A Comprehensive Gene-Environment Interaction Analysis in Ovarian Cancer using Genome-wide Significant Common Variants

Running head: G x E analysis in ovarian cancer

Sehee Kim[1†], Miao Wang[1†], Jonathan P. Tyrer[2], Allan Jensen[3], Ashley Wiensch[4], Gang Liu[5], Alice W. Lee[6], Roberta B. Ness[7], Maxwell Salvatore[1, 4], Shelley S. Tworoger[8, 9], Alice S. Whittemore[10, 11], Hoda Anton-Culver[12], Weiva Sieh[13], Sara H. Olson[14], Andrew Berchuck[15], Ellen L. Goode[16], Marc T. Goodman[17, 18], Jennifer Anne Doherty[19], Georgia Chenevix-Trench[20], Mary Anne Rossing[21, 22], Penelope M. Webb[23], Graham G. Giles[24-26], Kathryn L. Terry[27, 28], Argyrios Ziogas[12], Renée T. Fortner[29], Usha Menon[30], Simon A. Gayther[31-33], Anna H. Wu[31], Honglin Song[2], Angela Brooks-Wilson[34, 35], Elisa V. Bandera[36], Linda S. Cook[37, 38], Daniel W. Cramer[27, 28], Roger L. Milne[24, 25], Stacey J. Winham[16], Susanne K. Kjaer[3, 39], Francesmary Modugno[40-42], Pamela J. Thompson[17], Jenny Chang-Claude[29, 43], Holly R. Harris[21,], Joellen M. Schildkraut[44], Nhu D. Le[45], Nico Wentzensen[46], Britton Trabert[46], Estrid Høgdall[3, 47], David Huntsman[48, 49], Malcolm C. Pike[14, 50], Paul D.P. Pharoah[2, 51], Celeste Leigh Pearce[4, 32] and Bhramar Mukherjee[1].

Corresponding author: Bhramar Mukherjee, SPH Tower, 1415 Washington Heights, Ann Arbor MI 48109, Phone: 734-764-6544, Fax: 734-764-3192, Email: bhramar@umich.edu

†These authors contributed equally to this work

[1] Department of Biostatistics, University of Michigan School of Public Health, Ann Arbor, MI, USA.
[2] Centre for Cancer Genetic Epidemiology, Department of Oncology, University of Cambridge, Cambridge, UK.
[3] Department of Virus, Lifestyle and Genes, Danish Cancer Society Research Center, Copenhagen, Denmark.
[4] Department of Epidemiology, University of Michigan School of Public Health, Ann Arbor, MI, USA.
[5] Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, MA, USA.
[6] Department of Health Science, California State University, Fullerton, Fullerton, CA, USA.
[7] University of Texas MD Anderson Cancer Center, Houston, TX, USA.
[8] Department of Cancer Epidemiology, Moffitt Cancer Center, Tampa, FL, USA.
[9] Research Institute and Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA, USA.
[10] Department of Health Research and Policy - Epidemiology, Stanford University School of Medicine, Stanford, CA, USA.
[11] Department of Biomedical Data Science, Stanford University School of Medicine, Stanford, CA, USA.
[12] Department of Epidemiology, Genetic Epidemiology Research Institute, University of California Irvine, Irvine, CA, USA.

[13] Department of Genetics and Genomic Sciences, Department of Population Health Science and Policy, Icahn School of Medicine at Mount Sinai, New York, NY, USA.
[14] Department of Epidemiology and Biostatistics, Memorial Sloan-Kettering Cancer Center, New York, NY, USA.
[15] Department of Obstetrics and Gynecology, Duke University Medical Center, Durham, NC, USA.
[16] Department of Health Sciences Research, Mayo Clinic, Rochester, MN, USA.
[17] Cancer Prevention and Control, Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA.
[18] Community and Population Health Research Institute, Department of Biomedical Sciences, Cedars-Sinai Medical Center, Los Angeles, CA, USA.
[19] Geisel School of Medicine, Dartmouth College, Hanover, NH, USA.
[20] Department of Genetics and Computational Biology, QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia.
[21] Program in Epidemiology, Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA, USA.
[22] Department of Epidemiology, University of Washington, Seattle, WA, USA.
[23] Population Health Department, QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia.
[24] Cancer Epidemiology & Intelligence Division, Cancer Council Victoria, Melbourne, Victoria, Australia.
[25] Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Melbourne, Victoria, Australia.
[26] Department of Epidemiology and Preventive Medicine, Monash University, Melbourne, Victoria, Australia.
[27] Obstetrics and Gynecology Epidemiology Center, Brigham and Women's Hospital, Boston, MA, USA.
[28] Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA, USA.
[29] Division of Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany.
[30] Gynaecological Cancer Research Centre, Women's Cancer, Institute for Women's Health, University College London, London, UK.
[31] Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA.
[32] Center for Cancer Prevention and Translational Genomics, Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA.
[33] Department of Biomedical Sciences, Cedars-Sinai Medical Center, Los Angeles, CA, USA.
[34] Genome Sciences Centre, BC Cancer Agency, Vancouver, BC, Canada.
[35] Department of Biomedical Physiology and Kinesiology, Simon Fraser University, Burnaby, BC, Canada.
[36] Cancer Prevention and Control Program, Rutgers Cancer Institute of New Jersey, New Brunswick, NJ, USA.
[37] University of New Mexico Health Sciences Center, University of New Mexico, Albuquerque, NM, USA.

[38] Division of Cancer Care, Department of Population Health Research, Alberta Health Services, Calgary, AB, Canada.
[39] Department of Gynaecology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark.
[40] Ovarian Cancer Center of Excellence, Womens Cancer Research Program, Magee-Womens Research Institute and Hillman Cancer Center, Pittsburgh, PA, USA.
[41] Department of Epidemiology, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA, USA.
[42] Division of Gynecologic Oncology, Department of Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA.
[43] Research Group Genetic Cancer Epidemiology, University Cancer Center Hamburg (UCCH), University Medical Center Hamburg-Eppendorf, Hamburg, Germany.
[44] Department of Public Health Sciences, University of Virginia, Charlottesville, VA, USA.
[45] Cancer Control Research, BC Cancer Agency, Vancouver, BC, Canada.
[46] Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA.
[47] Molecular Unit, Department of Pathology, Herlev Hospital, University of Copenhagen, Copenhagen, Denmark
[48] British Columbia's Ovarian Cancer Research (OVCARE) program, Vancouver General Hospital, BC Cancer Agency and University of British Columbia
[49] Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada
[50] Department of Preventive Medicine, Keck School of Medicine, University of Southern California Norris Comprehensive Cancer Center, Los Angeles, CA, USA.
[51] Centre for Cancer Genetic Epidemiology, Department of Public Health and Primary Care, University of Cambridge, Cambridge, UK.

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Abbreviations:

AR = absolute risk
BMI = body mass index
BSO = bilateral salpingo-oophorectomy
CI = confidence interval
df = degrees of freedom
G x E = gene-environment interaction
GWAS = genome-wide association study
LRT = likelihood ratio test
OCAC = Ovarian Cancer Association Consortium
OCP = oral contraceptive pill
OR = odds ratio
RD = risk difference
SNP = single nucleotide polymorphism

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Novelty and Impact: Our paper conducts gene x environment interaction analysis on both additive and multiplicative scales using data from 9,971 ovarian cancer (OC) cases and 15,566 controls. Seven OC risk factors are considered with 28 variants identified from previous GWAS. The top interaction was between oral contraceptive pill (OCP) use (ever vs never) and rs13255292 (P-value=3.48×10^{-4}). The protective benefit of OCP use differs by genotype suggesting that prevention strategies need tailoring to an individual's genotypic profile.

ABSTRACT

As a follow-up to genome-wide association analysis of common variants associated with ovarian carcinoma (cancer), this study considers seven well-known ovarian cancer risk factors and their interactions with 28 genome-wide significant common genetic variants. The interaction analyses were based on data from 9,971 ovarian cancer cases and 15,566 controls from 17 case-control studies. Likelihood ratio and Wald tests for multiplicative interaction and for relative excess risk due to additive interaction were used. The top multiplicative interaction was noted between oral contraceptive pill (OCP) use (ever vs never) and rs13255292 (P-value = 3.48×10^{-4}). Among women with the TT genotype for this variant, the odds ratio for OCP use was 0.53 (95% CI=0.46-0.60) compared to 0.71 (95%CI=0.66-0.77) for women with the CC genotype. When stratified by duration of OCP use, women with 1-5 years of OCP use exhibited differential protective benefit across genotypes. However, no interaction on either the multiplicative or additive scale was found to be statistically significant after multiple testing correction. The results suggest that OCP use may offer increased benefit for women who are carriers of the T allele in rs13255292. On the other hand, for women carrying the C allele in this variant, longer (5+ years) use of OCP may reduce the impact of carrying the risk allele of this SNP. Replication of this finding is needed. The study presents a comprehensive analytic framework for conducting gene-environment analysis in ovarian cancer.

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INTRODUCTION

Ovarian carcinoma (cancer) is a disease with high mortality; most women are diagnosed with advanced stage disease where five-year survival is less than 50% [1]. Effective screening modalities have been elusive [2], and therefore primary prevention strategies remain the most promising avenue to minimize the incidence and mortality of ovarian cancer.

Several factors consistently associated with reduced or increased risk have been identified for ovarian cancer, including some that represent opportunities for chemoprevention or surgical intervention. Factors associated with reduced risk include oral contraceptive pill (OCP) [3] use aspirin use [4], tubal ligation [5], parity [3], salpingectomy [6-9] and bilateral salpingo-oophorectomy (BSO). Common germline genetic variation [10-20], first-degree family history of ovarian cancer [21, 22], menopausal hormone therapy use [23-25], greater body mass index (BMI) [26] and endometriosis [27] are risk factors for the disease. OCPs and aspirin use represent feasible chemoprevention strategies whereas salpingectomy is now recommended by many gynecologic societies as an ovarian cancer prevention approach for women seeking tubal sterilization, having a hysterectomy, or having other pelvic surgery.

Average lifetime risk of ovarian cancer diagnosis for women in the U.S. is 1.3% [28], but this number varies greatly depending on the composite exposure history of risk factors [29]. Pearce et al. estimated the lifetime risk for women in the general population ranges from 0.35% (95%CI = 0.29% to 0.42%) to 8.8% (95%CI = 7.1% to 10.9%) depending on exposure history for six factors: OCP use, parity, tubal ligation, endometriosis, first degree family history of ovarian cancer and genetic risk score quintile [29].

However, these lifetime risk estimates were limited to six risk factors and did not consider their interaction with individual genetic variants identified through genome-wide association studies (GWAS) [28]. The multiplicative scale is commonly used for gene-environment interaction (G x E) analysis. Additive interaction analysis has been suggested for case-control studies in many recent papers for a more mechanistic interpretation [30-34]. Validity of a truly multiplicative model implies existence of additive interaction when the two factors under consideration have non-null main effects [35]. Thus, failure to detect G x E interaction on multiplicative scale may imply there exists interaction on additive scale, but the ability to detect it depends on the sample size and the main and interaction effect sizes [35]. We present here our efforts to evaluate both multiplicative and additive gene-environment interactions in ovarian cancer using data from the international Ovarian Cancer Association Consortium (OCAC) comprising 17 case-control studies.

We have included 28 common genetic variants previously associated with risk of ovarian cancer in genome-wide association analyses for our G x E analyses [36]. Environmental factors included in our analysis are OCP use, parity, tubal ligation, breastfeeding, menopausal hormone therapy, usual adult BMI, and endometriosis. A small number of studies in OCAC had data available on aspirin use and thus we have not included this risk factor in our analysis here. Among our list of environmental factors, BMI, OCP use, tubal ligation, breastfeeding, and menopausal hormone therapy are of special interest because they are modifiable targets for prevention.

METHODS

Study Population

The OCAC is an international multidisciplinary consortium formed in 2005 (http://apps.ccge.medschl.cam.ac.uk/consortia/ocac/) with a goal of sharing data from worldwide ovarian cancer studies to establish reliable estimation of association between environmental and genetic factors related to risk of ovarian cancer [23, 37]. Cases were defined as women with ovarian carcinoma (i.e., invasive epithelial ovarian cancers), fallopian tube cancer and primary peritoneal cancer. Controls were women without ovarian cancer and who had at least one ovary. For both cases and controls, individuals with prior cancers except non-melanoma skin cancers were excluded.

Genetic Association Analysis

In total, 28 single nucleotide polymorphisms (SNPs) previously identified through GWAS were included from 75 OCAC sites (***Table 1***). The first 26 SNPs were found to be significantly associated with either ovarian cancer overall or one or more histotypes [36]. In addition, rs13255292 and rs10962643 were included because they were in the same region as two other significant SNPs but showed a strong independent association with ovarian cancer risk. The SNP at locus 15q26 (rs8037137), which was found to be genome-wide significant [13], was not included because not enough non-carriers were present in our analytic dataset for examining interactions. The genetic data included both genotyped and imputed variants (imputation being carried out using phase 2 Hapmap reference panel). More details regarding genotyping and imputation of the genetic data have been previously described [12, 17, 18, 20]. The methods for analyzing the SNP data in the OCAC have also been described previously [12, 17, 18, 20]. Briefly, logistic regression

models were fit to examine the association between ovarian cancer and each genetic variant under an additive model (using risk allele dosage). The models were adjusted for ethnicity, genotyping panel and the leading principal components for each ethnicity. The summary results are shown in ***Table 1*** and are also available through the OCAC website (http:/apps.ccge.medschl.cam.ac.uk/consortia/ocac/).

