrate distinct gene expression signatures and survival patterns, with C1.MES and C5.PRO having worse survival compared with C2.IMM and C4.DIF (29–38).

To date, only one previous study has evaluated associations between epidemiologic factors and gene expression subtypes accounting for intratumoral heterogeneity (39). Clarifying risk factor associations with HGSC subtypes is necessary to identify targets for risk reduction and develop risk prediction models. In this study, we evaluated associations of reproductive and hormonal characteristics and demographic and lifestyle factors with HGSC subtypes (35).

## Materials and Methods

### Study population

This analysis includes data from 11 case–control studies from North America ($n = 7$), Europe ($n = 3$), and Australia ($n = 1$; refs. 8–10, 12, 13, 16, 20, 40–50). For cases, formalin-fixed, paraffin-embedded tumor samples were assayed using a 518-marker or 340-marker NanoString CodeSet developed through a collaborative effort in the Ovarian Tumor Tissue Analysis consortium to identify markers associated with survival (35). All studies include HGSC cases confirmed through pathology evaluation following the 2014 World Health Organization guidelines (51), a comparable disease-free control group, data on epidemiologic factors, and a sample size larger than 30 cases and 30 controls. Case samples were included if they were of adnexal or presumed adnexal origin, did not receive neoadjuvant treatment, and NanoString data passed quality control measures. In the 11 studies with 2,261 eligible cases and 17,278 controls, we excluded 191 cases and 645 controls because they lacked data on OC use and pregnancy information. Thus, we were able to evaluate data from 2,070 cases and 16,633 controls for analyses. Additionally, we included data from a European study with 220 well-annotated cases (52), increasing the number for the case-only analysis to 2,290 cases (**Table 1**). Each study received institutional review board approval and obtained written consent from participants.

### Gene expression subtypes

All cases were assigned a gene expression subtype using the PrOTYPE assay, a validated 55-gene NanoString-based classifier described in detail elsewhere (35). PrOTYPE classifies HGSC cases into four gene expression subtypes: C1.MES, C2.IMM, C4.DIF, and C5.PRO. A probability of assignment is generated for each of the four HGSC subtypes (summing to 100%), and the final subtype assignment is the subtype with the highest probability. Each PrOTYPE prediction also generates an entropy score. Entropy is defined as the uncertainty inherent in the prediction of subtype classification and is inversely correlated with the probability of assignment.

### Exposures

Epidemiologic factors included reproductive and hormonal characteristics and demographic and lifestyle factors that have been associated with EOC, as well as modifiable factors that have been less consistently associated with EOC, primarily based on previous Ovarian Cancer Association Consortium (OCAC) studies. We included a previously derived and validated PRS(27), family history of breast or ovarian cancer defined as no known first-degree family history of breast or ovarian cancer, first-degree relative with a history of breast cancer (but not ovarian cancer), or first-degree relative with a history of ovarian cancer (with or without a first-degree relative with breast cancer). Reproductive and hormonal characteristics included: OC use (ever or never), duration of OC use (never, <6 months, 6 months to <5 years, 5 to <10 years, or ≥10 years), number of full-term pregnancies (0, 1, 2, or 3+), age at last pregnancy (<25, 25–29, 30–34, or ≥35 years), duration of breastfeeding (never, ≤6 months, >6 months–2 years, or >2 years), age at menarche (≤11, 12–13, or ≥14 years), menopausal hormone therapy use (never, estrogen only for <10 years, E only for ≥10 years, E and progestin for <10 years, E and progestin for ≥10 years, use of both, and use of unknown hormone therapies; not restricted to postmenopausal women), lifetime ovulatory years (23), endometriosis (yes or no), and tubal ligation (yes or no).

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Cancer Epidemiol Biomarkers Prev 2025;34:762–73

**doi:** 10.1158/1055-9965.EPI-24-1143

**Table 1.** Distributions of controls and cases by HGSC subtype for the 12 studies.

| Study | Full study name, reference | Country | Study design | Diagnosis years | Controls *n* | Cases *n* | C1.MES *n* (%) | C5.PRO *n* (%) | C2.IMM *n* (%) | C4.DIF *n* (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| AUS | Australian Ovarian Cancer Study (8) | Australia | Population-based | 2002–2006 | 1,505 | 309 | 69 (22) | 54 (17) | 99 (32) | 87 (28) |
| BAV | Bavarian Ovarian Cancer Cases and Controls (40) | Germany | Clinic-based | 1995–2008 | 339 | 45 | 7 (16) | 5 (11) | 14 (31) | 19 (42) |
| DOV | Diseases of the Ovary and their Evaluation (13) | United States | Population-based | 2002–2009 | 1,849 | 543 | 166 (31) | 83 (15) | 130 (24) | 164 (30) |
| HAW | Hawaii Ovarian Cancer Study (41) | United States | Population-based | 1994–2007 | 1,103 | 52 | 5 (9.6) | 5 (9.6) | 15 (29) | 27 (52) |
| HOP | Hormones and Ovarian Cancer Prediction (50) | United States | Population-based | 2003–2008 | 1,802 | 35 | 6 (17) | 9 (26) | 8 (23) | 12 (34) |
| NEC | New England Case-Control Study (42, 43) | United States | Population-based | 1992–2008 | 2,100 | 110 | 30 (27) | 20 (18) | 29 (26) | 31 (28) |
| NCO | North Carolina Ovarian Cancer Study (44, 45) | United States | Population-based | 1999–2008 | 1,050 | 399 | 109 (27) | 59 (15) | 114 (29) | 117 (29) |
| MAY | Mayo Clinic Ovarian Cancer Case Control Study (20, 46) | United States | Clinic-based | 1999–2008 | 2,167 | 404 | 142 (35) | 73 (18) | 81 (20) | 108 (27) |
| POL | Polish Ovarian Cancer Case-Control Study (47) | Poland | Population-based | 2001–2003 | 1,116 | 38 | 8 (21) | 2 (5.3) | 11 (29) | 17 (45) |
| UKO | UK Ovarian Cancer Population Study (48) | United Kingdom | Population-based | 2006–2007 | 1,012 | 87 | 8 (9.2) | 20 (23) | 20 (23) | 39 (45) |
| USC | Study of Lifestyle and Women's Health (9, 12, 16) | United States | Population-based | 1993–2005 | 2,590 | 48 | 6 (13) | 10 (21) | 17 (35) | 15 (31) |
| | **Total, case–control analyses** | | | | **16,633** | **2,070** | **556 (27)** | **340 (16)** | **538 (26)** | **636 (31)** |
| SEA | UK Study of Epidemiology and Risk Factors in Cancer Heredity Ovarian Cancer Study (52) | United Kingdom | Population-based | 1998–2013 | | 220 | 31 (14) | 45 (20) | 44 (20) | 100 (45) |
| | **Total, case-only analyses** | | | | | **2,290** | **587 (26)** | **385 (17)** | **582 (25)** | **736 (32)** |

Demographic and lifestyle characteristics were also evaluated including age at diagnosis (<49, 50–54, 55–59, 60–64, 65–69, or ≥70 years; case-only analysis), BMI at 18 years of age and within 5 years of diagnosis/interview data (<18.5, 18.5–24.9, 25–29.9, or ≥30 kg/m$^2$), regular aspirin use (nonregular use or regular use), frequency of aspirin use (nonregular use, use <30 days/month, and use ≥30 days/month), genital powder use (never or ever), smoking status (never, former, or current), lifetime alcohol use (never, ever, or former), and recent alcohol use (no alcohol use in 5 years before reference date or any). Aspirin use, smoking status, and lifetime alcohol use were defined at the time of diagnosis for cases or interview/comparable reference date for the controls. Supplementary Table S1 illustrates variables included for each study site. If data were missing, then that study was excluded from the analyses of that variable.

### Statistical analysis

We used polytomous logistic regression to compute ORs and 95% confidence intervals (95% CI) associating epidemiologic factors with HGSC subtypes. Models were adjusted for age at diagnosis (cases) or at interview/reference date (controls), number of full-term pregnancies, OC use, and study site. For the two studies that did not collect information on the number of full-term pregnancies, we used number of live births as a proxy. As this study aimed to estimate heterogeneity in the associations between epidemiologic factors and HGSC subtypes based on the magnitude of the estimates and their associated precision, no hypothesis testing was performed (53–57).

We performed two analyses to account for potential misclassification from assigning the subtype with the highest probability. First, we repeated the case–control analyses, restricting to cases with >80% probability of subtype assignment ($n$ = 1,228 cases; 59%). Second, we conducted a case-only analysis to compute ORs and 95% CIs associating epidemiologic risk factors with HGSC subtypes with adjustment for the entropy score using polytomous regression. This OR approximates the ratio of two subtype-specific ORs to elucidate the subtype-specific heterogeneity (58). We compared the C5.PRO, C2.IMM, and C4.DIF categories with C1.MES because C1.MES had the largest sample size and lowest entropy scores.

To examine patterns of associations across HGSC subtypes, we performed unsupervised hierarchical clustering of the four subtypes with normalized β-estimates for the associations between dichotomized factors of interest and HGSC subtypes using complete linkage and uncentered correlation (Pearson coefficient; refs. 9, 59).

Nearly all of the cases and controls were self-reported White race or of European decent (93.3% cases and 90.4% controls). In a sensitivity analysis, we restricted the cases and controls to White individuals.