Environmental Association Analysis

Environmental Variables (E): A total of seven established environmental risk factors for ovarian cancer were of primary interest (***Table 2***), including four associated with decreased risk and three with increased risk for ovarian cancer or one specific histotype. These included: OCP use (measured as both ever/never and duration of OCP use (never users including <1 one year of use, 1-<5, 5+yr), tubal ligation (yes/no), breastfeeding (ever/never), parity (0, 1-2, 3+ full-term births (i.e., those lasting ≥6 months), type of menopausal hormone therapy use for more than 1 year after age 50 (never user, menopausal estrogen therapy only, any use of menopausal estrogen + progestin therapy), BMI (<25, 25-<30, 30+), and a history of endometriosis (yes/no).

Four other environmental variables were included in our analysis, as covariates: baseline age (<50, 50-<55, 55-<60, 60-<65, 65-70, 70+ years), race (non-Hispanic white, Hispanic White, Black, Other), education (less than high school, high school graduate, some college, college graduate) and first-degree family history of ovarian cancer (yes/no). In addition to these four covariates, study site, OCP use, tubal ligation, parity, BMI and endometriosis were also included in all models for the environmental association analysis and gene by environment interaction analysis.

Harmonization and Imputation of Environmental Data: A brief description of environmental data harmonization across OCAC study sites is provided in ***eMethod 1*** in the ***Supplementary Material***. To optimize power and enhance the chance for discovery, we carried out multiple imputation of the environmental data. The maximal amount of data was used for imputation (see ***eMethod 1*** and ***eFigure 1*** in the ***Supplementary Material*** for details). A total of 19 studies comprising 13,722 cases and 22,975 controls with partially missing data were included for imputation. Of these 19 studies, 12 were from the US, 4 from Europe, 2 from Canada and 1 from Australia (see ***eTable 1*** for a description of study sites). Further details for these 19 studies have been previously described (see ***Supplementary Material***). The environmental variables included in our analysis were multiply imputed by chained equations (MICE) to produce ten imputed datasets. See details of imputation model in ***eMethod 2.1*** in the ***Supplementary Material***.

All analyses were performed on each of the ten imputed datasets, and coefficients/test statistics were properly combined to account for uncertainty due to imputation, following the recommended combination rule for multiply imputed datasets [38] (see details in ***eMethod 2.3*** in the ***Supplementary Material***). Our marginal environmental association analysis was based on combined inference from the ten imputed versions of this harmonized E data. Logistic regression models were used for evaluating marginal associations between the environmental risk factors with ovarian cancer after adjusting for covariate. The estimated ORs, their 95% CIs, as well as two-sided Wald tests after accounting for imputation uncertainty are presented in ***Table 2*** along with summary statistics of complete cases before imputation. Full results of the complete cases analysis using logistic regression models are presented in ***eTable 2***.

Gene by Environment Interaction Analysis

After marginal analysis of the genetic and environmental risk factors, we considered gene by environment (G x E) interaction analysis both on the multiplicative (odds ratio/relative risk) and the additive (relative excess risk due to interaction/absolute risk) scale [39]. From the 19 studies with imputed environmental data, a subset of 17 case-control studies with 9,971 cases and 15,566 controls had available genetic data, thus G x E analyses were carried out on these 17 studies. Each imputed environmental dataset was merged with the genetic data for subsequent G x E analyses. Interaction analyses were then carried out separately on the ten imputed G x E datasets, and then all tests and coefficients reported were combined using appropriate multiple imputation combination rules [38].

For both multiplicative and additive interaction analysis, we started with global likelihood ratio tests (LRTs) for each G x E pair as several environmental factors had multiple categories resulting in tests for interactions with multiple degrees of freedom (df). These global joint tests, serving as a screening step for G x E interactions, were carried out for a total of 196 (7×28=196) G x E pairs. After the global tests, we then followed up on the suggestive interactions (with global test P-value < 0.2) and carried out a two-sided Wald test for interactions involving each separate category of an environmental risk factor.

For the *k*-th SNP G_k $(\mathrm{k} = 1, \ldots, 28)$, coded as a continuous allelic dosage, the *j*-th environmental risk factor E_j $(j = 1, \ldots, 7)$, and a set of confounders/covariates $\{C_q\}$ $(q = 1, \ldots, Q)$, the basic fitted model for the probability of ovarian cancer of the *i*-th subject, namely, π_i, is of the following form:

$$logit(\pi_i \mid G_{ki}, E_{ji}, C_{1i}, \dots, C_{Qi})$$

$$= \beta_0 + \beta_G G_{ki} + \sum_{l=1}^{L} \beta_{El} I(E_{ji} = l) + \sum_{l=1}^{L} \beta_{GEl} I(E_{ji} = l)\, G_{ki} + \sum_{q=1}^{Q} \sum_{m=1}^{M_q} \beta_{C_q m} I(C_{qi} = m),$$

[M1]

where *L* = (levels of E_j) – 1, M_q = (levels of C_q) – 1, and *Q* is the number of adjusted covariates.

Multiplicative Interaction Tests: For testing the multiplicative interaction between G_k and E_j, we first used the global LRT with *L* degrees of freedom to test for the joint null hypothesis $H_0: \beta_{GE1} = \beta_{GE2} = \cdots = \beta_{GEL} = 0$. If the global test P-value < 0.2, we further assessed the multiplicative interaction at each level of E_j by using a Wald test with one degree of freedom for the null hypothesis $H_0: \beta_{GEl} = 0$ for the *l*-th level.

Additive Interaction Tests: Due to limitations of existing software (CGEN) [40] for testing additive interactions with continuous dosage data, we used the maximal probable genotype for imputed SNPs. We further conducted the LRTs with binary collapsing of SNPs assuming a dominant genetic susceptibility model (given the constraints in software) [31]. For a given SNP G_k and an environmental risk factor E_j with *L* categories, a global LRT with L df was used for the following joint null hypothesis

$$H_0: \frac{\{\exp(\beta_{E1}) + \exp(\beta_G) - 1\}}{\exp(\beta_{E1} + \beta_G)} = \exp(\beta_{GE1}), \dots, \frac{\{\exp(\beta_{EL}) + \exp(\beta_G) - 1\}}{\exp(\beta_{EL} + \beta_G)} = \exp(\beta_{GEL}),$$

where the regression coefficients (β) are log odds ratio parameters described in model [M1]. This null hypothesis is based on a rare disease assumption [41], which is tenable for our study (lifetime risk of ovarian cancer in the US is approximately 1.3%) [42]. If the global LRT P-value < 0.2, we further assessed the additive interaction at each level of E_j through the relative excess risk due to interaction (RERI) [41]. At the *l*-h level of E_j, a Wald

test with one degree of freedom (35) was used to test for the null hypothesis:

$H_0: RERI_{GEl} = 0$, where $RERI_{GEl} = \exp(\beta_{El} + \beta_{GEl} + \beta_G) - \exp(\beta_{El}) - \exp(\beta_G) + 1$.

After the screening step, we further explored the structure of the most promising interactions (defined as global test P-value < 0.01). This was accomplished by exploring odds ratios corresponding to E in sub-groups defined by G (for the multiplicative interaction) or absolute risks for ovarian cancer in each configuration of the values of (G, E) (for the additive interaction). To better understand these two different scales of interaction, we also compared the observed joint ORs with the corresponding expected ORs under the multiplicative and the additive nulls.

To estimate sub-group specific absolute risk (AR) for each stratum defined by a given SNP G_k and environmental risk factor, we need the relative risk and the joint distribution of G_k and E_j. The former was estimated from the fitted model [M1], and the latter was empirically estimated from the observed joint frequency of E_j and G_k in the control population (*details in* ***eMethod3*** from the ***Supplementary Material)***. ***Table 4*** presents the bootstrap confidence intervals for the estimated ARs and the risk differences (RDs) (see details in ***eMethod4*** in the ***Supplementary Material***). The results for G x E analysis are presented in ***Table 3*** (multiplicative interaction), ***Table 4*** (additive interaction) and ***eTable 5*** (observed and expected joint OR under the two different nulls). All calculations were performed in the statistical software R [30, 40].

RESULTS

The marginal G analysis was carried out on 26,864 cases and 48,034 controls and the results are shown in ***Table 1***. These results are available through the OCAC website (http://apps.ccge.medschl.cam.ac.uk/consortia/ocac/). A total of 36,697 women with

13,722 ovarian cancer cases from 19 sites were included in the marginal E analysis using the imputed datasets. All seven environmental risk factors were associated with ovarian cancer in the expected direction (***Table 2***). OCP use for five or more years was associated with a 52% decrease in risk of ovarian cancer compared to never users (OR=0.48, 95%CI = 0.45 to 0.51). Tubal ligation (OR=0.73, 95%CI = 0.69 to 0.78) and breastfeeding (OR=0.76, 95%CI = 0.71 to 0.80) showed similar magnitudes of decreased risk. Also, having more than 3 children (versus none) was associated with a 50% (OR=0.5, 95%CI = 0.46 to 0.53) reduction in risk of ovarian cancer. Using menopausal estrogen therapy only for more than one year (OR=1.22, 95%CI = 1.12 to 1.34), being obese (OR=1.15, 95%CI = 1.08 to 1.22), and history of endometriosis (OR=1.60, 95%CI = 1.46 to 1.75) were all associated with increased risk of ovarian cancer. The inference remained robust before and after imputation (***eTable 2***.).

Gene by Environment Interaction Results

Global Likelihood Ratio Tests: The global LRT essentially serves as a screening approach to identify a list of potentially interesting interactions. All interactions with global LRT P-value < 0.2 (40 on multiplicative scale and 41 on additive scale) are listed in ***eTable 3***, while more detailed analysis of the top interactions, which showed the strongest significance (P-value < 0.01; 4 on multiplicative and 2 on additive scale), are shown in ***Table 3*** and ***Table 4,*** respectively.

According to Global LRT results, the top interaction on the multiplicative scale was identified with the SNP rs13255292 and OCP use (ever and never use: P-value = 3.48 x 10^{-4}; duration of use [<1 yr, 1-5 yr, 5+ yr]: P-value = 7.26 x 10^{-3}) (***Table 3***). None of the

observed interactions were significant based on a Bonferroni threshold of 0.05/(28 x 7)= 2.55 x 10^{-4}.

Wald Tests for Multiplicative interactions: For the most promising multiplicative interactions reported in ***Table 3*** we carried out an in-depth analysis to better understand the structure of interactions by estimating the ORs (with accompanying Wald CIs and tests) corresponding to E in strata defined by G. For example, the OR for OCP use among women with the TT genotype for rs13255292 is estimated to be 0.53 (95%CI = 0.46 to 0.60), whereas for the CC genotype the estimated OR is 0.71 (95%CI = 0.66 to 0.77) suggesting a stronger protective effect of OCP use among TT genotypes (***Table 3***, ***Figure 1A***).

When OCP use was further stratified by duration, we observed an interesting pattern in its interaction with rs13255292. The estimated OR corresponding to 1-5 year of OCP use vs < 1 year use in the TT genotype group was 0.58 (95%CI = 0.50 to 0.69) compared to an OR of 0.79 (95%CI = 0.72 to 0.87) among women with CC genotype, showing effect modification by the risk allele (C) of rs13255292 (***Table 3***, ***Figure 1B***). This is akin to the result with ever/never user. However, the OR corresponding to 5+ years of OCP use vs < 1 year of use for the TT genotype group was 0.43 (95%CI = 0.37 to 0.50) and for the CC genotype was 0.53 (95%CI = 0.49 to 0.58) (***Table 3***, ***Figure 1C***). With overlapping confidence intervals, there is no significant difference in the odds ratios for long-term OCP users across genotype sub-groups. ***Table 3*** shows that the P-value of the Wald test for interaction of rs13255292 and 1-5 years of OCP use (vs < 1 yr) was lower (P-value = 4.74 x 10^{-3}), when compared to the P-value for interaction of the same variant with 5+ years of OCP use (vs < 1 yr) (P-value = 2.43 x 10^{-2}).

Wald Test for Additive interaction/RERI: For the most statistically significant additive interactions in ***Table 4***, we estimated the sub-group specific absolute risks (ARs) and risk differences (RDs) in each E by G stratum. For example, for the strongest additive interaction based on the global likelihood ratio tests in Table 4, there was suggestive evidence that rs11658063 modified the effect of menopausal estrogen therapy use, compared to never use of menopausal hormone therapy (P-value = 3.01×10^{-2}). Among women with the GG genotype, never users of menopausal hormone therapy had an estimated AR of 1.33% (95%CI =1.26% to 1.40%) while women who used menopausal estrogen therapy had an estimated AR of 1.96% (95%CI = 1.59% to 2.33%), leading to an absolute risk increase of 0.63% (95%CI = 0.24% to 1.02%) (***Table 4, eFigure 2***).
For women with the CC genotype, the estimated AR was 1.27% (95%CI = 1.23% to 1.32%) for never receiving menopausal hormone therapy and 1.36% (95%CI = 1.15% to 1.57%) for receiving menopausal estrogen only therapy. This implies virtually no increased risk from taking menopausal estrogen only therapy among women with the CC genotype (95%CI = -0.14% to 0.31%; ***Table 4, eFigure 2***). The results on the additive interactions were in general weaker in terms of the strength of P-values.

DISCUSSION

We have conducted a comprehensive multiplicative and additive interaction analysis of previously identified common genetic variants and environmental factors unequivocally associated with ovarian cancer risk. We observed six suggestive interactions (with P-value < 0.01), four on the multiplicative scale and two on the additive scale. The lack of statistical significance of interactions after multiple testing correction from a large collection of data and well-curated studies enable us to conclude that it is

unlikely that there are substantive interactions with single variants and environmental factors regardless of the choice of scale. This is consistent with what has been observed for other cancers. One may argue that the Bonferroni threshold for multiple comparisons is likely to be conservative for this set of correlated environmental factors, but the general pattern of findings remains consistent with smaller magnitude of interaction effect sizes. However, there are several interesting findings from this analysis that may be worthwhile to follow-up in future G x E studies of ovarian cancer.

Mechanistic Insight: In addition to guiding targeted prevention strategies, G x E analysis has the potential to provide mechanistic insight into the complex multifactorial structure of the underlying biological pathway. One issue complicating observed gene-environment interactions of even confirmed susceptibility loci is that the true casual alleles and the biological impact of the variants are unknown. Our top interaction is between OCP use and rs13255292. This variant lies in the 8q24 region which harbors several risk loci for ovarian cancer [18] and other cancers [43, 44]. The SNP is in the *PVT1* gene which interacts with the oncogene *MYC* [45]. *MYC* has long been reported to be at least in part under hormonal control [46, 47] thus an interaction with OCP use is plausible. Conversely, our top additive interaction is between menopausal estrogen use and rs11658063 which falls in *HNF1B*. To our knowledge there is no relationship between *HNF1B* and hormones thus underscoring the difficulty of understanding these gene-environment interactions given our limited understanding of the function of the variants and even more broadly the biological role of the genes.

Exposure Pathways and Potential for Targeted Prevention: The strongest interactions are observed with OCP use or menopausal estrogen use which are

modifiable exposures. Our most promising finding is the potential interaction between SNP rs13255292 and OCP use. This finding, if replicated could potentially lead to improved understanding of exposure pathways.

Analytic Architecture and the Choice of Scale for Measuring Interaction: We present a comprehensive analytical framework to carry out post-GWAS G x E analysis on both multiplicative and additive scale. Our framework starting with data harmonization and imputation followed by Global likelihood ratio tests and single df Wald tests provides a principled analytic architecture for such analysis. Our analysis reiterates the well-known fact that testing the additive and multiplicative nulls are very similar when the marginal associations are weak but could depart when both marginal associations are large in magnitude and the sample size is finite. In ***eTable 5***, we present observed joint odds ratios for strata defined by G and E along with the expected odds ratios under the multiplicative null and the additive null. We use our top hit rs13255292 and OCP use (ever versus never) and length of OCP use (<1yr, 1-<5 yrs, 5+ yrs) as an illustration. One can note that the expected ORs are fairly close under both models. However, their estimated departure from the observed joint OR is more pronounced for the 1-<5 yrs sub-group when compared to 5+ yrs, explaining the suggestive evidence for rejecting the null.

We discussed the multiplicative interaction results for rs13255292 and OCP use in the previous section. We now explore the structure of additive interaction for this G x E result (***Figure 2A-2C***). Marginally, without including any genetic information, from a pure environmental association analysis we observed a relationship between duration of OCP use and risk reduction for ovarian cancer. For 1-5 years of OCP use (vs <1 year) the estimated absolute risk difference was 0.47% (95%CI = 0.37% to 0.56%), while the

estimated absolute risk difference for long-term use of OCPs (5+ year vs <1 year) was 0.84% (95%CI = 0.77% to 0.92%) (***Figure 2B-2C, eTable 4***), in agreement with previous findings that longer duration of OCP use is associated with larger risk reduction in ovarian cancer [3]. However, when stratified by rs13255292 genotype, we observed an interesting pattern. Among individuals with TT genotype, the corresponding absolute risk difference estimate for 1-5 year of OCP use (vs <1 year) was 0.69% (95%CI = 0.49% to 0.88%), whereas among individuals with CC genotypes the corresponding risk reduction estimate was 0.36% (95%CI = 0.22% to 0.50%), implying potential effect modification by the C allele at locus rs13255292 (P-value = 1.12×10^{-2}) (***Figure 2B, eTable 4)***. In contrast, the absolute risk difference is estimated at 0.95% (95%CI = 0.78% to 1.12%) for women with TT genotype and at 0.79% (95%CI = 0.69% to 0.90%) in women with CC genotype. This indicates that longer OC use is associated with greater risk reduction overall and the risk reduction might be even greater for women with the TT genotype than those with the CC genotype. From ***Figure 2B-2C*** we observe the interplay between "nature vs nurture" with risk due to germline genetic mutations offset by long-term use of a modifiable protective factor. This analysis also highlights the benefit of measuring duration of exposure as opposed to a coarse indicator of ever/never use.

Prior work in G x E for ovarian cancer has focused solely on multiplicative interactions. We previously reported no departures from a multiplicative model with the first six risk loci identified through GWAS with a reduced set of exposures [3]. Follow-up work identified an interaction with menopausal estrogen therapy use and rs10069690 in the *TERT* gene [48], but that finding was not replicated in the present analysis which included a larger set of studies. Fridley and colleagues have reported on G x E taking a

candidate gene approach with several promising findings [49]. There are several studies in other cancers examining G x E on the multiplicative scale with limited success in identifying interactions, but to our knowledge, only prostate cancer and bladder cancer have been studied on the additive scale. In prostate cancer, suggestive additive interactions between vitamin D, confirmed genetic variants and risk have been identified [50]. In bladder cancer, additive interaction has been explored between confirmed genetic loci and smoking with risk of disease [31]. In this work the authors were able to demonstrate that the absolute risk of bladder cancer for current smokers varied from 2.9% to 9.9% based on the polygenetic risk score quartile. These results are similar to our findings on the additive scale with absolute risk differing based on genetics and hormone therapy use; an interesting next step for our work is to consider the polygenetic risk score for all of these confirmed ovarian cancer susceptibility alleles.

There are several limitations of the current analysis. Though we considered both multiplicative and additive interactions, the logistic model in (M1) is linear in covariates and exposures. We ignored potential non-linearity and exposure x exposure as well as exposure x covariate interactions. Similarly, we ignored any higher order interactions. A completely non-parametric machine learning approach, based on a recursive partition of the predictor space may avoid misspecification of the model, but would lack interpretability from an epidemiologic and public health perspective. We also acknowledge that this exploration of interaction is purely statistical, a more causal interpretation in a biological sense will require functional validation. One may also want to explore G x E interaction with loci that are not significant at genome-wide threshold but are significant at a less stringent threshold or even conduct genome-wide G x E scans.

The associations between ovarian cancer risk and some of the variants included here were limited to specific histotypes of ovarian cancer, however we have only presented results for all epithelial ovarian cancers combined. Developing histotype-specific risk stratification approaches is not feasible because for any given histotype the absolute risk is unlikely to ever reach an actionable threshold on a population level. In addition, risk reducing strategies are the same across histotypes and thus there is little benefit to considering histotype specific results from a precision prevention perspective. Heterogeneous associations between environmental risk factors and ovarian cancer risk by histology has previously been well characterized [3, 23, 27]. There is value in understanding histotype associations for disease etiology and mechanisms and this will be the focus of future work.

The analyses presented here offer insight into potential biological mechanisms, opportunities for ovarian cancer risk stratification, and approaches to studying gene-environment interactions. Ideally, replication for the six promising findings would be undertaken, but this is challenging with ovarian cancer given that most studies with the relevant data are included here. Functional studies for the regions harboring our most promising findings are underway and it is possible that the association described here may help inform those investigations [51]. Also, gene-environment interaction analyses can also be used to identify novel genetic associations [51] and thus a deeper evaluation of variants that are still borderline significant, but do not exactly achieve a genome-wide threshold is warranted for subsequent G x E analysis. Of particular interest will be to conduct risk stratification and risk prediction analysis using a summative polygenic risk score and to conduct an agnostic genome-wide search for G x E interaction. Despite the

limitations the comprehensive framework of data harmonization, imputation, screening test followed by characterization of effect and risk estimates that has been used in this analysis can serve as a robust model for future gene-environment interaction analyses.

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Figure Legends

Figure 1A-1C. ORs of oral contraceptive (OCP) use, marginally, or stratified by number of risk allele of rs13255292. The ORs were calculated from a logistic regression model assuming log-additive effect of SNPs. (A) OR of OCP (ever vs never) (B) OR of 1 to 5 years of OCP use (vs < 1 year) (B) OR of more than 5 years of OCP use (vs < 1 year).

Figure 2A-2C. Estimated absolute risk (AR) of ovarian cancer given OCP use and number of copies of C allele, among non-Hispanic white college graduates aged below 50 with no family history of ovarian cancer, BMI below 25, no tubal ligation, no endometriosis, with one child. The ARs were calculated from a logistic regression model assuming log-additive effect of SNPs while all covariates fixed at their most frequent level as described above. (A) ARs stratified by OCP (ever vs never) and genotype (B) ARs stratified by 1 to 5 years of OCP use (vs < 1 year) and genotype (F) ARs stratified by more than 5 years of OCP use (vs < 1 year) and genotype. Risk differences were also reported as the solid black bar.

Figure 1

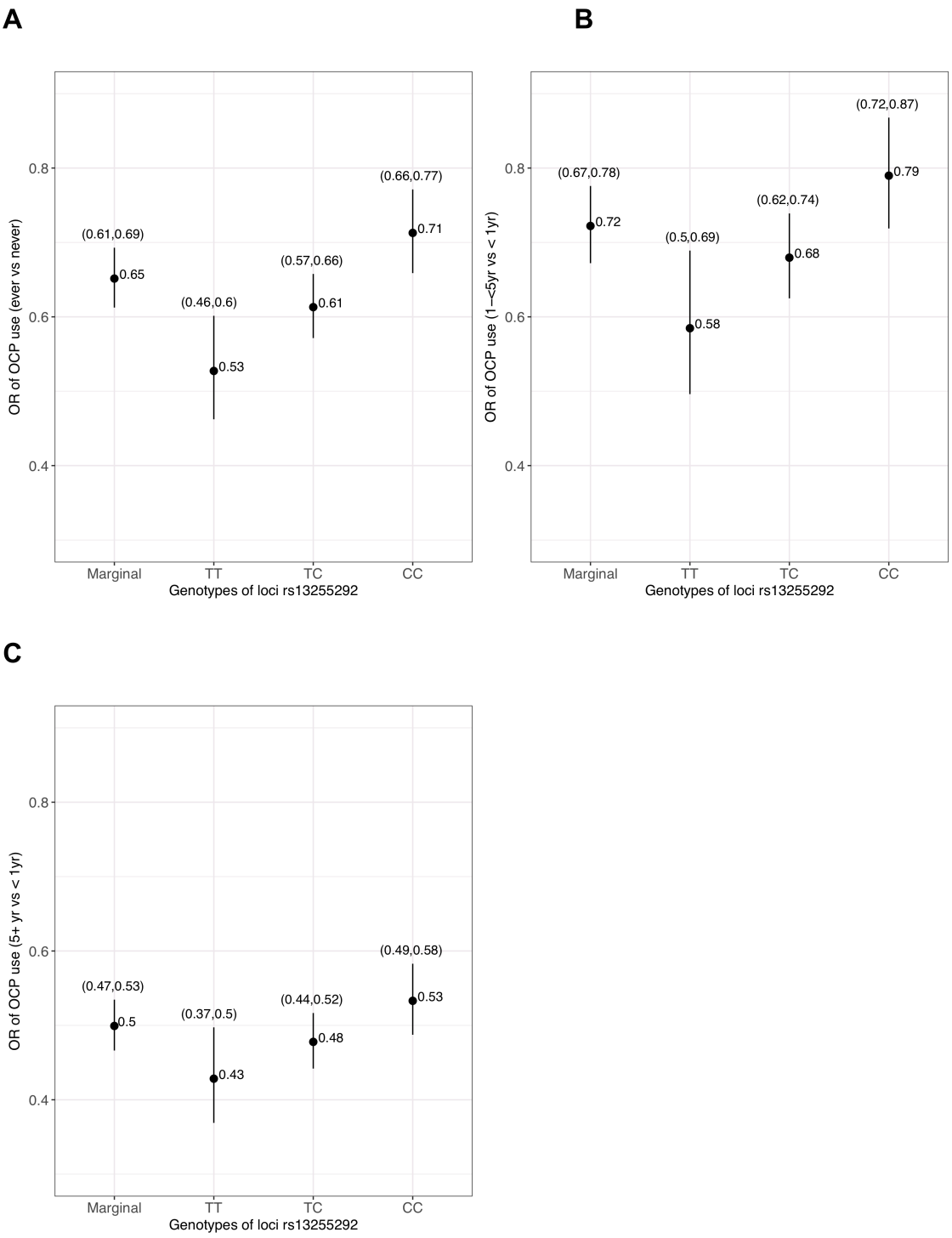
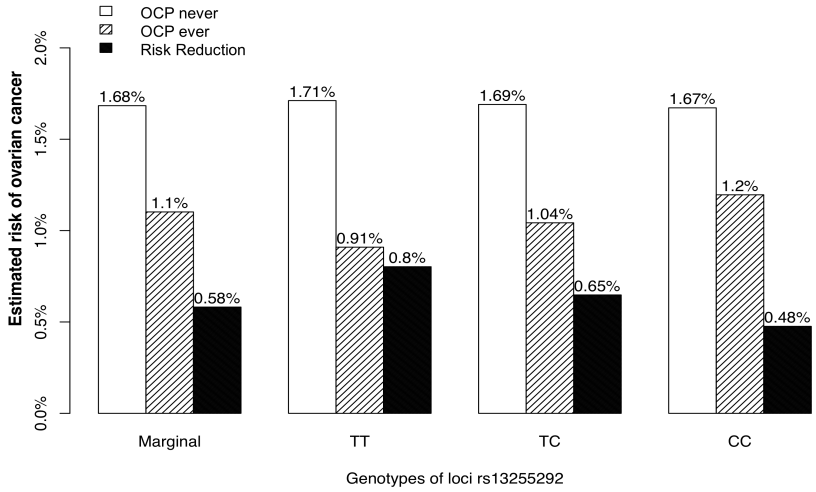
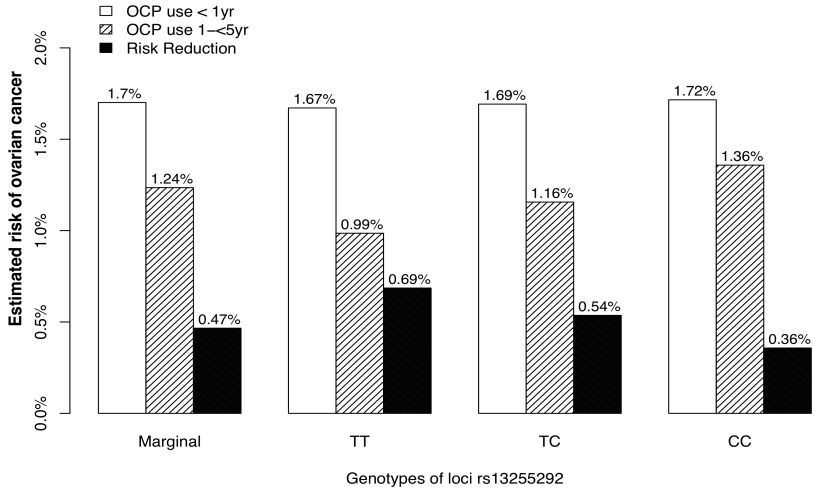