Analyses were conducted using STATA (version 16.1, STATA Corporation) and R v4.0.

### Data availability

The full individual patient data are not publicly available but can be requested through the existing data request processes of the OCAC (https://ocac.ccge.medschl.cam.ac.uk/).

## Results

Among the 2,070 HGSC cases, 556 (27%) were classified as C1.MES, 340 (16%) as C5.PRO, 538 (26%) as C2.IMM, and 636 (31%) as C4.DIF (**Table 1**). The distribution of HGSC subtypes was similar across study sites; however, there was larger variation among study sites with relatively few (<100) cases.

### Reproductive and hormonal characteristics

Similar associations were observed across all HGSC subtypes for having a first-degree relative with a history of ovarian cancer (ORs > 2), OC use (ORs ~ 0.6), 10+ years of OC use (ORs ~ 0.45), 3+ full-term pregnancies (ORs = 0.63–0.78), >2 years breastfeeding (ORs = 0.40–0.61), and lifetime ovulatory years (OR ~ 1.0; **Table 2**). We observed heterogeneity in associations across HGSC subtypes for several factors. A one-SD increase in the PRS was associated with increased odds of all subtypes (OR = 1.4) but most pronounced among C5.PRO (OR = 1.58; 95% CI = 1.41, 1.77). Having a first-degree relative with a history of breast cancer only was associated with higher odds of C4.DIF (OR = 1.44; 95% CI = 1.16, 1.78). Age at last pregnancy of 35+ was associated with lower odds of all subtypes (ORs ranged 0.72–0.85); however, for C2.IMM, ages at last pregnancy of 25+ were also associated with reduced odds (ages 25–29: OR = 0.73; 95% CI = 0.54, 0.97; ages 30–34: OR = 0.69; 95% CI = 0.51, 0.94). For menopausal hormone therapy use, ≥10 years of E-only hormone therapy was associated with increased odds of C1.MES (OR = 2.14), C2.IMM (OR = 2.00), and C4.DIF (OR = 1.90) but was attenuated for C5.PRO (OR = 1.18). History of endometriosis was associated with increased odds of C5.PRO (OR = 1.46; 95% CI = 0.98, 2.16) and C4.DIF (OR = 1.27; 95% CI = 0.94, 1.71) but not C1.MES or C2.IMM (OR = 0.92; 95% CI = 0.64, 1.32 and OR = 1.03; 95% CI = 0.72, 1.48, respectively).

The mean age at diagnosis was similar for C1.MES, C5.PRO, and C2.IMM, ranging from 60.0 to 61.4 years, whereas C4.DIF tended to be younger at diagnosis (57.5 years; **Table 3**). In case-only analyses with C1.MES as the comparison group, results were similar to those from the case–control analyses. Having a first-degree history of breast cancer only was associated with increased odds of C4.DIF (OR = 1.39; 95% CI = 1.01, 1.90). One SD unit increase in the PRS was associated with higher odds of C5.PRO (OR = 1.14; 95% CI = 0.99, 1.31). Ages at last pregnancy of 25 to 29 and 30 to 34 were associated with lower odds of C2.IMM (OR = 0.65; 95% CI = 0.42, 0.99 and OR = 0.69; 95% CI = 0.44, 1.08, respectively) and a less pronounced but similar pattern for C5.PRO (ORs ranged 0.83–0.86). E-only hormone use for 10+ years was associated with lower odds of C5.PRO (OR = 0.49; 95% CI = 0.25, 0.95), with less pronounced associations for C2.IMM (OR = 0.84; 95% CI = 0.48, 1.45) and C4.DIF (OR = 0.81; 95% CI = 0.47, 1.40). History of endometriosis was associated with increased odds of C5.PRO (OR = 1.40; 95% CI = 0.81, 2.44) and C4.DIF (OR = 1.23; 95% CI = 0.76, 1.97).

### Demographic and lifestyle characteristics

Regular reported aspirin use was associated with lower odds of C5.PRO (OR = 0.72; 95% CI = 0.48, 1.06) and was stronger for daily aspirin use (OR = 0.54; 95% CI = 0.32, 0.93) but was not associated with C1.MES, C2.IMM, or C4.DIF (**Table 2**). Genital powder use was associated with increased odds of C2.IMM (OR = 1.42; 95% CI = 1.08, 1.86) but not C1.MES, C4.DIF, or C5.PRO. Current smoking was associated with increased odds of C4.DIF only (OR = 1.40; 95% CI = 1.11, 1.76). Former smoking was associated with higher odds of C2.IMM (OR = 1.25; 95% CI = 1.03, 1.51). Ever alcohol use was associated with lower odds of all subtypes (ORs = 0.42–0.69).

Results from the case-only analyses with C1.MES as the comparison group were generally consistent with the case–control analyses. Regular aspirin use was associated with lower odds of C5.PRO (OR = 0.70; 95% CI = 0.43, 1.15) and was stronger for daily use (OR = 0.48; 95% CI = 0.25, 0.91). Genital powder use was associated with increased odds of C2.IMM (OR = 1.42; 95% CI = 0.94, 2.14). Current smoking was associated only with C4.DIF (OR = 1.34; 95% CI = 0.94, 1.91), and former smoking was associated with higher odds of C5.PRO and C2.IMM (OR = 1.29; 95% CI = 0.95, 1.76 and OR = 1.52; 95% CI = 1.16, 2.00, respectively). Ever use of alcohol was associated with increased odds of C5.PRO, C2.IMM, and C4.DIF (ORs = 1.31–1.46).

### Sensitivity analysis

The proportion of HGSC cases that were assigned their subtype with >80% probability varied by primary assignment; 74% of C1.MES, 60% of C4.DIF, 50% of C2.IMM, and 49% of C5.PRO cases reached that threshold. The mixtures of subtype proportions also differed by primary assignment (Supplementary Fig. S1). For example, for C1.MES and C5.PRO, the next most common subtype proportion was C2.IMM, and C1.MES samples had very low proportions of C4.DIF. C4.DIF samples had larger proportions of C1.MES and also substantial proportions of C2.IMM. C2.IMM tended to have a higher proportion of C5.PRO than the other subtypes. Results from sensitivity analyses restricting to HGSC cases to those assigned a subtype with >80% are presented in Supplementary Table S2 and were generally comparable with those presented in **Table 2**, albeit with less statistical precision due to reduced sample size, suggesting that intratumoral heterogeneity of subtypes had minimal effect on the results. The results restricted to White individuals were unchanged (Supplementary Table S3).

### Patterns of epidemiologic factor associations across HGSC subtypes

**Figure 1** illustrates a heatmap for patterns of associations of dichotomized epidemiologic risk factors by HGSC subtype. Hierarchical clustering split the four subtypes into two major groups. C2.IMM and C4.DIF subtypes clustered together most closely (Pearson correlation of 0.87). C5.PRO showed the most different pattern of associations compared with the other subtypes, although the overall Pearson correlation between C5.PRO and C2.IMM/C4.DIF was still high at 0.82.

The heatmap shows consistent, strong increased odds of all subtypes associated with family history of ovarian cancer and strong decreased odds of all subtypes associated with OC use, three or more pregnancies, having breastfed for more than 2 years, and ever use of alcohol. For C1.MES, the only additional factor associated with increased odds was E-only hormone therapy use for 10+ years. For C5.PRO, duration of breastfeeding and regular aspirin use were associated with decreased odds and endometriosis was associated with increased odds. The association between E-only hormone therapy and C5.PRO was much weaker than for the other subtypes. Genital powder use and former smoking were uniquely associated with increased odds of C2.IMM, and E-only hormone therapy was also strongly associated with increased odds of C2.IMM. For C4.DIF, current smoking and family history of breast cancer were uniquely associated with increased odds and similar to C5.PRO, endometriosis was associated with increased odds. Like C1.MES and C2.IMM, E-only hormone therapy was strongly associated with increased odds of C4.DIF.

## Discussion

In this study, several epidemiologic factors were similarly associated across HGSC subtypes, including increased risk

**Table 2.** Associations between epidemiologic factors and HGSC C1.MES, C5.PRO, C2.IMM, and C4.DIF subtypes among the 2,070 cases and 16,633 controls from 11 case–control studies.