A
B
C
OR of OCP use (ever vs never)
OR of OCP use (1 –<5yr vs < 1yr)
OR of OCP use (5+ yr vs < 1yr)
0.8
0.6
0.4
(0.61,0.69)
0.65
(0.46,0.6)
0.53
(0.57,0.66)
0.61
(0.66,0.77)
0.71
(0.67,0.78)
0.72
(0.5,0.69)
0.58
(0.62,0.74)
0.68
(0.72,0.87)
0.79
(0.47,0.53)
0.5
(0.37,0.5)
0.43
(0.44,0.52)
0.48
(0.49,0.58)
0.53
Marginal
TT
TC
CC
Genotypes of loci rs13255292

Figure 2:

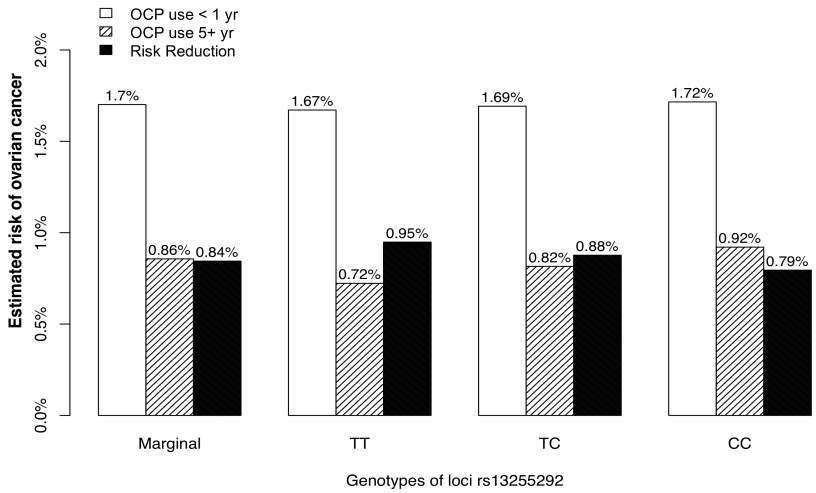
A


OCP never
OCP ever
Risk Reduction
Estimated risk of ovarian cancer
0.0%
0.5%
1.0%
1.5%
2.0%
1.68%
1.1%
0.58%
1.71%
0.91%
0.8%
1.69%
1.04%
0.65%
1.67%
1.2%
0.48%
Marginal
TT
TC
CC
Genotypes of loci rs13255292

B


OCP use < 1yr
OCP use 1–<5yr
Risk Reduction
Estimated risk of ovarian cancer
0.0%
0.5%
1.0%
1.5%
2.0%
1.7%
1.24%
0.47%
1.67%
0.99%
0.69%
1.69%
1.16%
0.54%
1.72%
1.36%
0.36%
Marginal
TT
TC
CC
Genotypes of loci rs13255292

C


OCP use < 1 yr
OCP use 5+ yr
Risk Reduction
Estimated risk of ovarian cancer
0.0%
0.5%
1.0%
1.5%
2.0%
1.7%
0.86%
0.84%
1.67%
0.72%
0.95%
1.69%
0.82%
0.88%
1.72%
0.92%
0.79%
Marginal
TT
TC
CC
Genotypes of loci rs13255292

Tables

Table 1. Odds ratios for marginal associations of 28 genetic susceptibility variants with ovarian cancer. Analysis used data with 26864 cases and 48034 controls from 75 study sites.

SNP	Previously published best hit[a]	Chr	Position	Risk Allele	Baseline Allele	RAF	OR[b]	P-value[b]
rs12023270	rs58722170[15]	1	38086578	T	C	0.264	1.08 (1.05,1.10)	2.65×10^{-8}
chr2:111818658	rs2165109 [18]	2	111818658	C	A	0.277	1.06 (1.04,1.09)	2.03×10^{-6}
rs874898	rs752590 [14]	2	113974196	C	G	0.262	1.00 (0.98,1.03)	7.36×10^{-1}*
rs1562314	rs711830 [14]	2	177045560	T	A	0.638	1.10 (1.07,1.13)	2.84×10^{-14}
rs112071820 [18]		3	138849110	allele 1	G	0.270	1.03 (1.00,1.06)	5.17×10^{-2}*
chr3:156397692	rs62274041 [17]	3	156397692	T	C	0.048	1.47 (1.39,1.55)	7.73×10^{-47}*
rs9870207 [18]		3	190525516	A	G	0.666	1.05 (1.03,1.08)	2.95×10^{-5}
rs7705526	rs10069690[10]	5	1285974	A	C	0.343	1.10 (1.07,1.12)	5.52×10^{-14}
chr5:66121089	rs555025179 [18]	5	66121089	allele2	G	0.526	1.03 (1.00,1.05)	2.61×10^{-2}*
chr8:82653644	8:82668818 [17]	8	82653644	G	A	0.064	1.18 (1.12,1.23)	3.25×10^{-12}*
rs9886651 [18]		8	128817883	G	A	0.435	1.06 (1.03,1.08)	2.89×10^{-6}*
rs13255292 [18]	NA	8	129076573	C	T	0.700	1.07 (1.05,1.10)	3.57×10^{-8}*
rs10103314	rs1400482 [12]	8	129560744	A	C	0.883	1.15 (1.11,1.20)	5.76×10^{-15}*
chr9:16915105	rs10962692[20]	9	16915105	C	G	0.834	1.24 (1.20,1.28)	4.54×10^{-41}*
rs10962643	NA	9	16857403	C	A	0.699	1.17 (1.14,1.20)	1.13×10^{-35}*
rs320203 [18]		9	104943226	C	A	0.842	1.03 (1.00,1.06)	5.21×10^{-2}
chr9:136138765 [15]		9	136138765	G	allele 3	0.176	1.12 (1.08,1.15)	1.49×10^{-12}*
rs7084454	rs144962376[17]	10	21821274	A	G	0.301	1.07 (1.05,1.10)	3.32×10^{-8}*
rs7902587 [18]		10	105694301	T	C	0.091	1.08 (1.03,1.12)	4.54×10^{-4}*
chr12:121403724	rs7953249 [18]	12	121403724	A	G	0.570	1.05 (1.03,1.07)	2.58×10^{-5}*
chr15:91531995	rs8037137[13]	15	91531995	C	T	0.829	1.08 (1.05,1.12)	1.18×10^{-6}*
rs11658063	rs7405776 [19]	17	36103872	G	C	0.614	1.04 (1.02,1.07)	2.98×10^{-4}*
chr17:43552537	rs1879586 [17]	17	43552537	A	G	0.164	1.12 (1.08,1.15)	2.22×10^{-12}*

rs7217120	rs7207826[16]	17	46484755	C	T	0.275	1.10 (1.07,1.13)	$8.69\times10^{-13*}$
rs8098244[18]		18	21405553	G	A	0.741	1.04 (1.01,1.07)	$4.23\times10^{-3*}$
rs4808075 [11]		19	17390291	C	T	0.268	1.13 (1.10,1.16)	$1.49\times10^{-20*}$
rs74597329	rs688187[14]	19	39739155	G	T	0.301	1.02 (0.99,1.04)	2.63×10^{-1}
rs6005807 [18]		22	28934313	T	C	0.095	1.09 (1.04,1.13)	$3.35\times10^{-5*}$

Abbreviations: SNP, single-nucleotide polymorphism; RAF, risk allele frequency; Chr, chromosome; OR, odds ratio; allele1, GCCAGATTCAGAAT; allele2, GACACACAC; allele3, GCGCCCACCACTA.

[a]: If not specified, the previously published best hit is the same as the current best hit.

[b]: Logistic regression for ovarian cancer overall (regardless of histology), adjusted for ethnicity, study panel and leading principal components for each ethnicity (using a total of 47 principal components).

*: P-value > 0.01.

Table 2. Odds ratios for marginal associations of seven environmental risk factors with ovarian cancer risk with 13722 cases and 22975 controls from 19 study sites.

Environmental risk factor	Before Imputation[a]		After Imputation[b]			
	Control	Case	Control	Case	OR[c]	P-value[c]
OCP use						
Never	0.347	0.444	0.351	0.452	Ref	
Ever	0.645	0.536	0.649	0.548	0.62 (0.59,0.66)	5.24×10^{-73}
(missing)	0.008	0.020				
Duration of OCP use						
Never users (including <1 year)	0.425	0.542	0.430	0.554	Ref	
1-<5 year	0.229	0.208	0.232	0.215	0.70 (0.66,0.74)	8.23×10^{-32}
5+ year	0.332	0.222	0.338	0.231	0.48 (0.45,0.51)	2.20×10^{-133}
(missing)	0.014	0.028				
Tubal ligation						
No	0.693	0.777	0.762	0.824	Ref	
Yes	0.208	0.160	0.238	0.176	0.73 (0.69,0.78)	1.81×10^{-23}
(missing)	0.098	0.063				
Breastfeeding						
No	0.239	0.294	0.380	0.515	Ref	
Yes	0.532	0.410	0.620	0.485	0.76 (0.71,0.80)	4.80×10^{-21}
(missing)	0.229	0.296				
Parity (number of full-term births)						
0	0.148	0.241	0.149	0.243	Ref	
1-2	0.487	0.434	0.489	0.438	0.59 (0.55,0.63)	1.94×10^{-65}
3+	0.359	0.315	0.362	0.319	0.50 (0.46,0.53)	4.91×10^{-90}
(missing)	0.006	0.011				
Type of HT using more than 1 year after age 50						
Never use	0.687	0.647	0.789	0.782	Ref	
ET only	0.060	0.075	0.066	0.084	1.22 (1.12,1.34)	2.65×10^{-5}
Any EPT	0.131	0.118	0.145	0.134	0.97 (0.90,1.04)	3.55×10^{-1}

(missing)	0.121	0.160				
BMI						
< 25	0.392	0.370	0.516	0.485	Ref	
25-<30	0.209	0.213	0.284	0.286	1.03 (0.98,1.09)	2.55×10^{-1}
30+	0.144	0.174	0.200	0.229	1.15 (1.08,1.22)	6.11×10^{-6}
(missing)	0.255	0.243				
Endometriosis						
No	0.703	0.695	0.937	0.902	Ref	
Yes	0.047	0.076	0.063	0.098	1.60 (1.46,1.75)	3.41×10^{-23}
(missing)	0.250	0.230				

Abbreviations: OR, odds ratio; OCP, oral contraceptive pills; BMI, body mass index; HT, menopausal hormone therapy; ET, menopausal estrogen therapy; EPT, menopausal estrogen + progestin therapy; Ref, reference group.

[a]: Harmonized environmental data before imputation. Results of the complete cases analysis are provided in eTable 2.

[b]: Based on ten imputed E datasets.

[c]: Logistic regression model adjusted for reference age, race, education, family history, OCP use, tubal ligation, parity, BMI, endometriosis and study site.

Table 3. Results from Multiplicative Interaction Analysis: Odds ratios corresponding to environmental risk factors, stratified by genotype (for G-E pairs with global likelihood ratio test p-value < 0.01. Analysis used the G×E data with 9971 cases and 15566 controls from 17 study sites).

SNP	Environmental risk factor		N (cases/controls)[a]			Estimated OR[b] for E stratified by G (95%CI)			Global[c] LRT	Wald[d] Test
Risk/Baseline allele	Variable	Category	Genotype			Genotype			(df)	(df)
			TT	TC	CC	TT	TC	CC		
rs13255292 C/T	OCP use	Never	396/503	1758/2175	2077/2570	Ref			Ref	Ref
		Ever	446/1069	2286/4336	2768/4750	0.53 (0.46,0.60)	0.61 (0.57,0.66)	0.71 (0.66,0.77)	3.48×10^{-4} (1)	3.47×10^{-4} (1)
		Missing	24/15	96/56	120/96					
			TT	TC	CC	TT	TC	CC		
rs13255292 C/T	Duration of OCP use	< 1 yr	451/636	2213/2670	2546/3145	Ref			Ref	Ref
		1-<5 yr	171/362	854/1522	1082/1662	0.58 (0.50,0.69)	0.68 (0.63,0.74)	0.79 (0.72,0.87)	7.26×10^{-3} (2)	4.74×10^{-3} (1)
		5+ yr	209/568	945/2269	1178/2470	0.43 (0.37,0.5)	0.48 (0.44,0.52)	0.53 (0.49,0.58)		2.43×10^{-2} (1)
		Missing	35/21	128/106	159/135					

			AA	AC	CC	AA	AC	CC		
rs10962643 C/A	**Parity (full term birth)**	0	230/220	940/940	1194/1080	Ref			Ref	Ref
		1-2	398/835	1741/3184	2202/3536	0.52 (0.44,0.61)	0.56 (0.51,0.6)	0.60 (0.54,0.66)	7.52×10^{-3} (2)	1.99×10^{-1} (1)
		3+	243/579	1242/2459	1664/2614	0.38 (0.32,0.46)	0.46 (0.42,0.5)	0.55 (0.49,0.61)		2.86×10^{-3} (1)
		Missing	11/15	47/58	59/46					
			GG	GC	CC	GG	GC	CC		
chr9:16915105 C/G	**Parity (full term birth)**	0	73/72	624/649	1667/1519	Ref			Ref	Ref
		1-2	111/300	1129/2285	3101/4970	0.46 (0.36,0.58)	0.52 (0.47,0.59)	0.60 (0.55,0.65)	5.25×10^{-3} (2)	5.10×10^{-2} (1)
		3+	70/220	749/1679	2330/3753	0.33 (0.26,0.43)	0.42 (0.37,0.48)	0.53 (0.48,0.58)		1.25×10^{-3} (1)
		missing	2/7	37/36	78/76					

Abbreviation: SNP, single-nucleotide polymorphism; OR, odds ratio; OCP, oral contraceptive pills; yr, year; Ref, reference group; df, degree of freedom, LRT, likelihood ratio test.

[a]: Number of cases and controls were estimated from the original merged G×E data (before imputation) with 9971 cases and 15566 controls from 17 study sites, using maximal probable genotypes for imputed SNPs.

[b]: ORs were estimated from the logistic regression model with SNP, E variable, SNP×E variable.

[c]: LRT was performed for jointly testing multiplicative interactions.

[d]: Wald test for individual multiplicative interaction.

All models were estimated from the logistic regression model with SNP, E variable, SNP×E variable, assuming log-additive model, using dosage data for imputed SNPs, adjusted for reference age, race, education, family history, OCP use, tubal ligation, parity, BMI, endometriosis and study site and were performed on imputed datasets of G-E (9971 cases, 15566 controls) with proper pooling.

Table 4. Absolute risks and risk differences stratified by levels of environmental risk factor and levels of genotype (for G-E pairs with global likelihood ratio test p-value < 0.01 on additive scale. Analysis used the G×E data with 9971 cases and 15566 controls from 17 study sites).

SNPs	Environmental risk factor		N (cases/controls)[a]			Estimated ARs or RDs for E stratified by SNPs (95%CI)[c]			Global LRT[d]	Wald Test[e]
risk/baseline allele	variable	category	Genotype			Genotype			(df)	(df)
			CC	CG	GG	CC	CG	GG		
rs11658063 G/C	Type of HT	Neither	589/1142	2609/4518	3310/4956	1.27% (1.23%,1.32%)	1.30% (1.28%,1.33%)	1.33% (1.26%,1.40%)	Ref	Ref
		ET only	66/98	281/409	416/454	1.36% (1.15%,1.57%)	1.63% (1.46%,1.79%)	1.96% (1.59%,2.33%)		
		RD[b]				0.09% (-0.14%,0.31%)	0.33% (0.15%,0.50%)	0.63% (0.24%,1.02%)	3.29×10^{-3} (2)	3.01×10^{-2} (1)
		Any EPT	105/207	498/952	606/1046	1.16% (1.04%,1.28%)	1.21% (1.12%,1.30%)	1.27% (1.09%,1.44%)		
		RD				-0.12% (-0.26%,0.03%)	-0.09% (-0.20%,0.01%)	-0.06% (-0.26%,0.13%)		7.04×10^{-1} (1)
		missing	122/202	582/762	787/820					
			AA	AG	GG	AA	AG	GG		
rs9886651 G/A	OCP use	Never	1278/1718	2053/2502	900/1028	1.52% (1.42%,1.62%)	1.70% (1.64%,1.76%)	1.91% (1.77%,2.04%)	Ref	Ref
		Ever	1666/3105	2640/4978	1194/2072	1.07% (1.02%,1.12%)	1.10% (1.07%,1.13%)	1.14% (1.07%,1.21%)		
		RD				-0.45% (-0.57%, -0.33%)	-0.60% (-0.69%, -0.51%)	-0.77% (-0.93%, -0.60%)	5.32×10^{-3} (2)	9.90×10^{-3} (1)
		missing	70/47	113/79	57/37					

Abbreviation: SNP, single-nucleotide polymorphism; AR, absolute risk; RD, risk difference; OCP, oral contraceptive pills; HT, menopausal hormone therapy; ET, menopausal estrogen therapy; EPT, menopausal estrogen + progestin therapy; Ref, reference group; df, degree of freedom.

[a]: Number of cases and controls were estimated from the original merged G×E data (before imputation) with 9971 cases and 15566 controls from 17 study sites, using maximal probable genotypes for imputed SNPs.

[b]: The risk difference corresponds to given category compared to the reference group, stratified by SNP.

[c]: ARs were estimated from logistic regression model by empirically estimated distribution of E and SNPs, while fixing all other covariates at their mode (determined from the original data).

[d]: LRT was performed for jointly testing additive interactions, assuming dominant effect model of SNPs (due to limitation of software).

[e]: 1-df Wald test corresponds to the test individual RERI term (SNP = 2 vs SNP = 0, E = k vs E = reference group) is zero or not.

All models were estimated from logistic regression model with SNP, E variable, SNP×E variable, assuming log-additive model (except for additive LRT which assumes dominant effect), using maximal probable genotypes for imputed SNPs, adjusted for reference age, race, education, family history, OCP use, tubal ligation, parity, BMI, endometriosis and study site and were performed on imputed datasets of G-E (9971 cases, 15566 controls) with proper pooling.

Supplementary Material

eMethods

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eMethod 2 – Imputation procedures and imputed-data analysis
eMethod 3 – Estimation of absolute risk (AR) from case-control data
eMethod 4 – Confidence Intervals for the estimated absolute risk (AR) and risk difference (RD)

eMethods References

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eMethod 1. Data harmonization and preparation for imputation of E data

Proper data harmonization is essential for a practical imputation and reliable analysis. A brief description for the harmonization process of environmental variables follows. Initially, the Epidemiology Working Group in OCAC established a set of core variables that were requested from each OCAC study. A detailed codebook was provided to each site. In addition, each OCAC study provided their questionnaire to the Epidemiology Working Group. Core variables were assigned to members of the Epidemiology Working Group for harmonization and review. This included questionnaire review to ensure similarity in the way questions were asked and logic checks for the data provided. For example, for the oral contraceptive variables, checks were run to make sure that any individual coded as never having used oral contraceptives was likewise coded as zero months for oral contraceptive use duration. When an expansion of the original core data was desired for a particular analysis, an epidemiologist reviewed the questionnaires, developed a harmonization plan, and created a codebook. Similar logic checks were carried out for new variables brought into the OCAC dataset.

For imputation, we originally started with an interest of 10 environmental risk factors (1-10) with 4 (10-13) confounders from 21 study sites:

1) Oral contraceptive usage
    a. Oral contraceptive pills (OCP) use (ever/never)
    b. Duration of OCP use (<1, 1-<5, 5+ yr)
2) Tubal ligation (y/n)
3) Breastfeeding (y/n)

4) Parity (0,1-2,3+ births)
5) Type of menopausal hormone therapy (HT) 1+ year after aged 50 (never user, menopausal estrogen therapy only, any use of menopausal estrogen + progestin therapy)
6) BMI (< 25, 25-<30, 30+)
7) Endometriosis (y/n)
8) Age at menarche (12-15, < 12, 15+ yr)
9) Alcohol consumption in last 5 years(y/n)
10) Talc powder use on genital area (ever/never)
11) Reference age (<50, 50-<55, 55-<60, 60-<65, 65-70, 70+ years)
12) Race (Non-Hispanic white, Hispanic White, Black, Others)
13) Education (< high school, high school graduate, some college, college graduate)
14) first-degree family history of ovarian cancer (y/n)

Missingness of the environmental risk factors varied by study-site and its pattern is presented in ***eFigure 1A***. To avoid discarding a large number of subjects who had missing data at least one of the variables, we imputed missing values. Simultaneously, to reduce errors due to the imputation, we excluded some study sites and variables that had a high proportion of missingness.

1.1 Exclusion of environmental risk factors

Originally, we had environmental information from 45,966 subjects (15,833 cases and 30,083 controls) from 21 study sites. Examining the study site-specific missing data patterns showed that the two variables, alcohol use within 5 years (y/n) genital power use (ever/never), were not reported by more than 50% of subjects (***eFigure 1A***).

Moreover, more than half of study sites did not collect any information on at least one of these two variables. Therefore, these two risk factors were excluded from the entire analysis.

1.2 Exclusion of study sites

Moreover, we found two study sites, Melbourne Collaborative Cohort Study in Australia (MCC) and UK Studies of Epidemiology and Risk Factors in Cancer Heredity (SEARCH) Ovarian Cancer Study (SEA), did not collect 6-7 variables. To improve the validity of imputation, we excluded MCC and SEA. Since this study focused on the effects in the general population (not a specific study site), we kept all the remaining 19 study sites (***eFigure 1B***), some of which may have no information on a few variables. However, including as many subjects as possible could improve power to identify any potential G-E interaction effect.

After the above exclusions, the final E dataset consisted of 36,697 subjects with 13,722 cases from 19 study sites (see study characteristics in ***eTable 1***). All the 19 study sites have been previously described (1-18).

eMethod 2. Imputation procedures and imputed-data analysis

In our G-E interaction analysis, imputation of E data was a key element because analysis restricted to the complete data might not provide enough power and could also lead to biased results(19). Multiple imputation is one way to keep all the data by "filling in missing values multiple times and thus created multiple 'complete' datasets"(20). In contrast to single imputation methods (such as plug in a mean of the variable), multiple imputation methods can properly account for the missing data uncertainty(20). Specifically, we used multiple imputation by chained equations (MICE)(21).

2.1 Building imputation models for E data

We imputed the following 13 variables: continuous variables of BMI, duration of OCP use, and reference age as well as categorical/binary variables of parity, endometriosis, age at menarche, type of menopausal hormone therapy for 1+ year, breastfeeding, OCP use, tubal ligation, race, education, family history of ovarian. Because the collection of OCP use (ever vs never) and duration of OCP use were acquired through two different questions in the survey, we decided to impute both variables because they convey slightly different information.

Using regression models, we sequentially imputed missing values for the above 13 risk factors, starting with the variable with least missing and progressing in order of increasing missing proportions. Each imputation model included case/control status, height, interview year (>=1976-<1986, >=1986-<1996, >=1996-<2006, >=2006-<2016), age at diagnosis/interview and study site as covariates for adjustment, in addition to the remaining 13 imputation variables. We used the R package MICE to implement the imputation procedures above (21) .

2.2 Ten imputed E data and ten imputed G-E data

Ten imputed E datasets were created by MICE, each of which consisted of 13,722 cases and 22,975 controls. We compared the association between case-control status and each imputed variable before and after imputation to verify the validity of imputation (***eTable 2***). For each imputed E dataset, G data from 17 case-control studies (a total of 9,971 cases and 15,566 controls) were merged to create a G×E dataset.

menopausal hormone therapy

2.3 Combining multiple imputation results

Environmental association analysis and G × E interaction analysis were repeatedly carried out with each of the 10 imputed E datasets and each of the 10 imputed G×E datasets, respectively.

Odds Ratio. Individual estimates of the log odds ratio and the corresponding individual standard errors from each of the 10 imputed datasets were combined using Rubin's rule(22). Suppose *D* imputed datasets yield the log odds ratio estimates $(Q_1, \dots, Q_D)$ and their variance estimates $(U_1, \dots, U_D)$. Then, the pooled estimate is given by $\bar{Q} = \frac{1}{D}\sum_{m=1}^{D} Q_m$ and its variance estimate is given by $T = \bar{U} + \left(1 + \frac{1}{D}\right) B$, where $\bar{U} = \frac{1}{D}\sum_{m=1}^{D} U_m$, $B = \frac{1}{D-1}\sum_{m=1}^{D}(Q_m - \bar{Q})^2$. Note $(Q - \bar{Q})T^{-\frac{1}{2}}$ approximately follows a t-distribution (22,23) with the degrees of freedom $v_D^* = \left(\frac{1}{v_D} + \frac{1}{v_{obs}}\right)^{-1}$,

where $v_D = (D-1)\left(1 + \frac{\bar{U}}{(1+D^{-1})B}\right)^2$, $v_{obs} = \frac{v_0+1}{v_0+3} v_0(1-\gamma)$, $\gamma = \frac{(1+D^{-1})B}{T}$. In our analysis, as the sample size is over 20,000 and the number of covariates in each model is small, by central limit theorem, we assumed that $\bar{Q}$ is normal with mean *Q* and variance *T*.