| | Controls | C1.MES | | | C5.PRO | | | C2.IMM | | | C4.DIF | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | (*n* = 16,633) | (*n* = 556) | | | (*n* = 340) | | | (*n* = 538) | | | (*n* = 636) | | |
| **Characteristic** | ***n* (%)** | ***n* (%)** | **OR** | **(95% CI)** | ***n* (%)** | **OR** | **(95% CI)** | ***n* (%)** | **OR** | **(95% CI)** | ***n* (%)** | **OR** | **(95% CI)** |
| Family history of breast/ovarian cancer | | | | | | | | | | | | | |
| No known family history | 13,710 (84) | 429 (78) | 1.00 | (Ref.) | 261 (78) | 1.00 | (Ref.) | 411 (78) | 1.00 | (Ref.) | 462 (75) | 1.00 | (Ref.) |
| First-degree history of breast cancer only | 2,120 (13) | 82 (15) | 0.98 | (0.77–1.26) | 54 (16) | 1.09 | (0.81–1.48) | 72 (14) | 0.99 | (0.76–1.29) | 115 (19) | 1.44 | (1.16–1.78) |
| First-degree history of ovarian cancer | 464 (2.8) | 38 (6.9) | 2.45 | (1.71–3.51) | 20 (6) | 2.12 | (1.32–3.40) | 41 (7.8) | 2.86 | (2.02–4.04) | 40 (6.5) | 2.42 | (1.71–3.43) |
| Stepwise PRS (per 1 SD) | | | 1.40 | (1.28–1.53) | | 1.58 | (1.41–1.77) | | 1.43 | (1.30–1.56) | | 1.41 | (1.30–1.54) |
| Reproductive and hormonal characteristics | | | | | | | | | | | | | |
| OC use[a] | | | | | | | | | | | | | |
| No | 5,688 (34) | 207 (37) | 1.00 | (Ref.) | 142 (42) | 1.00 | (Ref.) | 216 (40) | 1.00 | (Ref.) | 232 (36) | 1.00 | (Ref.) |
| Yes | 10,945 (66) | 349 (63) | 0.60 | (0.49–0.74) | 198 (58) | 0.55 | (0.43–0.70) | 322 (60) | 0.56 | (0.45–0.68) | 404 (64) | 0.57 | (0.47–0.68) |
| Duration of OC use[a] | | | | | | | | | | | | | |
| Never used | 5,606 (35) | 201 (37) | 1.00 | (Ref.) | 141 (42) | 1.00 | (Ref.) | 208 (40) | 1.00 | (Ref.) | 218 (36) | 1.00 | (Ref.) |
| <6 months | 678 (4.2) | 23 (4.2) | 0.87 | (0.55–1.38) | 18 (5.4) | 1.08 | (0.64–1.82) | 27 (5.2) | 1.01 | (0.66–1.56) | 30 (4.9) | 0.97 | (0.64–1.47) |
| 6 months to <5 years | 4,388 (27) | 164 (30) | 0.72 | (0.57–0.91) | 92 (28) | 0.64 | (0.48–0.85) | 145 (28) | 0.64 | (0.51–0.82) | 196 (32) | 0.74 | (0.59–0.92) |
| 5 to <10 years | 2,651 (16) | 81 (15) | 0.58 | (0.44–0.78) | 35 (10) | 0.39 | (0.26–0.58) | 64 (12) | 0.45 | (0.34–0.62) | 91 (15) | 0.54 | (0.42–0.71) |
| ≥10 years | 2,864 (18) | 74 (14) | 0.47 | (0.35–0.63) | 49 (15) | 0.48 | (0.34–0.68) | 73 (14) | 0.45 | (0.33–0.60) | 75 (12) | 0.39 | (0.30–0.52) |
| Missing | 107 | 6 | | | 0 | | | 7 | | | 7 | | |
| Number of full-term pregnancies[b] | | | | | | | | | | | | | |
| 0 | 2,198 (16) | 68 (17) | 1.00 | (Ref.) | 38 (15) | 1.00 | (Ref.) | 81 (18) | 1.00 | (Ref.) | 96 (19) | 1.00 | (Ref.) |
| 1 | 2,058 (15) | 64 (16) | 0.98 | (0.69–1.41) | 32 (12) | 0.92 | (0.57–1.50) | 72 (16) | 0.94 | (0.68–1.31) | 77 (15) | 0.83 | (0.60–1.13) |
| 2 | 4,781 (34) | 124 (30) | 0.71 | (0.52–0.97) | 94 (36) | 0.98 | (0.66–1.44) | 133 (30) | 0.65 | (0.49–0.87) | 163 (32) | 0.67 | (0.52–0.88) |
| 3+ | 5,090 (36) | 151 (37) | 0.70 | (0.52–0.95) | 98 (37) | 0.78 | (0.53–1.16) | 157 (35) | 0.63 | (0.48–0.85) | 173 (34) | 0.68 | (0.52–0.89) |
| Age at last pregnancy (years)[c] | | | | | | | | | | | | | |
| <25 | 1,989 (16) | 67 (19) | 1.00 | (Ref.) | 43 (18) | 1.00 | (Ref.) | 90 (23) | 1.00 | (Ref.) | 84 (19) | 1.00 | (Ref.) |
| 25–29 | 3,901 (31) | 125 (35) | 1.05 | (0.77–1.45) | 85 (36) | 1.02 | (0.69–1.50) | 123 (31) | 0.73 | (0.54–0.97) | 166 (37) | 1.10 | (0.83–1.46) |
| 30–34 | 3,919 (31) | 105 (30) | 1.00 | (0.72–1.40) | 72 (30) | 0.95 | (0.64–1.43) | 105 (27) | 0.69 | (0.51–0.94) | 117 (26) | 0.83 | (0.61–1.12) |
| ≥35 | 2,894 (23) | 59 (17) | 0.85 | (0.58–1.25) | 38 (16) | 0.77 | (0.48–1.23) | 73 (19) | 0.72 | (0.51–1.02) | 79 (18) | 0.80 | (0.57–1.13) |
| Missing | 164 | 6 | | | 3 | | | 8 | | | 14 | | |
| Duration of breastfeeding[d] | | | | | | | | | | | | | |
| Never breastfed | 4,035 (34) | 144 (42) | 1.00 | (Ref.) | 95 (43) | 1.00 | (Ref.) | 138 (38) | 1.00 | (Ref.) | 146 (35) | 1.00 | (Ref.) |
| ≤6 months | 3,203 (27) | 88 (26) | 0.73 | (0.55–0.97) | 68 (31) | 0.82 | (0.59–1.15) | 116 (32) | 1.01 | (0.77–1.32) | 134 (32) | 1.02 | (0.79–1.32) |
| >6 months to 2 years | 3,372 (29) | 84 (25) | 0.75 | (0.55–1.02) | 47 (21) | 0.54 | (0.37–0.80) | 90 (25) | 0.80 | (0.59–1.08) | 107 (26) | 0.79 | (0.60–1.04) |
| >2 years | 1,165 (9.9) | 23 (6.8) | 0.61 | (0.37–1.00) | 12 (5.4) | 0.40 | (0.21–0.76) | 21 (5.8) | 0.59 | (0.36–0.98) | 27 (6.5) | 0.55 | (0.35–0.87) |
| Missing | 418 | 7 | | | 6 | | | 10 | | | 17 | | |
| Age at menarche (years) | | | | | | | | | | | | | |
| ≤11 | 3,221 (20) | 99 (18) | 1.00 | (Ref.) | 61 (18) | 1.00 | (Ref.) | 94 (18) | 1.00 | (Ref.) | 130 (21) | 1.00 | (Ref.) |
| 12–13 | 8,490 (52) | 307 (56) | 1.08 | (0.86–1.37) | 182 (54) | 1.07 | (0.79–1.44) | 290 (54) | 1.09 | (0.86–1.39) | 321 (51) | 0.91 | (0.73–1.12) |
| ≥14 | 4,767 (29) | 140 (26) | 0.96 | (0.73–1.25) | 91 (27) | 0.99 | (0.71–1.38) | 148 (28) | 1.04 | (0.79–1.36) | 176 (28) | 0.93 | (0.74–1.18) |
| Missing | 155 | 10 | | | 6 | | | 6 | | | 9 | | |
| Menopausal hormone therapy Never user | 8,412 (65) | 187 (50) | 1.00 | (Ref.) | 136 (55) | 1.00 | (Ref.) | 220 (54) | 1.00 | (Ref.) | 260 (56) | 1.00 | (Ref.) |
| E only <10 years | 856 (6.7) | 27 (7.2) | 1.02 | (0.67–1.57) | 19 (7.8) | 1.03 | (0.62–1.69) | 33 (8) | 1.24 | (0.84–1.83) | 34 (7.3) | 1.14 | (0.78–1.67) |
| E only ≥10 years | 503 (3.9) | 43 (11) | 2.14 | (1.46–3.14) | 17 (6.9) | 1.18 | (0.69–2.03) | 38 (9.3) | 2.00 | (1.36–2.95) | 34 (7.3) | 1.90 | (1.27–2.83) |
| E and progestin <10 years | 1,612 (13) | 63 (17) | 1.10 | (0.80–1.51) | 31 (13) | 0.79 | (0.52–1.20) | 47 (11) | 0.80 | (0.57–1.12) | 65 (14) | 0.96 | (0.71–1.29) |
| E and progestin ≥10 years | 625 (4.9) | 28 (7.4) | 0.98 | (0.63–1.51) | 15 (6.1) | 0.77 | (0.44–1.35) | 35 (8.5) | 1.24 | (0.84–1.85) | 31 (6.7) | 1.22 | (0.81–1.85) |
| Used both hormone therapies | 448 (3.5) | 11 (2.9) | 0.71 | (0.37–1.34) | 11 (4.5) | 1.08 | (0.57–2.06) | 16 (3.9) | 1.08 | (0.63–1.86) | 20 (4.3) | 1.25 | (0.77–2.05) |
| Used any unknown hormone therapies | 406 (3.2) | 17 (4.5) | 1.69 | (0.98–2.92) | 16 (6.5) | 1.53 | (0.87–2.69) | 21 (5.1) | 1.49 | (0.91–2.44) | 21 (4.5) | 1.32 | (0.81–2.15) |
| Missing | 149 (0) | 23 | | | 15 | | | 22 | | | 27 | | |
| Lifetime ovulatory years | | | 1.03 | (1.01–1.06) | | 1.03 | (1.01–1.06) | | 1.04 | (1.02–1.06) | | 1.05 | (1.03–1.07) |
| Endometriosis | | | | | | | | | | | | | |
| No | 13,868 (93) | 486 (94) | 1.00 | (Ref.) | 287 (91) | 1.00 | (Ref.) | 456 (93) | 1.00 | (Ref.) | 519 (91) | 1.00 | (Ref.) |
| Yes | 1,058 (7.1) | 34 (6.5) | 0.92 | (0.64–1.32) | 29 (9.2) | 1.46 | (0.98–2.16) | 35 (7.1) | 1.03 | (0.72–1.48) | 52 (9.1) | 1.27 | (0.94–1.71) |
| Missing | 591 | 28 | | | 22 | | | 36 | | | 48 | | |

*(Continued on the following page)*

**Table 2.** Associations between epidemiologic factors and HGSC C1.MES, C5.PRO, C2.IMM, and C4.DIF subtypes among the 2,070 cases and 16,633 controls from 11 case–control studies. (Cont'd)

| | Controls | C1.MES | | | C5.PRO | | | C2.IMM | | | C4.DIF | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | (*n* = 16,633) | (*n* = 556) | | | (*n* = 340) | | | (*n* = 538) | | | (*n* = 636) | | |
| **Characteristic** | ***n* (%)** | ***n* (%)** | **OR** | **(95% CI)** | ***n* (%)** | **OR** | **(95% CI)** | ***n* (%)** | **OR** | **(95% CI)** | ***n* (%)** | **OR** | **(95% CI)** |
| Tubal ligation | | | | | | | | | | | | | |
| No | 9,767 (75) | 305 (77) | 1.00 | (Ref.) | 204 (79) | 1.00 | (Ref.) | 330 (77) | 1.00 | (Ref.) | 378 (77) | 1.00 | (Ref.) |
| Yes | 3,174 (25) | 93 (23) | 0.86 | (0.67–1.11) | 55 (21) | 0.76 | (0.55–1.04) | 100 (23) | 0.87 | (0.69–1.12) | 114 (23) | 0.81 | (0.65–1.02) |
| Missing | 70 | 1 | | | 1 | | | 2 | | | 0 | | |
| Demographic and lifestyle characteristics | | | | | | | | | | | | | |
| BMI at 18 years of age (kg/m$^2$) | | | | | | | | | | | | | |
| 18.5–24.9 | 10,135 (73) | 276 (70) | 1.00 | (Ref.) | 180 (71) | 1.00 | (Ref.) | 313 (73) | 1.00 | (Ref.) | 327 (67) | 1.00 | (Ref.) |
| <18.5 | 2,406 (17) | 89 (22) | 1.06 | (0.83–1.36) | 50 (20) | 0.99 | (0.72–1.36) | 82 (19) | 0.92 | (0.72–1.19) | 102 (21) | 1.10 | (0.88–1.39) |
| 25–29.9 | 978 (7.1) | 25 (6.3) | 0.95 | (0.62–1.45) | 20 (7.8) | 1.12 | (0.70–1.80) | 31 (7.2) | 0.98 | (0.67–1.44) | 45 (9.2) | 1.35 | (0.97–1.87) |
| ≥30 | 309 (2.2) | 5 (1.3) | 0.64 | (0.26–1.58) | 5 (2) | 0.98 | (0.39–2.42) | 5 (1.2) | 0.53 | (0.21–1.30) | 15 (3.1) | 1.43 | (0.82–2.47) |
| Missing | 299 | 12 | | | 7 | | | 12 | | | 20 | | |
| Recent BMI (kg/m$^2$)[e] | | | | | | | | | | | | | |
| 18.5–24.9 | 6,471 (46) | 212 (43) | 1.00 | (Ref.) | 132 (44) | 1.00 | (Ref.) | 206 (44) | 1.00 | (Ref.) | 234 (44) | 1.00 | (Ref.) |
| <18.5 | 268 (1.9) | 8 (1.6) | 0.90 | (0.43–1.86) | 4 (1.3) | 0.70 | (0.25–1.92) | 11 (2.4) | 1.20 | (0.64–2.26) | 10 (1.9) | 1.03 | (0.54–1.99) |
| 25–29.9 | 4,146 (30) | 140 (28) | 0.90 | (0.72–1.13) | 103 (35) | 1.05 | (0.81–1.37) | 136 (29) | 0.92 | (0.74–1.15) | 135 (25) | 0.84 | (0.68–1.05) |
| ≥30 | 3,164 (23) | 137 (28) | 1.10 | (0.88–1.38) | 59 (20) | 0.77 | (0.56–1.05) | 114 (24) | 0.97 | (0.76–1.23) | 151 (29) | 1.14 | (0.92–1.41) |
| Missing | 117 | 36 | | | 15 | | | 26 | | | 31 | | |
| Aspirin use | | | | | | | | | | | | | |
| Nonregular use | 6,948 (80) | 270 (81) | 1.00 | (Ref.) | 179 (85) | 1.00 | (Ref.) | 297 (83) | 1.00 | (Ref.) | 336 (81) | 1.00 | (Ref.) |
| Regular use | 1703 (20) | 64 (19) | 0.97 | (0.73–1.30) | 31 (15) | 0.72 | (0.48–1.06) | 62 (17) | 0.98 | (0.73–1.31) | 78 (19) | 1.09 | (0.84–1.42) |
| Missing | 4,360 | 65 | | | 50 | | | 73 | | | 78 | | |
| Frequency of aspirin use | | | | | | | | | | | | | |
| Nonregular use | 5,475 (80) | 256 (81) | 1.00 | (Ref.) | 161 (85) | 1.00 | (Ref.) | 267 (83) | 1.00 | (Ref.) | 301 (82) | 1.00 | (Ref.) |
| <30 days/month regular use | 321 (4.7) | 14 (4.4) | 0.84 | (0.48–1.48) | 12 (6.3) | 1.23 | (0.67–2.26) | 18 (5.6) | 1.17 | (0.71–1.92) | 30 (8.1) | 1.58 | (1.06–2.37) |
| ≥30 days/month regular use (daily use) | 1,075 (16) | 47 (15) | 1.04 | (0.74–1.45) | 16 (8.5) | 0.54 | (0.32–0.93) | 38 (12) | 0.93 | (0.64–1.33) | 38 (10) | 0.81 | (0.56–1.15) |
| Missing | 3,028 | 44 | | | 31 | | | 60 | | | 53 | | |
| Genital powder use | | | | | | | | | | | | | |
| No | 5,811 (74) | 182 (73) | 1.00 | (Ref.) | 109 (74) | 1.00 | (Ref.) | 181 (66) | 1.00 | (Ref.) | 233 (73) | 1.00 | (Ref.) |
| Yes | 2,012 (26) | 66 (27) | 1.04 | (0.77–1.41) | 39 (26) | 0.93 | (0.63–1.36) | 95 (34) | 1.42 | (1.08–1.86) | 86 (27) | 1.10 | (0.84–1.44) |
| Missing | 1,586 | 137 | | | 82 | | | 119 | | | 119 | | |
| Smoking status | | | | | | | | | | | | | |
| Never | 8,498 (56) | 319 (59) | 1.00 | (Ref.) | 196 (59) | 1.00 | (Ref.) | 277 (53) | 1.00 | (Ref.) | 343 (54) | 1.00 | (Ref.) |
| Current | 2,034 (13) | 64 (12) | 1.05 | (0.79–1.39) | 32 (9.6) | 0.93 | (0.63–1.37) | 58 (11) | 1.00 | (0.74–1.35) | 106 (17) | 1.40 | (1.11–1.76) |
| Former | 4,753 (31) | 159 (29) | 0.87 | (0.71–1.06) | 105 (32) | 0.95 | (0.74–1.21) | 193 (37) | 1.25 | (1.03–1.51) | 181 (29) | 0.97 | (0.80–1.16) |
| Missing | 1,348 | 14 | | | 7 | | | 10 | | | 6 | | |
| Lifetime alcohol use | | | | | | | | | | | | | |
| Never | 3,560 (41) | 45 (40) | 1.00 | (Ref.) | 33 (33) | 1.00 | (Ref.) | 53 (33) | 1.00 | (Ref.) | 69 (34) | 1.00 | (Ref.) |
| Ever | 4,007 (46) | 56 (50) | 0.42 | (0.27–0.67) | 55 (55) | 0.62 | (0.38–1.02) | 86 (53) | 0.69 | (0.46–1.03) | 109 (54) | 0.68 | (0.47–0.97) |
| Former | 1,184 (14) | 11 (9.8) | 0.50 | (0.25–1.00) | 12 (12) | 0.87 | (0.44–1.72) | 22 (14) | 1.16 | (0.69–1.96) | 25 (12) | 0.84 | (0.52–1.36) |
| Missing | 2,544 | 132 | | | 73 | | | 90 | | | 102 | | |
| Recent alcohol use[e] | | | | | | | | | | | | | |
| No recent alcohol use | 5,423 (48) | 147 (42) | 1.00 | (Ref.) | 101 (47) | 1.00 | (Ref.) | 144 (38) | 1.00 | (Ref.) | 173 (42) | 1.00 | (Ref.) |
| Any recent alcohol use | 5,813 (52) | 204 (58) | 0.91 | (0.72–1.15) | 113 (53) | 0.73 | (0.55–0.98) | 232 (62) | 1.07 | (0.85–1.35) | 241 (58) | 0.95 | (0.77–1.18) |
| Missing | | | | | | | | | | | | | |

NOTE: All models are adjusted for age at reference, number of live births, ever used OCs, and study site unless otherwise noted.
[a]Models are adjusted for age at reference, number of live births, and study site.
[b]Models are adjusted for age at reference, ever used OCs, and study site.
[c]Restricted to those who have had a pregnancy. Models are adjusted for age at reference, number of pregnancies, ever used OCs, and study site.
[d]Restricted to those who had a live birth.
[e]Reference defined as 5 years prior to diagnosis/interview date or 1 year prior to interview date.

associated with family history of ovarian cancer and decreased risk associated with OC use, having three or more pregnancies, having breastfed for more than 2 years, and ever use of alcohol—suggesting common biological effects. Still, the magnitude of associations for some factors varied across HGSC subtypes, specifically highlighting the potential role of past smoking history and genital powder use in risk of C2.IMM; older age at diagnosis and history of endometriosis in increased risk and daily aspirin use in reduced risk of C5.PRO; and younger age at diagnosis, smoking history, family history of breast cancer only, and endometriosis in risk of C4.DIF. We also illustrate that risk factor profiles for C4.DIF and C2.IMM are more similar to each other than to those for C1.MES and C5.PRO.