RERI-statistics. We combine RERI estimate by the same way as combining the estimated log-OR mentioned above.

LRT-statistic. Suppose *(LR_1,…, LR_D)* are the individual LRT-statistics from *D* imputed G×E datasets. Let $\overline{LR}$ be the sample mean of $(LR_1, \ldots, LR_D)$ and v be the sample variance of $\left(\sqrt{LR_1}, \ldots \sqrt{LR_D}\right)$. Then, the pooled LRT-statistic is calculated by

$$\widehat{LR} = \frac{\overline{LR}/k - (1 - D^{-1})v}{1 + (1 + D^{-1})v}$$

and the corresponding overall p-value is obtained by

$$P - value = Pr\left(F_{k,b} > \widehat{LR}\right),$$

where $F_{k,b}$ is an reference distribution with k = degrees of freedom for each LRT test and $b = k^{-\frac{3}{D}}(D-1)\{1 + [(1 + D^{-1})v]^{-1}\}^2$ (22,24). This is a simplest way of combining p-values which only requires the chi-square statistics from each analysis, yet it performs pretty well when $D \geq 5$ (24).

eMethod 3. Estimation of absolute risk (AR) from case-control data

This section describes how the AR in each G×E stratum was estimated from case-control studies with aid of external knowledge that the incidence rate of ovarian cancer is 1.3% (25).

Let $L = (levels\ of\ E_j) - 1$, $M_q = (levels\ of\ C_q) - 1$, and Q be the number of adjusted covariates. For a given SNP $G_k = g\ (k = 1, \dots, 28)$ and environmental risk factor $E_j = l\ (j = 1, \dots, 7)$, the AR of ovarian cancer was calculated by

$$Prob(D = 1 | G_k = g, E_j = l)$$

$$= \frac{\exp\left(\hat{\beta}_0^* + \hat{\beta}_G g + \hat{\beta}_{El} I(E_j = l) + \hat{\beta}_{GEl} I(E_j = l) g + \sum_{q=1}^{Q} \sum_{m=1}^{M_q} \hat{\beta}_{C_q m} I(C_q = m)\right)}{1 + \exp\left(exp\left(\hat{\beta}_0^* + \hat{\beta}_G g + \hat{\beta}_{El} I(E_j = l) + \hat{\beta}_{GEl} I(E_j = l) g + \sum_{q=1}^{Q} \sum_{m=1}^{M_q} \hat{\beta}_{C_q m} I(C_q = m)\right)\right)}$$

where $\hat{\beta}_G, \hat{\beta}_{El}, \hat{\beta}_{GEl}, \hat{\beta}_{C_{11}}, \dots, \hat{\beta}_{C_O M_O}$ are estimated from the logistic regression model [M1] in main manuscript. However, in general the intercept term $\hat{\beta}_0^*$ cannot be directly estimated from case-control studies unless one knows the sampling proportion of cases and controls. In our analysis, to estimate $\hat{\beta}_0^*$, we used external knowledge, $Prob(D = 1) = 1.3\%$. Specifically, we view

$$logit(\pi_i | G_{ki}, E_{ji}, C_{1i}, \dots, C_{Oi}; \beta_0^*)$$

$$= \beta_0^* + \beta_G G_{ki} + \sum_{l=1}^{L} \beta_{El} I(E_{ji} = l) + \sum_{l=1}^{L} \beta_{GEl} I(E_{ji} = l) G_{ki} + \sum_{q=1}^{Q} \sum_{m=1}^{M_q} \beta_{C_q m} I(C_{qi} = m)$$

as a function of β_0^*, and we assume G and E are independent and

$$Prob(D = 1 | C_1 = m_1, C_Q = m_Q; \beta_0^*)$$

$$= \sum_{l=1}^{L} \sum_{g=0}^{2} Prob\left(D = 1 \middle| G = g, E = l, C_1 = m_1, C_Q = m_Q; \beta_0^*\right) * Prob(G = g) * Prob(E = l)$$

$$= 1.3\%$$

where m_q is the mode of $C_q$covariate, and $Prob(G = g)$ and $Prob(E = l)$ are estimated from controls only. Then, the solution of the above equation for β_0^* is the estimate $\hat{\beta}_0^*$.

eMethod 4. Confidence Intervals for the estimated absolute risk (AR) and risk difference (RD)

To obtain confidence intervals for ARs and RDs in ***Table 4*** and ***eTable 6***, we used a nonparametric bootstrapping method. For each of the *D* imputed datasets, we first generated *b* (set *b* = 1000) bootstrap samples and calculated the imputation-specific estimate of AR (or RD), denoted by Q_d^* $(d = 1, \dots, D)$, as the sample mean of *b* bootstrap estimates and the within-imputation variance, U_d^*, as the sample variance of *b* bootstrap estimates. Then, we pooled the *D* imputation-specific estimates using Robin's rule (22) (see **eMethod 2.3**), where the between-imputation variance, B^*, was estimated as the sample variance of $(Q_1^*, \dots, Q_D^*)$.

eFigure Legends

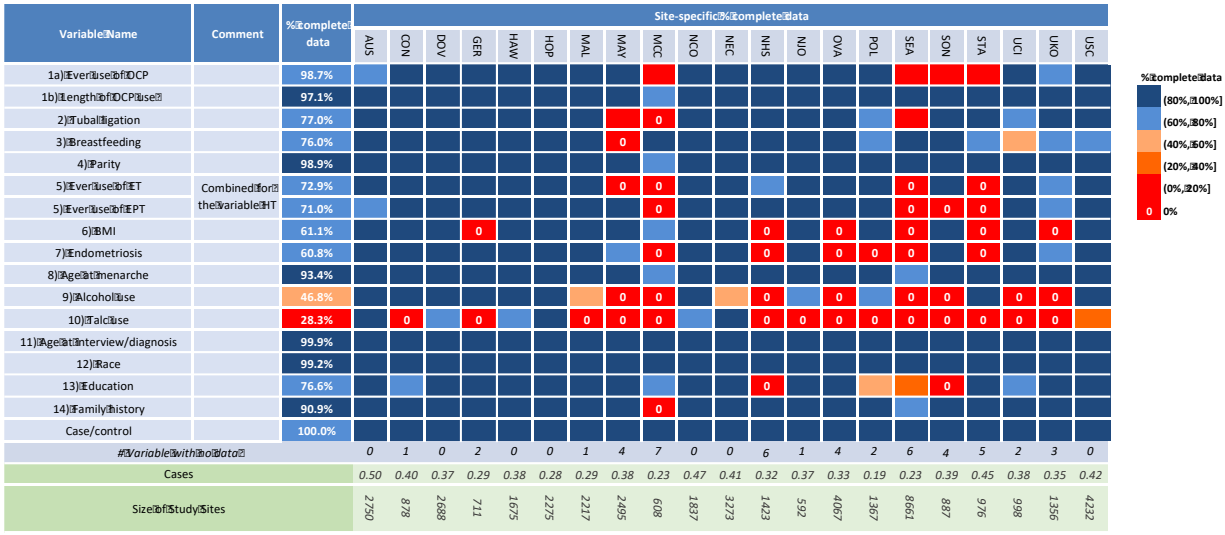
eFigure 1. Site-specific missing data stricture of 13 variables (A) for raw E data with 15833 cases and 30083 controls from 21 study sites (B) for harmonized E data with 13722 cases and 22975 controls from 19 study sites.

eFigure 2. Estimated absolute risk (AR) of ovarian cancer given type of menopausal hormone therapy (never user [neither], menopausal estrogen therapy only [ET only]) and number of risk allele of rs11658063, among non-Hispanic white college graduates aged below 50, ever used OCP with no family history of ovarian cancer, BMI below 25, no tubal ligation, no endometriosis, with one child. The ARs were calculated from a logistic regression model assuming log-additive effect of SNPs, while all the rest covariates fixed at their most frequent level.

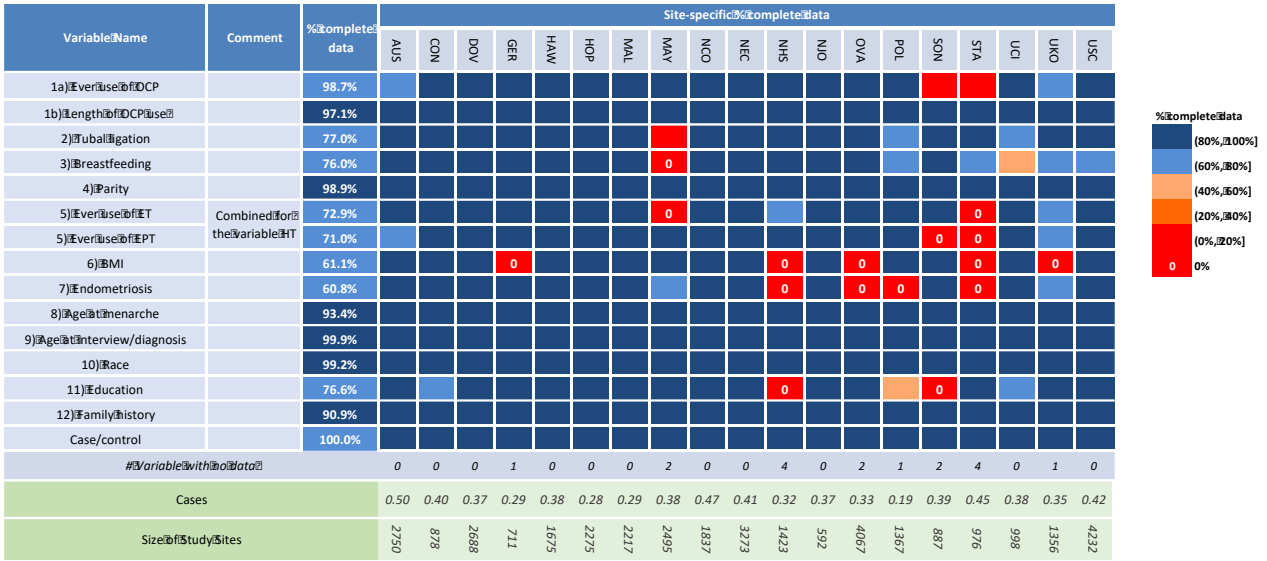
eFigures

eFigure1

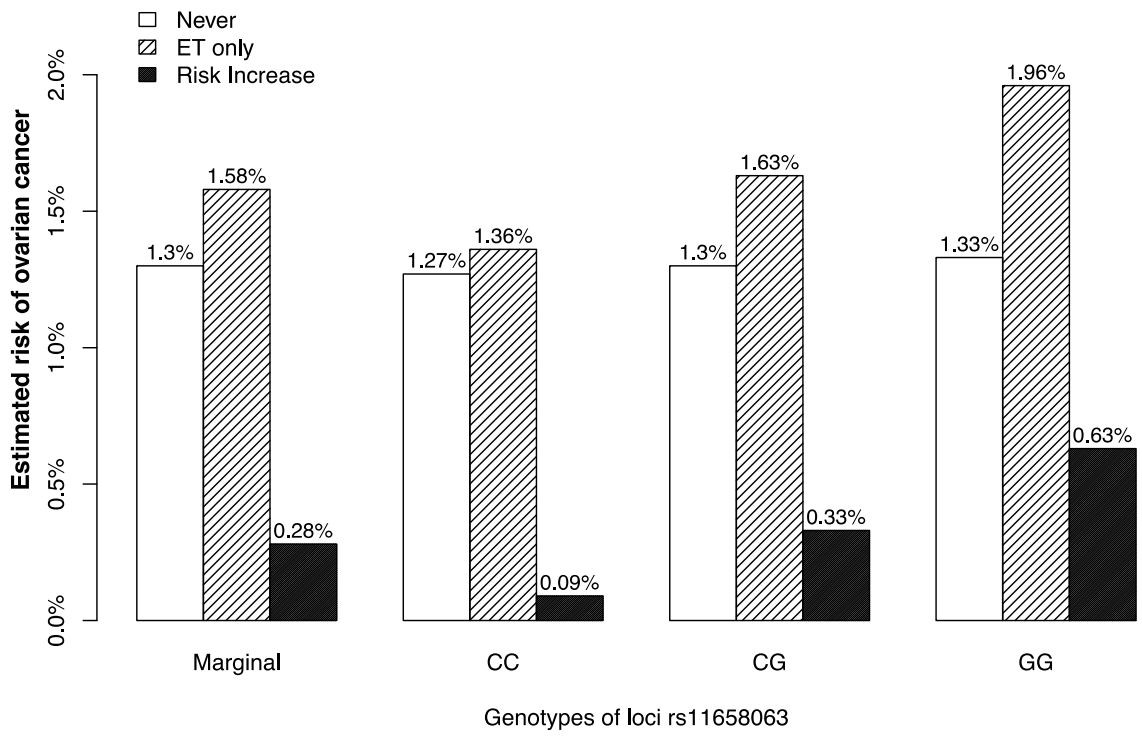
A



B



eFigure 2


Never
ET only
Risk Increase
Estimated risk of ovarian cancer
0.0%
0.5%
1.0%
1.5%
2.0%
1.3%
1.58%
0.28%
1.27%
1.36%
0.09%
1.3%
1.63%
0.33%
1.33%
1.96%
0.63%
Marginal
CC
CG
GG
Genotypes of loci rs11658063

eTable 1. Characteristics of 19 Case-Control Studies from the Ovarian Cancer Association Consortium (OCAC) included in the analyses