**Table 3.** Case-only associations between epidemiologic factors and HGSC subtypes (C1.MES referent) accounting for the probability of subtype classification (entropy) among the 2,290 cases from 12 studies.

| | C1.MES (Ref.) | C5.PRO | | | C2.IMM | | | C4.DIF | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | (*n* = 587) | (*n* = 385) | | | (*n* = 582) | | | (*n* = 736) | | |
| **Factor** | ***n* (%)** | ***n* (%)** | **OR** | **(95% CI)** | ***n* (%)** | **OR** | **(95% CI)** | ***n* (%)** | **OR** | **(95% CI)** |
| Family history of breast/ovarian cancer | | | | | | | | | | |
| No known family history | 456 (79) | 301 (79) | 1.00 | (Ref.) | 446 (79) | 1.00 | (Ref.) | 536 (75) | 1.00 | (Ref.) |
| First-degree history of breast cancer only | 86 (15) | 56 (15) | 1.04 | (0.71, 1.54) | 78 (14) | 1.01 | (0.71, 1.44) | 131 (18) | 1.39 | (1.01, 1.90) |
| First-degree history of ovarian cancer | 38 (6.6) | 23 (6.1) | 0.93 | (0.53, 1.63) | 44 (7.7) | 1.16 | (0.72, 1.87) | 50 (7) | 1.08 | (0.68, 1.71) |
| Stepwise PRS (per 1 SD) | | | 1.14 | (0.99, 1.31) | | 1.00 | (0.88, 1.13) | | 0.98 | (0.87, 1.10) |
| Reproductive and hormonal characteristics | | | | | | | | | | |
| OC use[a] | | | | | | | | | | |
| No | 225 (38) | 164 (43) | 1.00 | (Ref.) | 242 (42) | 1.00 | (Ref.) | 288 (39) | 1.00 | (Ref.) |
| Yes | 362 (62) | 221 (57) | 0.98 | (0.72, 1.33) | 340 (58) | 0.94 | (0.71, 1.24) | 448 (61) | 0.98 | (0.76, 1.28) |
| Duration of OC use[a] | | | | | | | | | | |
| Never used | 219 (38) | 163 (43) | 1.00 | (Ref.) | 234 (42) | 1.00 | (Ref.) | 274 (39) | 1.00 | (Ref.) |
| <6 months | 23 (4) | 20 (5.3) | 1.54 | (0.78, 3.09) | 27 (4.8) | 1.25 | (0.66, 2.37) | 32 (4.5) | 1.20 | (0.66, 2.20) |
| 6 months to <5 years | 168 (29) | 99 (26) | 0.93 | (0.65, 1.35) | 154 (27) | 0.90 | (0.65, 1.25) | 211 (30) | 1.04 | (0.76, 1.41) |
| 5 to <10 years | 84 (15) | 42 (11) | 0.75 | (0.47, 1.20) | 67 (12) | 0.75 | (0.50, 1.14) | 106 (15) | 0.94 | (0.65, 1.37) |
| ≥10 years | 79 (14) | 56 (15) | 1.09 | (0.70, 1.70) | 79 (14) | 0.96 | (0.64, 1.44) | 87 (12) | 0.87 | (0.59, 1.28) |
| Missing | 7 | 0 | | | 7 | | | 7 | | |
| Number of full-term pregnancies[b] | | | | | | | | | | |
| 0 | 72 (16) | 42 (14) | 1.00 | (Ref.) | 86 (18) | 1.00 | (Ref.) | 115 (19) | 1.00 | (Ref.) |
| 1 | 68 (16) | 39 (13) | 0.94 | (0.53, 1.67) | 76 (16) | 0.88 | (0.48, 1.42) | 89 (15) | 0.75 | (0.48, 1.18) |
| 2 | 136 (31) | 113 (37) | 1.42 | (0.87, 2.30) | 156 (32) | 0.98 | (0.64, 1.48) | 206 (34) | 0.91 | (0.62, 1.34) |
| 3+ | 162 (37) | 113 (37) | 1.15 | (0.71, 1.86) | 169 (35) | 0.90 | (0.60, 1.36) | 199 (33) | 0.89 | (0.61, 1.31) |
| Age at last pregnancy (years)[c] | | | | | | | | | | |
| <25 | 70 (18) | 49 (18) | 1.00 | (Ref.) | 94 (22) | 1.00 | (Ref.) | 91 (17) | 1.00 | (Ref.) |
| 25–29 | 136 (35) | 93 (33) | 0.83 | (0.51, 1.35) | 133 (31) | 0.65 | (0.42, 0.99) | 192 (36) | 0.99 | (0.66, 1.49) |
| 30–34 | 111 (29) | 90 (32) | 0.86 | (0.52, 1.42) | 124 (29) | 0.69 | (0.44, 1.08) | 142 (27) | 0.84 | (0.55, 1.31) |
| ≥35 | 66 (17) | 47 (17) | 0.88 | (0.49, 1.57) | 79 (18) | 0.86 | (0.52, 1.42) | 101 (19) | 1.05 | (0.65, 1.72) |
| Missing | 6 | 3 | | | 8 | | | 15 | | |
| Duration of breastfeeding[d] | | | | | | | | | | |
| Never breastfed | 149 (41) | 108 (41) | 1.00 | (Ref.) | 148 (37) | 1.00 | (Ref.) | 170 (34) | 1.00 | (Ref.) |
| ≤6 months | 99 (27) | 79 (30) | 0.95 | (0.63, 1.45) | 126 (31) | 1.21 | (0.83, 1.77) | 168 (34) | 1.30 | (0.91, 1.86) |
| >6 months to 2 years | 89 (24) | 60 (23) | 0.83 | (0.52, 1.31) | 106 (26) | 1.23 | (0.82, 1.86) | 125 (25) | 1.08 | (0.73, 1.59) |
| >2 years | 26 (7.2) | 14 (5.4) | 0.59 | (0.27, 1.29) | 23 (5.7) | 0.95 | (0.48, 1.90) | 30 (5.7) | 0.91 | (0.48, 1.72) |
| Missing | 10 | 8 | | | 11 | | | 19 | | |
| Age at menarche (years) | | | | | | | | | | |
| ≤11 | 107 (19) | 72 (19) | 1.00 | (Ref.) | 105 (18) | 1.00 | (Ref.) | 152 (21) | 1.00 | (Ref.) |
| 12–13 | 318 (55) | 201 (53) | 1.02 | (0.71, 1.48) | 308 (54) | 1.01 | (0.72, 1.40) | 361 (50) | 0.87 | (0.64, 1.18) |
| ≥14 | 151 (26) | 105 (28) | 0.91 | (0.60, 1.38) | 162 (28) | 0.96 | (0.66, 1.40) | 210 (29) | 0.91 | (0.65, 1.28) |
| Missing | 11 | 7 | | | 7 | | | 13 | | |
| Menopausal hormone therapy | | | | | | | | | | |
| Never user | 187 (50) | 136 (55) | 1.00 | (Ref.) | 220 (54) | 1.00 | (Ref.) | 260 (56) | 1.00 | (Ref.) |
| E only <10 years | 27 (7.2) | 19 (7.8) | 1.00 | (0.51, 1.96) | 33 (8) | 1.24 | (0.69, 2.24) | 34 (7.3) | 1.10 | (0.62, 1.96) |
| E only ≥10 years | 43 (11) | 17 (6.9) | 0.49 | (0.25, 0.95) | 38 (9.3) | 0.84 | (0.48, 1.45) | 34 (7.3) | 0.81 | (0.47, 1.40) |
| E and progestin <10 years | 63 (17) | 31 (13) | 0.74 | (0.43, 1.27) | 47 (12) | 0.73 | (0.45, 1.17) | 65 (14) | 0.90 | (0.58, 1.39) |
| E and progestin ≥10 years | 28 (7.4) | 15 (6.1) | 0.82 | (0.40, 1.69) | 35 (8.5) | 1.26 | (0.69, 2.30) | 31 (6.7) | 1.32 | (0.73, 2.42) |
| Used both hormone therapies | 11 (2.9) | 11 (4.5) | 1.44 | (0.58, 3.59) | 16 (3.9) | 1.44 | (0.62, 3.35) | 20 (4.3) | 1.67 | (0.75, 3.70) |
| Used any unknown hormone therapies | 17 (4.5) | 16 (6.5) | 0.78 | (0.35, 1.75) | 21 (5.1) | 0.68 | (0.32, 1.44) | 21 (4.5) | 0.71 | (0.34, 1.50) |
| Missing | 23 | 15 | | | 22 | | | 27 | | |
| Lifetime ovulatory years | | | 1.00 | (0.96, 1.03) | | 1.00 | (0.97–1.03) | | 1.01 | (0.98, 1.03) |
| Endometriosis | | | | | | | | | | |
| No | 486 (94) | 287 (91) | 1.00 | (Ref.) | 456 (93) | 1.00 | (Ref.) | 519 (91) | 1.00 | (Ref.) |
| Yes | 34 (6.5) | 29 (9.2) | 1.40 | (0.81, 2.44) | 35 (7.1) | 0.98 | (0.58, 1.65) | 52 (9.1) | 1.23 | (0.76, 1.97) |
| Missing | 28 | 22 | | | 36 | | | 48 | | |
| Tubal ligation | | | | | | | | | | |
| No | 333 (78) | 240 (80) | 1.00 | (Ref.) | 367 (78) | 1.00 | (Ref.) | 458 (78) | 1.00 | (Ref.) |
| Yes | 95 (22) | 62 (20) | 0.93 | (0.63, 1.39) | 105 (22) | 1.01 | (0.71, 1.42) | 127 (22) | 1.01 | (0.73, 1.40) |
| Missing | 2 | 3 | | | 4 | | | 7 | | |

*(Continued on the following page)*

**Table 3.** Case-only associations between epidemiologic factors and HGSC subtypes (C1.MES referent) accounting for the probability of subtype classification (entropy) among the 2,290 cases from 12 studies. (Cont'd)

| | C1.MES (Ref.) | C5.PRO | | | C2.IMM | | | C4.DIF | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | (*n* = 587) | (*n* = 385) | | | (*n* = 582) | | | (*n* = 736) | | |
| **Factor** | ***n* (%)** | ***n* (%)** | **OR** | **(95% CI)** | ***n* (%)** | **OR** | **(95% CI)** | ***n* (%)** | **OR** | **(95% CI)** |
| Demographic and lifestyle characteristics | | | | | | | | | | |
| Age at diagnosis (years)[e,f] | | | | | | | | | | |
| Mean age (SD) | 60.7 (10) | 61.4 (9.6) | | | 60 (10) | | | 57.5 (9.6) | | |
| <49 | 93 (16) | 42 (11) | 1.00 | (Ref.) | 91 (16) | 1.00 | (Ref.) | 159 (22) | 1.00 | (Ref.) |
| 50–54 | 66 (11) | 44 (11) | 1.56 | (0.90, 2.69) | 77 (13) | 1.29 | (0.82, 2.05) | 126 (17) | 1.16 | (0.77, 1.75) |
| 55–59 | 96 (16) | 67 (17) | 1.52 | (0.92, 2.51) | 104 (18) | 1.16 | (0.76, 1.77) | 153 (21) | 0.97 | (0.66, 1.41) |
| 60–64 | 131 (22) | 81 (21) | 1.37 | (0.84, 2.22) | 125 (21) | 1.04 | (0.70, 1.56) | 119 (16) | 0.54 | (0.37, 0.79) |
| 65–69 | 92 (16) | 76 (20) | 1.82 | (1.09, 3.04) | 87 (15) | 1.02 | (0.65, 1.60) | 98 (13) | 0.61 | (0.40, 0.92) |
| ≥70 | 109 (19) | 75 (19) | 1.78 | (1.04, 3.06) | 98 (17) | 1.14 | (0.72, 1.81) | 81 (11) | 0.50 | (0.32, 0.78) |
| BMI at 18 years of age (kg/m$^2$) | | | | | | | | | | |
| 18.5–24.9 | 297 (70) | 215 (72) | 1.00 | (Ref.) | 346 (73) | 1.00 | (Ref.) | 397 (69) | 1.00 | (Ref.) |
| <18.5 | 93 (22) | 55 (18) | 0.99 | (0.66, 1.47) | 87 (18) | 0.93 | (0.66, 1.33) | 113 (20) | 1.07 | (0.77, 1.49) |
| 25–29.9 | 28 (6.6) | 23 (7.7) | 1.06 | (0.58, 1.94) | 34 (7.2) | 0.99 | (0.57, 1.71) | 51 (8.8) | 1.32 | (0.80, 2.18) |
| ≥30 | 6 (1.4) | 6 (2) | 1.41 | (0.42, 4.70) | 5 (1.1) | 0.65 | (0.19, 2.26) | 17 (2.9) | 1.80 | (0.68, 4.78) |
| Missing | 14 | 8 | | | 15 | | | 31 | | |
| Recent BMI (kg/m$^2$)[e] | | | | | | | | | | |
| 18.5–24.9 | 212 (43) | 132 (44) | 1.00 | (Ref.) | 206 (44) | 1.00 | (Ref.) | 234 (44) | 1.00 | (Ref.) |
| <18.5 | 8 (1.6) | 4 (1.3) | 0.88 | (0.25, 3.17) | 11 (2.4) | 1.50 | (0.55, 4.09) | 10 (1.9) | 1.21 | (0.45, 3.26) |
| 25–29.9 | 140 (28) | 103 (35) | 1.17 | (0.82, 1.66) | 136 (29) | 1.01 | (0.73, 1.40) | 135 (26) | 0.90 | (0.66, 1.23) |
| ≥30 | 137 (28) | 59 (20) | 0.73 | (0.49, 1.09) | 114 (24) | 0.95 | (0.68, 1.33) | 151 (29) | 1.05 | (0.76, 1.44) |
| Missing | 36 | 15 | | | 26 | | | 31 | | |
| Aspirin use | | | | | | | | | | |
| Nonregular use | 270 (81) | 179 (85) | 1.00 | (Ref.) | 297 (83) | 1.00 | (Ref.) | 336 (81) | 1.00 | (Ref.) |
| Regular use | 65 (19) | 31 (15) | 0.70 | (0.43, 1.15) | 62 (17) | 0.97 | (0.64, 1.47) | 78 (19) | 1.11 | (0.75, 1.65) |
| Missing | 65 | 50 | | | 73 | | | 78 | | |
| Frequency of aspirin use | | | | | | | | | | |
| Nonregular use | 256 (81) | 161 (85) | 1.00 | (Ref.) | 267 (83) | 1.00 | (Ref.) | 301 (83) | 1.00 | (Ref.) |
| <30 days/month regular use | 14 (4.4) | 12 (6.3) | 1.34 | (0.59, 3.08) | 18 (5.6) | 1.23 | (0.58, 2.63) | 30 (8.1) | 1.79 | (0.91, 3.54) |
| ≥30 days/month regular use (daily use) | 47 (15) | 16 (8.5) | 0.48 | (0.25, 0.91) | 38 (12) | 0.85 | (0.51, 1.40) | 38 (10) | 0.77 | (0.47, 1.25) |
| Missing | 44 | 31 | | | 60 | | | 53 | | |
| Genital powder use | | | | | | | | | | |
| No | 182 (73) | 109 (74) | 1.00 | (Ref.) | 183 (66) | 1.00 | (Ref.) | 234 (73) | 1.00 | (Ref.) |
| Yes | 66 (27) | 39 (26) | 0.94 | (0.57, 1.56) | 96 (34) | 1.42 | (0.94, 2.14) | 87 (27) | 1.05 | (0.70, 1.58) |
| Missing | 143 | 92 | | | 133 | | | 132 | | |
| Smoking status | | | | | | | | | | |
| Never | 337 (59) | 218 (58) | 1.00 | (Ref.) | 306 (54) | 1.00 | (Ref.) | 403 (55) | 1.00 | (Ref.) |
| Current | 67 (12) | 35 (9.3) | 0.95 | (0.59, 1.52) | 59 (13) | 1.00 | (0.67, 1.51) | 114 (16) | 1.34 | (0.94, 1.91) |
| Former | 169 (30) | 125 (33) | 1.29 | (0.95, 1.76) | 207 (36) | 1.52 | (1.16, 2.00) | 213 (29) | 1.15 | (0.88, 1.49) |
| Missing | 14 | 7 | | | 10 | | | 6 | | |
| Lifetime alcohol use | | | | | | | | | | |
| Never | 45 (40) | 33 (33) | 1.00 | (Ref.) | 53 (33) | 1.00 | (Ref.) | 69 (34) | 1.00 | (Ref.) |
| Ever | 56 (50) | 55 (55) | 1.31 | (0.64, 2.67) | 86 (53) | 1.34 | (0.70, 2.54) | 109 (54) | 1.46 | (0.79, 2.68) |
| Former | 11 (9.8) | 12 (12) | 1.12 | (0.39, 3.17) | 22 (14) | 1.46 | (0.58, 3.64) | 25 (12) | 1.04 | (0.43, 2.52) |
| Missing | 132 | 73 | | | 90 | | | 102 | | |
| Recent alcohol use[e] | | | | | | | | | | |
| No recent alcohol use | 148 (42) | 101 (47) | 1.00 | (Ref.) | 146 (38) | 1.00 | (Ref.) | 174 (41) | 1.00 | (Ref.) |
| Any recent alcohol use | 209 (58) | 116 (53) | 0.86 | (0.59, 1.25) | 242 (62) | 1.20 | (0.87, 1.67) | 252 (59) | 1.06 | (0.77, 1.44) |
| Missing | | | | | | | | | | |

NOTE: Models include SEA (case-only study). All models are adjusted for age at diagnosis, number of live births, ever used OCs, entropy, and study site unless otherwise noted.