Study acronym	Study Name	Country	Year of interview	Size[a]		Mean age (+/- sd)
AUS [9]	Australian Ovarian Cancer Study	Australia	2001-2006	case	1381	59.0 (48.0-69.9)
				control	1369	55.5 (43.0-68.0)
CON [13]	Connecticut Ovary Study	USA	1999-2003	case	352	59.1 (48.3-69.9)
				control	526	52.8 (42.4-63.1)
DOV [1]	Diseases of the Ovary and their Evaluation	USA	2002-2009	case	1001	55.8 (46.9-64.7)
				control	1687	56.0 (46.8-65.3)
GER [14]	Germany Ovarian Cancer Study	Germany	1993-1998	case	209	55.9 (44.3-67.6)
				control	502	54.7 (42.4-67.1)
HAW [7]	Hawaii Ovarian Cancer Study	USA	1993-2008	case	641	56.4 (43.9-69.0)
				control	1034	54.8 (40.2-69.3)
HOP [11]	Hormones and Ovarian Cancer Prediction	USA	2003-2009	case	645	60.0 (47.7-72.4)
				control	1630	57.6 (45.3-70.0)
MAL [3]	Danish Malignant Ovarian Tumor Study	Denmark	1994-1999	case	653	59.3 (48.6-69.9)
				control	1564	57.1 (45.8-68.4)
MAY [5]	Mayo Clinic Ovarian Cancer Case Control Study	USA	1999-2014	case	956	61.6 (49.1-74.1)
				control	1539	59.4 (44.9-73.8)
NCO [10]	North Carolina Ovarian Cancer Study	USA	1999-2008	case	856	56.9 (46.3-67.5)
				control	981	54.7 (42.9-66.5)
NEC [16]	New England-based Case-Control Study of Ovarian Cancer	USA	1992-2008	case	1327	55.1 (44.0-66.2)
				control	1946	53.1 (40.6-65.7)

NHS [15]	Nurses' Health Study	USA	1976-2009	case	450	62.4 (51.5-73.3)
				control	973	62.5 (52.1-72.9)
NJO [26]	New Jersey Ovarian Cancer Study	USA	2002-2009	case	219	56.2 (45.9-66.5)
				control	373	63.3 (52.1-74.5)
OVA [6]	Ovarian Cancer in Alberta and British Columbia Study	Canada	2002-2012	case	1355	58.6 (47.7-69.5)
				control	2712	56.7 (47.0-66.4)
POL [2]	NCI Ovarian Case-Control Study in Poland	Poland	2000-2004	case	260	56.2 (45.5-66.9)
				control	1107	55.6 (45.1-66.2)
SON [4]	Southern Ontario Ovarian Cancer Study	Canada	1990-1993	case	345	57.7 (46.4-69.0)
				control	542	56.7 (44.4-69.0)
STA [8]	Genetic Epidemiology of Ovarian Cancer	USA	1997-2002	case	436	49.8 (40.5-59.0)
				control	540	47.0 (36.8-57.1)
UCI [18]	UC Irvine Ovarian Cancer Study	USA	1994-2005	case	384	57.6 (45.4-69.9)
				control	614	53.7 (41.2-66.2)
UKO [27]	UK Ovarian Cancer Population Study	UK	2006-2009	case	477	60.0 (48.7-71.2)
				control	879	64.8 (58.9-70.7)
USC [12,17]	Los Angeles County Case-Control Studies of Ovarian Cancer	USA	1993-2010	case	1775	57.1 (45.2-68.9)
				control	2457	54.0 (41.8-66.3)
Total study population for marginal environmental association analysis			1976-2014	Case	13722	57.7 (46.3-69.1)
				Control	22975	56.3 (44.2-68.3)
Total study population for gene by environmental interaction analysis[b]			1976-2014	Case	9971	57.9 (46.5-69.2)
				Control	15566	56.5 (44.6-68.4)

[a] Size refers to the number of individuals included for marginal E analysis.
[b] Subsets in harmonized environmental data with available genetic data were included in the interaction analysis.

eTable 2. Odds ratios for marginal associations of seven environmental risk factors in complete cases analysis and multiple imputation analysis.

Environmental risk factor	Complete Cases Analysis[a] (5803 cases, 10190 controls, 11 sites)				Multiple Imputation Analysis[b] (13722 cases, 22975 controls, 19 sites)			
	Control	Case	OR[c]	P-value[c]	Control	Case	OR[c]	P-value[c]
OCP use								
Never	0.309	0.414	Ref		0.351	0.452	Ref	
Ever	0.691	0.586	0.66 (0.61, 0.71)	1.24×10^{-24}	0.649	0.548	0.62 (0.59,0.66)	5.24×10^{-73}
Duration of OCP use								
Never users (including <1 year)	0.415	0.546	Ref		0.430	0.554	Ref	
1-<5 year	0.254	0.232	0.71 (0.65, 0.77)	1.39×10^{-14}	0.232	0.215	0.70 (0.66,0.74)	8.23×10^{-32}
5+ year	0.331	0.222	0.50 (0.46, 0.55)	3.92×10^{-52}	0.338	0.231	0.48 (0.45,0.51)	2.20×10^{-133}
Tubal ligation								
No	0.755	0.814	Ref		0.762	0.824	Ref	
Yes	0.245	0.186	0.71 (0.65, 0.77)	5.85×10^{-15}	0.238	0.176	0.73 (0.69,0.78)	1.81×10^{-23}
Breastfeeding								
No	0.351	0.457	Ref		0.380	0.515	Ref	
Yes	0.649	0.543	0.79 (0.73, 0.85)	3.27×10^{-9}	0.620	0.485	0.76 (0.71,0.80)	4.80×10^{-21}
Parity (number of full-term births)								
0	0.049	0.075	Ref		0.149	0.243	Ref	
1-2	0.536	0.543	0.64 (0.55, 0.74)	6.31×10^{-10}	0.489	0.438	0.59 (0.55,0.63)	1.94×10^{-65}
3+	0.415	0.382	0.50 (0.43, 0.58)	3.50×10^{-20}	0.362	0.319	0.50 (0.46,0.53)	4.91×10^{-90}
Type of HT using more than 1 year after age 50								
Never use	0.775	0.745	Ref		0.789	0.782	Ref	
ET only	0.067	0.099	1.31 (1.15, 1.49)	3.26×10^{-5}	0.066	0.084	1.22 (1.12,1.34)	2.65×10^{-5}
Any EPT	0.158	0.156	0.94 (0.85, 1.04)	2.29×10^{-1}	0.145	0.134	0.97 (0.90,1.04)	3.55×10^{-1}
BMI								
< 25	0.517	0.487	Ref		0.516	0.485	Ref	
25-<30	0.283	0.289	1.03 (0.95, 1.11)	4.76×10^{-1}	0.284	0.286	1.03 (0.98,1.09)	2.55×10^{-1}
30+	0.200	0.224	1.11 (1.02, 1.21)	2.20×10^{-2}	0.200	0.229	1.15 (1.08,1.22)	6.11×10^{-6}

Endometriosis								
No	0.940	0.908	Ref		0.937	0.902	Ref	
Yes	0.060	0.092	1.55 (1.37, 1.76)	7.02×10^{-12}	0.063	0.098	1.60 (1.46,1.75)	3.41×10^{-23}

Abbreviations: OR, odds ratio; OCP, oral contraceptive pills; BMI, body mass index; HT, menopausal hormone therapy; ET, menopausal estrogen therapy; EPT, menopausal estrogen + progestin therapy; Ref, reference group.

[a]: Harmonized environmental data with no missing values in all included variables.

[b]: Based on ten imputed E datasets.

[c]: Logistic regression model adjusted for reference age, race, education, family history, OCP use, tubal ligation, parity, BMI, endometriosis and study site.

eTable 3. Likelihood Ratio Tests for multiplicative and additive interactions between 28 SNP and 9 risk factor (showing P-value < 0.2) with 9971 cases, 15566 controls from 17 study sites

On Multiplicative scale							On additive scale					
Interaction Term			LRT[a]		1-df Wald Test [c]		Interaction Term		LRT[b]		1-df RERI Test [d]	
No.	Risk Factor	SNPs	P-value	df	P-value[e]	P-value[f]	Risk Factor	SNPs	P-value	df	P-value[e]	P-value[f]
1	OCP ever	rs13255292	3.48×10^{-4}	1	3.47×10^{-4}	NA	HRT	rs11658063	3.29×10^{-3}	2	3.01×10^{-2}	7.04×10^{-1}
2	Parity	chr9:16915105	5.25×10^{-3}	2	5.10×10^{-2}	1.25×10^{-3}	OCP ever	rs9886651	5.32×10^{-3}	1	9.90×10^{-3}	NA
3	Length of OCP	rs13255292	7.26×10^{-3}	2	4.74×10^{-3}	2.43×10^{-2}	Parity	rs74597329	1.90×10^{-2}	2	1.88×10^{-1}	8.12×10^{-1}
4	Parity	rs10962643	7.52×10^{-3}	2	1.99×10^{-1}	2.86×10^{-3}	Length of OCP	chr17:43552537	1.95×10^{-2}	2	7.25×10^{-1}	4.27×10^{-2}
5	OCP ever	rs9886651	1.97×10^{-2}	1	1.97×10^{-2}	NA	Length of OCP	chr9:16915105	2.13×10^{-2}	2	1.43×10^{-1}	1.25×10^{-4}
6	OCP ever	rs10962643	2.76×10^{-2}	1	2.76×10^{-2}	NA	Length of OCP	rs10103314	2.16×10^{-2}	2	1.27×10^{-1}	2.62×10^{-2}
7	HRT	chr9:16915105	3.08×10^{-2}	2	6.08×10^{-2}	1.10×10^{-1}	OCP ever	rs13255292	2.65×10^{-2}	1	2.85×10^{-3}	NA
8	Parity	rs74597329	4.04×10^{-2}	2	4.51×10^{-2}	8.38×10^{-1}	Tubal ligation	chr:9:136138765	2.71×10^{-2}	1	7.69×10^{-2}	NA
9	breastfeeding	rs7084454	4.14×10^{-2}	1	4.14×10^{-2}	NA	Parity	chr12:121403724	3.20×10^{-2}	2	4.76×10^{-1}	1.21×10^{-1}
10	Parity	chr12:121403724	6.82×10^{-2}	2	4.21×10^{-1}	3.08×10^{-2}	Parity	rs11658063	3.46×10^{-2}	2	2.54×10^{-1}	9.91×10^{-2}
11	breastfeeding	rs7705526	6.88×10^{-2}	1	6.88×10^{-2}	NA	OCP ever	rs10962643	3.49×10^{-2}	1	1.91×10^{-3}	NA
12	Tubal ligation	rs1562314	7.13×10^{-2}	1	7.07×10^{-2}	NA	Parity	rs9886651	3.85×10^{-2}	2	4.38×10^{-1}	7.28×10^{-1}
13	Parity	rs7902587	7.16×10^{-2}	2	2.67×10^{-1}	4.64×10^{-1}	OCP ever	rs4808075	5.05×10^{-2}	1	1.58×10^{-1}	NA
14	Length of OCP	rs10962643	7.81×10^{-2}	2	2.42×10^{-1}	2.69×10^{-2}	Length of OCP	rs7705526	5.09×10^{-2}	2	1.86×10^{-1}	3.25×10^{-3}
15	Length of OCP	rs7705526	7.98×10^{-2}	2	3.47×10^{-1}	2.51×10^{-2}	breastfeeding	chr2:111818658	5.41×10^{-2}	1	9.52×10^{-2}	NA