[a]Models are adjusted for age at diagnosis, number of live births, and study site.

[b]Models are adjusted for age at diagnosis, ever used OCs, and study site.

[c]Restricted to those who have had a pregnancy. Models are adjusted for age at diagnosis, number of pregnancies, ever used OCs, and study site.

[d]Restricted to those women who had a live birth.

[e]Reference defined as 5 years prior to diagnosis date or 1 year prior to interview date.

[f]Models are adjusted for number of live births, ever used OCs, and study site.

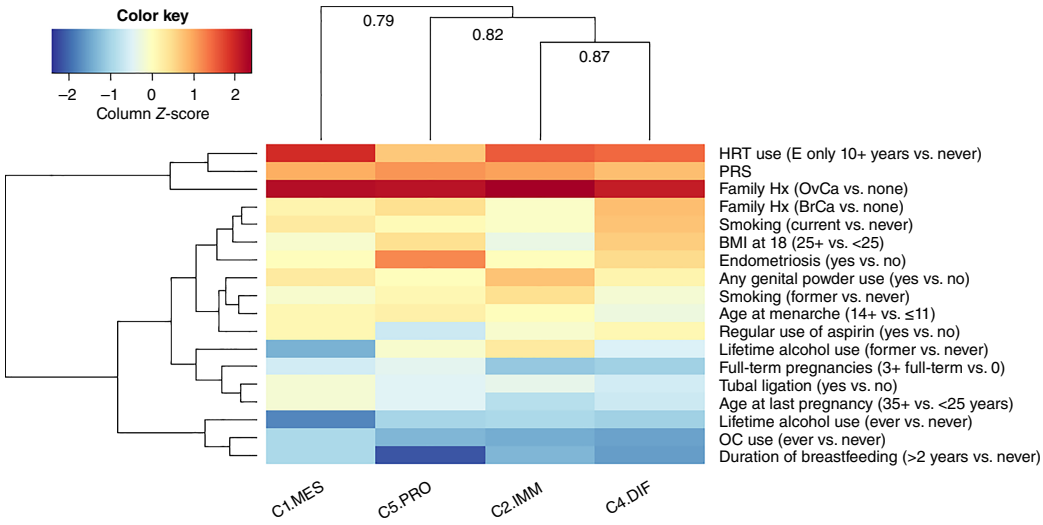


**Figure 1.**

Heatmap associating epidemiologic factors with HGSC subtypes among 2,070 cases and 16,633 disease-free controls in the 11 pooled case–control studies. The dichotomized variables included OC use (ever vs. never), number of full-term pregnancies (3+ vs. none), age at last pregnancy (35+ vs. < 25 years), breastfeeding (2+ years vs. never), age at menarche (14+ vs. ≤11 years), E-only hormone therapy (10+ years vs. never), endometriosis (yes vs. no), tubal ligation (yes vs. no), BMI at 18 years of age (≥25 vs. < 25 kg/m$^2$), regular aspirin use (any vs. none), genital powder use (any vs. none), current smoking (vs. never), former smoking (vs. never), alcohol use (ever vs. never), first-degree family history of ovarian cancer (vs. none), and first-degree family history of breast cancer only (vs. none). BrCa, breast cancer; HRT, hormone replacement therapy; Hx, history; OvCa, ovarian cancer.

In the only other study on epidemiologic factors by HGSC subtypes, which was smaller ($n = 193$) and was not restricted to HGSC, OC use and pregnancy history were similarly not strongly differentially associated with HGSC subtypes (39). In that study, Schildkraut and colleagues accounted for intratumoral heterogeneity by using the probability of subtype assignment in their modeling approach. They reported that family history of breast or ovarian cancer was more likely to be observed among C2.IMM, and we also observed that family history of ovarian cancer with or without breast cancer was most strongly associated with C2.IMM. These findings are consistent with the observation that a family history of either ovarian or breast cancer is associated with less aggressive disease (defined as surviving for five or more years after diagnosis), as survival is better for C2.IMM (28). George and colleagues (60) reported that C2.IMM is more common in tumors with germline or somatic aberrations in *BRCA1* than in *BRCA2*. We observed that history of breast cancer without ovarian cancer was associated with C4.DIF only (39), which aligns with the observations from the original PrOTYPE article that *BRCA1* and *BRCA2* mutations were most common (~37%) in C4.DIF (35). Indeed, our reanalysis of the data in George and colleagues (60) shows that the prevalence of germline *BRCA1* and *BRCA2* mutations was highest in C4.DIF (25%). Chen and colleagues (31) reported that patients with HGSC classified as C4.DIF were on average approximately 4 to 9 years younger at diagnosis than the other subtypes, which is consistent with tumors diagnosed among women with *BRCA* mutations. In the current article, we also observed that C4.DIF was diagnosed approximately 3 years younger on average than the other HGSC subtypes.

Many of the subtype-specific associations observed may be due to inflammation-related pathways. Current smoking has been reported to be associated with highly aggressive disease (death within 1 year of diagnosis; ref. 28), but our study suggests associations with C2.IMM and C4.DIF, which have better survival. We also observed different associations for current versus former smokers, which may relate to the recovery of the immune response among former smokers, misclassification with self-reported data, differences in smoking duration, or time since smoking cessation (15, 18, 61). A recent study using data from the Nurses' Health Study reported that early-life exposure to cigarette smoke was associated with changes to the tumor immune microenvironment (including activation of cytotoxic T cells; ref. 62), which may explain the results observed for the association between smoking history and C2.IMM. Smoking has also been associated with certain cytokines and MUC16 expression (63–65), which are characteristic of C4.DIF. Recent evidence from cohort studies has indicated increased risk of HGSC among those with a history of endometriosis (26, 66, 67), complementing the results observed in the current study between C5.PRO and C4.DIF subtypes. We observed that ever use of alcohol was associated with decreased risk of all HGSC subtypes, but prior studies of alcohol consumption have been mixed. In the pooled analyses in Kelemen and colleagues (68), several of the larger studies, included in that article and in the current analysis, observed a decreased risk of EOC associated with ever use [e.g., Diseases of the Ovary and their Evaluation (DOV), Australian Ovarian Cancer Study (AUS), and New England Case-Control Study (NEC)]. Results for ever use of alcohol were not presented by histotype, but they reported a 12% decreased risk of HGSC associated with consumption of more than two drinks per day. An inflammation-related risk score was developed in OCAC using 12 epidemiologic factors (alcohol use, aspirin use, other NSAID use, BMI, environmental smoke exposure, history of pelvic inflammatory disease, polycystic ovarian syndrome, endometriosis, menopausal hormonal therapy, physical inactivity, smoking status, and talc use), which was shown to be associated with ovarian cancer mortality (69). This study provides further evidence that prediagnostic behavioral and lifestyle factors likely affect inflammation, the development of the tumor immune microenvironment, and the immune response, which are also reflected in the results of the current article.

Intratumoral heterogeneity is a concern. The PrOTYPE algorithm assigns a probability for each of the four HGSC subtypes for every tumor (summing to 100%), and the final subtype assignment was based on the subtype with the highest probability. Nearly all samples in our study show probabilities of multiple subtypes (Supplementary Fig. S1), which has been observed previously (31, 32, 35). We accounted for intratumoral heterogeneity by restricting analyses to samples with a probability of a subtype assignment of >80% and by performing case-only analyses controlling for the confidence of subtype assignment. Although a substantial proportion of cases were excluded in the sensitivity analysis (41%), Supplementary Fig. S1 demonstrates that the majority of HGSC cases are classified with a >50% probability for one subtype and that the contribution of the other three subtypes is typically low.

There has been some debate in the literature about the optimal number of HGSC molecular subtypes, with studies reporting two to five HGSC subtypes, using various methods and data sources (29–38). Konecny and colleagues compared survival patterns between classifications of three and four subtypes and observed that there were larger survival differences between the four subtypes, leading the authors to conclude that four subtypes were more clinically relevant. Indeed, most studies have reported similar differences in survival across the four subtypes, regardless of the methods used to define them. Improved precision in molecular subtyping will likely be addressed through analysis of single-cell RNA sequencing and will help clarify the relative contributions of gene expression patterns in the tumor versus the tumor microenvironment (29–38, 70).