16	breastfeeding	rs320203	8.01×10^{-2}	1	8.00×10^{-2}	NA	Parity	rs7705526	5.44×10^{-2}	2	9.78×10^{-3}	1.24×10^{-2}
17	Length of OCP	chr9:16915105	8.02×10^{-2}	2	1.00*	3.58×10^{-2}	breastfeeding	rs7084454	6.70×10^{-2}	1	1.62×10^{-1}	NA
18	breastfeeding	rs10962643	8.38×10^{-2}	1	8.33×10^{-2}	NA	OCP ever	chr3:156397692	7.93×10^{-2}	1	1.90×10^{-1}	NA
19	Parity	rs7705526	8.57×10^{-2}	2	3.08×10^{-2}	7.20×10^{-2}	Length of OCP	rs9886651	7.93×10^{-2}	2	6.27×10^{-1}	3.86×10^{-2}
20	Parity	chr8:82653644	9.46×10^{-2}	2	9.43×10^{-1}	9.69×10^{-2}	OCP ever	rs7705526	8.17×10^{-2}	1	5.24×10^{-2}	NA
21	breastfeeding	rs7217120	1.10×10^{-1}	1	1.10×10^{-1}	NA	breastfeeding	rs7217120	9.00×10^{-2}	1	3.08×10^{-1}	NA
22	Length of OCP	rs4808075	1.15×10^{-1}	2	9.88×10^{-2}	5.16×10^{-1}	Tubal ligation	rs8098244	1.00×10^{-1}	1	1.87×10^{-1}	NA
23	HRT	rs6005807	1.15×10^{-1}	2	4.68×10^{-2}	3.89×10^{-1}	HRT	rs6005807	1.07×10^{-1}	2	9.78×10^{-3}	4.29×10^{-1}
24	Tubal ligation	rs4808075	1.25×10^{-1}	1	1.23×10^{-1}	NA	breastfeeding	rs7705526	1.08×10^{-1}	1	2.79×10^{-2}	NA
25	OCP ever	rs7705526	1.28×10^{-1}	1	1.28×10^{-1}	NA	BMI	rs10103314	1.21×10^{-1}	2	6.54×10^{-1}	4.35×10^{-1}
26	Parity	rs11658063	1.28×10^{-1}	2	2.39×10^{-1}	4.41×10^{-2}	Tubal ligation	rs7084454	1.23×10^{-1}	1	2.37×10^{-1}	NA
27	breastfeeding	chr17:43552537	1.29×10^{-1}	1	1.29×10^{-1}	NA	Tubal ligation	rs6005807	1.24×10^{-1}	1	6.84×10^{-1}	NA
28	HRT	chr15:91531995	1.30×10^{-1}	2	1.41×10^{-1}	2.33×10^{-1}	OCP ever	rs320203	1.34×10^{-1}	1	8.35×10^{-1}	NA
29	HRT	chr12:121403724	1.30×10^{-1}	2	8.63×10^{-2}	2.49×10^{-1}	Length of OCP	chr15:91531995	1.43×10^{-1}	2	2.22×10^{-1}	8.54×10^{-1}
30	HRT	rs11658063	1.36×10^{-1}	2	4.45×10^{-2}	7.13×10^{-1}	breastfeeding	chr17:43552537	1.44×10^{-1}	1	3.66×10^{-1}	NA
31	HRT	chr:9:136138765	1.59×10^{-1}	2	1.71×10^{-1}	1.29×10^{-1}	Length of OCP	rs10962643	1.44×10^{-1}	2	3.84×10^{-2}	2.30×10^{-4}
32	breastfeeding	chr2:111818658	1.64×10^{-1}	1	1.64×10^{-1}	NA	Parity	rs7902587	1.51×10^{-1}	2	4.10×10^{-1}	9.39×10^{-1}
33	HRT	rs1562314	1.69×10^{-1}	2	1.95×10^{-1}	2.3×10^{-1}	OCP ever	chr:9:136138765	1.74×10^{-1}	1	2.46×10^{-1}	NA
34	Tubal ligation	chr15:91531995	1.72×10^{-1}	1	1.72×10^{-1}	NA	Tubal ligation	chr15:91531995	1.78×10^{-1}	1	2.99×10^{-1}	NA
35	OCP ever	chr9:16915105	1.79×10^{-1}	1	1.79×10^{-1}	NA	Tubal ligation	chr9:16915105	1.84×10^{-1}	1	4.92×10^{-1}	NA

36	breastfeeding	rs7902587	1.81×10^{-1}	1	1.81×10^{-1}	NA	Length of OCP	rs7084454	1.86×10^{-1}	2	4.95×10^{-1}	8.28×10^{-2}
37	breastfeeding	rs6005807	1.83×10^{-1}	1	1.83×10^{-1}	NA	Parity	chr15:91531995	1.87×10^{-1}	2	8.36×10^{-1}	6.91×10^{-1}
38	Tubal ligation	chr12:121403724	1.91×10^{-1}	1	1.91×10^{-1}	NA	HRT	rs9886651	1.89×10^{-1}	2	1.85×10^{-1}	7.50×10^{-1}
39	Parity	rs4808075	1.95×10^{-1}	2	8.37×10^{-1}	1.43×10^{-1}	Length of OCP	rs7902587	1.90×10^{-1}	2	7.99×10^{-1}	1.99×10^{-1}
40	OCP ever	chr2:111818658	1.95×10^{-1}	1	1.95×10^{-1}	NA	Endometriosis	rs4808075	1.91×10^{-1}	1	2.88×10^{-1}	NA
41							HRT	chr9:16915105	1.95×10^{-1}	2	7.94×10^{-3}	8.24×10^{-2}

Abbreviations: SNP, single-nucleotide polymorphism; OR, odds ratio; AR, absolute risk; OCP, oral contraceptive pills; BMI, body mass index; HT, menopausal hormone therapy; ET, menopausal estrogen therapy; EPT, menopausal estrogen + progestin therapy; Ref, reference group; Mult, multiplicative; Add, additive.

[a] LRT comparing two model: one with interaction, main effect of given SNP and risk factor E; the other model without the interaction, using dosage data for imputed SNPs

[b] LRT comparing two models: one with interaction, main effect of given SNP and risk factor E; the other model assumes no additive interactions, using maximal probable genotypes for imputed SNPs.

[c] Wald test of individual multiplicative interaction, using dosage data for imputed SNPs

[d] Wald test for individual RERI term (SNP = 2 vs SNP = 0), using maximal probable genotypes for imputed SNPs.

[e] comparing E = 1 vs E = 0.

[f] comparing E = 2 vs E = 0.

[*] without rounding 0.9998719084

Shaded: Significant interactions that were selected for further analysis

All models were from logistic regression models adjusted for reference age, race, education, family history, OCP use, tubal ligation, parity, BMI, endometriosis and study sites and were performed on ten imputed sets of G×E dataset (9971

cases, 15566 controls) with proper pooling. Except additive LRT (dominant effect model of SNPs), all the rest tests assume log-additive effect model of SNPs.

eTable4. Estimated ARs stratified by OCP use or duration of OCP use and number of risk allele of rs1325292

SNP	Environmental risk factor		Estimated AR[b] for E stratified by G (95%CI)				Global LRT[c]	Wald Test[d]
Risk/Baseline allele	Variable	Category	Marginal	Genotype1	Genotype2	Genotype3	(df)	(df)
rs13255292 C/T	OCP use			TT	TC	CC		
		Never	1.68% (1.63%,1.74%)	1.71% (1.55%,1.87%)	1.69% (1.62%,1.76%)	1.67% (1.59%,1.76%)	Ref	Ref
		Ever	1.10% (1.07%, 1.13%)	0.91% (0.84%,0.98%)	1.04% (1.01%,1.08%)	1.20% (1.15%,1.24%)		
		RD[a]	0.58% (0.49%, 0.67%)	0.80% (0.62%,0.99%)	0.65% (0.55%,0.74%)	0.48% (0.36%,0.59%)	2.65×10^{-2} (2)	2.85×10^{-3} (1)
rs13255292 C/T	Duration of OCP use			TT	TC	CC		
		< 1 yr	1.70% (1.66%,1.74%)	1.67% (1.53%,1.81%)	1.69% (1.63%,1.75%)	1.72% (1.64%,1.79%)	Ref	Ref
		1-<5 yr	1.24% (1.17%,1.30%)	0.99% (0.86%,1.11%)	1.16% (1.09%,1.23%)	1.36% (1.26%,1.45%)		
		RD	0.47% (0.37%,0.56%)	0.69% (0.49%,0.88%)	0.54% (0.43%,0.64%)	0.36% (0.22%,0.50%)	6.02×10^{-1} (2)	1.12×10^{-2} (1)
		5+ yr	0.86% (0.82%,0.90%)	0.72% (0.64%,0.81%)	0.82% (0.77%,0.86%)	0.92% (0.86%,0.98%)		
		RD	0.84% (0.77%,0.92%)	0.95% (0.78%,1.12%)	0.88% (0.79%,0.96%)	0.79% (0.69%,0.90%)		1.72×10^{-1} (1)

Abbreviation: SNP, single-nucleotide polymorphism; AR, absolute risk; RD, risk difference; OCP, oral contraceptive pills; Ref, reference group; df, degree of freedom.

[a] The risk reduction corresponds to given category compared to the reference group, stratified by SNP.

[b] ARs were estimated from logistic regression model by empirically estimated distribution of E and SNPs, fixing all other covariates at their mode (determined from original data).

[c] LRT were performed for jointly testing additive interactions, assuming dominant effect model of SNPs (due to limitation of software).

[d] 1-df Wald test corresponds to the test individual RERI term (SNP = 2 vs SNP = 0, E = I vs E = reference group) is zero or not.

All models were estimated from logistic regression model with SNP, E variable, SNP x E variable, assuming log-additive model (except for additive LRT which assumes dominant effect), using maximal probable genotypes for imputed SNPs, adjusted for reference age, race, education, family history, OCP use, tubal ligation, parity, BMI, endometriosis and study site and were performed on imputed datasets of G-E (9971 cases, 15566 controls) with proper pooling.

eTable5. Observed and expected OR under multiplicative and additive null for six gene-environment pairs with G×E data comprising of 9971 cases and 15566 controls from 17 study sites

Environment Risk Factor		Genetic Risk Factor		Observed ORs (95%CI)			Expected OR_{joint}[a]		$P_{interaction}$	
Variable Name	Category	SNP	Genotype	OR_E	OR_{SNP}	OR_{joint}	Mult	Add	Mult[b]	Add[c]
Use of OCP	ever (vs never)	**rs13255292**	TC (vs TT)	0.53 (0.46,0.6)	0.99 (0.93,1.05)	0.61 (0.54,0.68)	0.52	0.51	3.47×10^{-4}	4.49×10^{-3}
			CC (vs TT)		0.98 (0.86,1.11)	0.70 (0.62,0.78)	0.51	0.50		2.85×10^{-3}
Duration of OCP	1-5 yr (vs < 1yr)	**rs13255292**	TC (vs TT)	0.58 (0.5,0.69)	1.01 (0.96,1.07)	0.69 (0.61,0.77)	0.59	0.60	4.47×10^{-3}	1.15×10^{-2}
			CC (vs TT)		1.03 (0.91,1.15)	0.81 (0.72,0.91)	0.60	0.61		1.12×10^{-2}
	>5 yr (vs < 1yr)		TC (vs TT)	0.43 (0.37,0.5)	1.01 (0.96,1.07)	0.48 (0.43,0.54)	0.43	0.44	2.43×10^{-2}	1.88×10^{-1}
			CC (vs TT)		1.03 (0.91,1.15)	0.55 (0.49,0.61)	0.44	0.45		1.72×10^{-1}
Parity	1-2 births (vs 0 birth)	**rs10962643**	AC (vs AA)	0.52 (0.44,0.61)	1.05 (0.96,1.15)	0.59 (0.5,0.68)	0.55	0.57	1.99×10^{-1}	7.45×10^{-1}
			CC (vs AA)		1.11 (0.93,1.33)	0.66 (0.57,0.77)	0.57	0.63		7.13×10^{-1}
	3+ births (vs 0 birth)		AC (vs AA)	0.38 (0.32,0.46)	1.05 (0.96,1.15)	0.48 (0.41,0.56)	0.41	0.44	2.86×10^{-3}	3.15×10^{-1}
			CC (vs AA)		1.11 (0.93,1.33)	0.61 (0.52,0.71)	0.43	0.49		2.41×10^{-1}
Parity	1-2 births (vs 0 birth)	**chr9:169151055**	GC (vs GG)	0.46 (0.36,0.58)	1.09 (0.98,1.22)	0.57 (0.47,0.7)	0.50	0.55	5.10×10^{-2}	6.73×10^{-1}
			CC (vs GG)		1.19 (0.95,1.49)	0.71 (0.58,0.87)	0.55	0.65		5.83×10^{-1}

	3+ births (vs 0 birth)		GC (vs GG)	0.33 (0.26,0.43)	1.09 (0.98,1.22)	0.46 (0.37,0.57)	0.36	0.42	1.25×10^{-3}	4.90×10^{-1}
			CC (vs GG)		1.19 (0.95,1.49)	0.63 (0.52,0.77)	0.40	0.52		3.27×10^{-1}
Type of HT	ET only (vs never)	**rs11658063**	CG (vs CC)	1.07 (0.91,1.27)	1.02 (0.98,1.07)	1.28 (1.14,1.44)	1.10	1.10	4.45×10^{-2}	1.88×10^{-2}
			GG (vs CC)		1.05 (0.96,1.14)	1.52 (1.24,1.86)	1.12	1.12		3.01×10^{-2}
	Any EPT (vs never)		CG (vs CC)	0.91 (0.8,1.03)	1.02 (0.98,1.07)	0.95 (0.86,1.04)	0.93	0.93	7.13×10^{-1}	7.03×10^{-1}
			GG (vs CC)		1.05 (0.96,1.14)	0.99 (0.85,1.15)	0.95	0.95		7.04×10^{-1}
Use of OCP	ever (vs never)	**rs9886651**	AG (vs AA)	0.71 (0.64,0.77)	1.13 (1.06,1.20)	0.73 (0.67,0.79)	0.80	0.83	1.97×10^{-2}	7.79×10^{-3}
			GG (vs AA)		1.27 (1.13,1.43)	0.75 (0.68,0.83)	0.90	0.98		9.90×10^{-3}

Abbreviation: SNP, single-nucleotide polymorphism; OR, odds ratio; OCP, oral contraceptive pills; HT, menopausal hormone therapy; ET, menopausal estrogen therapy; EPT, menopausal estrogen + progestin therapy; yr, year; Mult, multiplicative; Add, additive.

[a] Under multiplicative null, expected $OR_{joint} = OR_E * OR_{SNP}$; under additive null, expected $OR_{joint} = OR_E + OR_{SNP} - 1$, where $OR_E = \exp(\beta_E)$, $OR_{SNP} = \exp(\beta_{SNP})$ are estimated from logistic regression model with SNP, E variable, SNP x E variable.

[b] Wald test for individual multiplicative interaction

[c] 1-df Wald test corresponds to the test individual RERI term is zero or not.

All models were estimated from logistic regression model with SNP, E variable, SNP x E variable, assuming log-additive model (except for additive LRT which assumes dominant effect), using dosage data for imputed SNPs (except for additive $P_{interaction}$ which uses maximal probable genotypes for imputed SNPs), adjusted for reference age, race, education, family history, OCP use, tubal ligation, parity, BMI, endometriosis and study site and were performed on imputed datasets of G x E (9971 cases, 15566 controls) with proper pooling

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