This study has additional limitations. First, we analyzed 11 case–control studies, all of which had variation in data collection, question administration for exposure information, patterns of missingness, and selection of controls, which may influence the reported results. However, because ovarian cancer is rare, pooled analyses and consortium-based studies have been highly effective at understanding ovarian cancer etiology. Second, despite this being the first and largest study to date, many of the estimates were statistically imprecise because of the small sample sizes within subtypes, potentially contributing to chance findings. Still, all of the epidemiologic factors evaluated have been previously studied in relation to ovarian cancer and have been shown to be associated with an increased risk or decreased risk of HGSC. These established relationships provide evidence of an association, which supports our approach to examine the estimates and their precision, as well as the decision to not account for multiple testing. Therefore, additional studies in larger populations will be necessary to replicate the findings. Third, there could be residual confounding because we only controlled for age, parity, OC use, and study site. Fourth, the PrOTYPE assay was implemented in more than one iteration, and there may be batch effects that could explain variation across study sites; however, Ovarian Tumor Tissue Analysis has previously demonstrated that samples are classified as the same subtypes across batches (71). Finally, our study includes primarily individuals self-reporting White race, which may not generalize to all populations. Future studies are needed to further clarify the observed patterns of associations and will benefit from more diverse study populations (38).

The observed patterns of similarities and differences in epidemiologic factors by biologically relevant subtypes provide information about the etiology of HGSC subtypes. Our study provides evidence that risk factor profiles could be important drivers of tumor heterogeneity that can influence survival and be used in risk modeling to identify individuals who are more likely to have aggressive tumors.

## Authors' Disclosures

L.J. Collin reports grants from NCI during the conduct of the study, as well as personal fees from Epidemiologic Research & Methods LLC outside the submitted work. K.L. Terry reports grants from NIH during the conduct of the study. N. Sasamoto reports grants from Department of Defense, Rivkin Foundation, American Cancer Society, Aspira Women's Health, and NIH outside the submitted work. F. Modugno reports grants from NCI and Department of Defense during the conduct of the study. P.A. Fasching reports grants and personal fees from Novartis and Pfizer, grants from BioNTech, and personal fees from Daiichi Sankyo, AstraZeneca, Eisai, Merck Sharp & Dohme, Cepheid, Eli Lilly and Company, Seagen, Roche, Agendia, Gilead Sciences, Mylan, Menarini Stemline, Veracyte, and Guardant Health outside the submitted work. C.L. Pearce reports grants from NIH and Department of Defense during the conduct of the study. U. Menon reports grants and personal fees from Mercy BioAnalytics, grants from Intelligent Lab on Fiber and RNA Guardian and other support from Synteny outside the submitted work. A. Gentry-Maharaj reports other support from Medical Research Council, Cancer Research UK, NIH Research, and The Eve Appeal during the conduct of the study; other support from Intelligent Lab on Fiber, RNA Guardian, Micronoma, and Mercy BioAnalytics outside the submitted work; research collaborations with academic institutions: University of Cambridge, QIMR Berghofer Medical Research Institute, Imperial College London, University of Innsbruck, and Dana-Farber USA; and being the Cancer Research UK ACED co-director of research domain trials. M.S. Anglesio reports grants from Health Research BC and NIH during the conduct of the study. D.D.L. Bowtell reports grants from Roche-Genentech (paid to institution) and AstraZeneca (paid to institution) and personal fees from Exo Therapeutics and Cartherics outside the submitted work. S.S. Tworoger reports grants from NIH/NCI during the conduct of the study; grants from NIH, state of Florida, Department of Defense, Bristol Myers Squibb, and American Cancer Society and personal fees from American Association for Cancer Research, Ponce Health Sciences University, Ovarian Cancer Research Alliance, University of North Carolina, UNC Lineberger Comprehensive Cancer Center, and Roswell Park Comprehensive Cancer Center outside the submitted work; and nonpaid relationships member of the external advisory committee of the California Teachers Study (City of Hope), The Tomorrow Project (Alberta Cancer Center), and TRANCE program at Fred Hutchinson Cancer Center. J.M. Schildkraut reports grants from NIH/NCI during the conduct of the study. P.M. Webb reports grants from US Army Medical Research and Materiel Command, National Health and Medical Research Council of Australia, cancer councils of New South Wales, Victoria, Queensland, South Australia, and Tasmania, and Cancer Foundation of Western Australia during the conduct of the study. J.A. Doherty reports grants from NIH during the conduct of the study. No disclosures were reported by the other authors.

## Disclaimer

The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

## Authors' Contributions

**L.J. Collin:** Conceptualization, formal analysis, methodology, writing–original draft, writing–review and editing. **K.L. Cushing-Haugen:** Data curation, software, formal analysis, validation, methodology, writing–review and editing. **K.L. Terry:** Resources, funding acquisition, writing–review and editing. **E.L. Goode:** Resources, funding acquisition, writing–review and editing. **A.H. Wu:** Resources, funding acquisition, writing–review and editing. **H.R. Harris:** Resources, funding acquisition, writing–review and editing. **N. Sasamoto:** Resources, funding acquisition, writing–review and editing. **D.W. Cramer:** Resources, funding acquisition, writing–review and editing. **F. Modugno:** Resources, funding acquisition, writing–review and editing. **E. Elishaev:** Resources, data curation, writing–review and editing. **Z. Fu:** Resources, data curation, validation, writing–review and editing. **K.B. Moysich:** Resources, funding acquisition, writing–review and editing. **P.A. Fasching:** Resources, funding acquisition, project administration, writing–review and editing. **C.L. Pearce:** Resources, data curation, funding acquisition, writing–review and editing. **U. Menon:** Resources, data curation, funding acquisition, writing–review and editing. **A. Gentry-Maharaj:** Resources, data curation, funding acquisition, writing–review and editing. **S.A. Gayther:** Resources, funding acquisition, writing–review and

editing. **N. Wentzensen:** Resources, formal analysis, supervision, methodology, writing–review and editing. **M.T. Goodman:** Resources, funding acquisition, writing–review and editing. **J. George:** Resources, data curation, writing–review and editing. **A. Talhouk:** Resources, data curation, validation, methodology, writing–review and editing. **M.S. Anglesio:** Resources, data curation, formal analysis, funding acquisition, methodology, writing–review and editing. **S.J. Ramus:** Resources, funding acquisition, writing–review and editing. **D.D.L. Bowtell:** Resources, funding acquisition, writing–review and editing. **S.S. Tworoger:** Resources, funding acquisition, writing–review and editing. **J.M. Schildkraut:** Resources, data curation, funding acquisition, writing–review and editing. **P.M. Webb:** Resources, funding acquisition, writing–review and editing. **J.A. Doherty:** Conceptualization, resources, formal analysis, supervision, funding acquisition, validation, investigation, methodology, writing–original draft, writing–review and editing.

## Acknowledgments

L.J. Collin was supported by K99CA277580 from the NCI of the NIH. D.D.L. Bowtell was supported by the US Army Medical Research and Materiel Command Ovarian Cancer Research Program (award numbers W81XWH-16-2-0010 and W81XWH-21-1-0401) and the National Health and Medical Research Council of Australia (1092856, 1117044, and 2008781). M.S. Anglesio was supported by a scholar award from Michael Smith Health Research BC (18274). S.A. Gayther is partly funded by an NIH/NCI R01 award #CA259058. Individual studies were supported as follows: Australian Ovarian Cancer Study (AUS): The Australian Ovarian Cancer Study was supported by the US Army Medical Research and Materiel Command (DAMD17-01-1-0729), National Health and Medical Research Council of Australia (199600, 400413, and 400281), cancer councils of New South Wales, Victoria, Queensland, South Australia, and Tasmania, and Cancer Foundation of Western Australia (multistate applications 191, 211, and 182). The Australian Ovarian Cancer Study gratefully acknowledges additional support from Ovarian Cancer Australia and the Peter MacCallum Cancer Foundation; NIH (R01-CA172404, principal investigator: S.J. Ramus). Bavarian Ovarian Cancer Cases and Controls (BAV): not applicable. Diseases of the Ovary and their Evaluation (DOV): NIH R01 CA112523 to Mary Anne Rossing, R01-CA87538 to M.A. Rossing, and R01 CA168758 to M.A. Rossing and J.A. Doherty. Hawaii Ovarian Cancer Study (HAW): NIH R01 CA58598, N01 CN-55424, and N01 PC-67001. Hormones and Ovarian Cancer Prediction (HOP): US NCI K07 CA80668 (Modugno), R01CA095023 (Ness/Modugno), R21CA267050 (Modugno), the US Army Medical Research and Materiel Command DAMD17-02-1-0669 (Ness/Modugno), OC200085 (Modugno), and the NIH National Center for Research Resources/General Clinical Research Center grant MO1 RR000056. New England Case-Control Study (NEC): NIH R01 CA54419 and P50 CA105009 and Department of Defense W81XWH-10-1-02802; P01 CA087969. North Carolina Ovarian Cancer Study (NCO): NIH R01 CA76016. Mayo Clinic Ovarian Cancer Case Control Study (MAY): NIH P30 CA015083, P50 CA136939, and R01 CA248288. Polish Ovarian Cancer Case-Control Study (POL): Intramural Research Program of the NCI; ZIA CP010126. UK Ovarian Cancer Population Study (UKO): The UK Ovarian Cancer Population study was funded by The Eve Appeal (The Oak Foundation) with contribution to authors' salary through MRC core funding MC_UU_00004/01 and the NIH Research University College London Hospitals Biomedical Research Centre. Study of Lifestyle and Women's Health (USC): NIH R01 CA58598 and R01 CA17054 and California Cancer Research Program (2II0200).

## Note

Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

Received August 3, 2024; revised October 29, 2024; accepted February 12, 2025; posted first February 26, 2025.

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