**The association between reproductive and hormonal factors and ovarian cancer by
estrogen- α and progesterone receptor status**

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Abstract

Objective: We assessed the association between reproductive and hormonal factors and ovarian cancer incidence characterized by estrogen receptor- α (ER α) and progesterone receptor (PR) status.

Methods: Tissue microarrays were used to assess ER α and PR expression among 197 Nurses' Health Study (NHS), 42 NHSII and 76 New England Case-Control Study (NECC) ovarian cancer cases. NHS/NHSII cases were matched to up to 4 controls (n=954) on diagnosis date and birth year. NECC controls (n=725) were frequency matched on age. Cases were considered receptor positive if $\geq 1\%$ of tumor cells stained positive. Associations by ER α and PR status were assessed using polytomous logistic regression. P-value for heterogeneity was calculated using a likelihood ratio test.

Results: 45% of ovarian tumors were PR+, 78% were ER α + and 45% were ER α +/PR+, while 22% were ER α -/PR-. Postmenopausal status was associated with an increased risk of PR- tumors (OR: 2.07; 95%CI: 1.15-3.75; p-heterogeneity=0.01) and age at natural menopause was inversely associated with PR- tumors (OR, per 5yr: 0.77; 95%CI: 0.61-0.96; p-het=0.01). Increasing duration of postmenopause was differentially associated by PR status (p-het=0.0009). Number of children and tubal ligation were more strongly associated with ER α - versus ER α + tumors (p-het=0.002 and 0.05, respectively). No differential associations were observed for oral contraceptive or hormone therapy use.

Conclusions: Postmenopausal women have an increased risk of developing PR- ovarian tumors compared to premenopausal women. The associations observed for ovarian cancer differ from those seen for breast cancer suggesting that the biology for tumor development through ER α and PR pathways may differ.

Introduction

One challenge in understanding ovarian cancer etiology is accounting for tumor heterogeneity. Ovarian cancer histologic subtypes have different gene and protein expression patterns in addition to morphological differences.[1-4] For example, p53 mutations are observed in most high-grade serous ovarian tumors but rarely observed in other histologies.[1, 2] Further, ovarian cancer risk factor associations differ by histology.[5] The inverse association observed for parity and tubal ligation generally was stronger for endometrioid and clear cell ovarian tumors than serous tumors.[5-7] Differential associations for ovarian cancer risk factors with other tumor characteristics, including dominance and aggressiveness, have also been reported.[8, 9] An alternate approach is to characterize ovarian tumors by hormone receptor expression patterns, specifically estrogen receptor- α (ER α) and progesterone receptor (PR). It is well established that differences in ER α and PR expression are important in breast cancer epidemiology and prognosis;[10-12] however research on this topic is limited for ovarian tumors.

We previously reported differential risk factor associations by ER α and PR status in a small study of 157 ovarian tumors in the Nurses' Health Study (NHS).[13] Increasing age was positively associated with ER α + tumors but inversely associated with ER α - tumors while postmenopausal versus premenopausal women had a decreased risk of PR+ but an increased risk of PR- ovarian cancer. In this study, we updated our previous analysis including 40 additional ovarian tumors from NHS, 42 tumors from NHSII and 76 tumors from the New England Case-Control (NECC) study, more than doubling our previous sample size.

Methods

Study population

The NHS began in 1976 when 121,700 US female nurses aged 30-55 completed baseline questionnaires collecting data on disease diagnoses and exposures. In 1989, NHSII began when 116,430 US female nurses, aged 25-42, completed baseline questionnaires. Women were excluded from the cohorts if they were unmarried (NHS), had a history of cancer (except non-melanoma skin cancer and carcinoma in-situ) (NHSII), or did not reside in the 11 (NHS) or 14 (NHSII) states with the largest number of nurse registrants. In both cohorts, updated information on exposure and disease diagnoses were obtained through mailed biennial questionnaires. Incident cases of ovarian cancer were identified by biennial questionnaire, linkage to the National Death Index[14], the postal service or family members. Follow-up for both cohorts has been 85-90%. The Brigham and Women's Hospital institutional review board (IRB) approved both studies.

The NECC is a population-based case-control study in Eastern Massachusetts and New Hampshire conducted in five waves between 1978 and 2008.[15, 16] We included epithelial ovarian cancer cases and controls from the final wave (2003-2008) recruited within Eastern Massachusetts as tumor blocks were not available for cases in the earlier study waves. Cases were recruited through area hospital tumor registries and controls through town books. Exclusion criteria included: <18 years old, moved, had no phone, did not speak English, died, physician declined permission to contact them (cases only), or had a prior bilateral oophorectomy (controls only). In the 2003-2008 wave, 68.3% of eligible cases and 51.2% of eligible controls enrolled.[15, 16] Cases and controls were frequency matched on age. The Brigham and Women's Hospital and Dartmouth Medical School IRBs approved the study.

Ovarian tumor block collection

We included cases diagnosed from 1976 to 2006 for NHS, 1989 to 2005 for NHSII and 2003 to 2008 for NECC. A gynecologic pathologist, blinded to exposure status, reviewed pathology reports to confirm the epithelial ovarian cancer diagnosis and classify tumors by behavior (invasive and borderline), histologic type (serous/poorly differentiated, mucinous, endometrioid, clear cell, other) and stage (I/II, III/IV). We requested representative paraffin-embedded tissue blocks of ovarian tumors for NHS and NHSII confirmed cases with a pathology report and surgery and for NECC cases with an invasive tumor, no neoadjuvant chemotherapy, and surgery at Brigham and Women's Hospital, Boston, MA. Of the 1,083 confirmed NHS cases through 2006 and 201 NHSII confirmed cases through 2005, 217 NHS and 46 NHSII cases were included on tissue microarrays (TMAs). In NECC, 564 confirmed invasive cases were identified, of whom 78 were included on the TMA. For case exclusion details see Supplemental Figure S1. The major reasons tissue blocks were not obtained included: (1) blocks had been destroyed, (2) the case was deceased, and (3) budget constraints.[17] In NHS and NHSII, up to four controls (n=954) with no prior bilateral oophorectomy or menopause due to irradiation, no prior diagnosis of cancer (except non-melanoma skin cancer) and alive at the time of case diagnosis were selected and matched to cases on birth year. In NECC, controls recruited in Eastern Massachusetts from 20003-2008 (n=725) were included.

Assays

For each case, the pathologist (JH, MG) selected a primary tumor block, confirmed histology and behavior, assessed grade (1, 2, 3),[18] circled the tumor on the slide, and sent the block/slide to

the Dana-Farber/Harvard Cancer Center Specialized Histopathology Services Core for TMA construction. Three cores per case were extracted using 0.6 mm (NHS/NHSII) or 1 mm (NECC) diameter hollow needles. Cores were transferred to a recipient paraffin-embedded block and sections were cut to create array slides, which were processed and stained within 2 weeks. Details of the immunohistochemistry process have been described previously.[13] Briefly, slides were soaked in Xylene overnight, deparaffinized and antigens were retrieved and stained with the primary antibodies: ER α (rabbit monoclonal; clone SP1; Neomarkers; dilution 1:40) and PR (mouse monoclonal; clone PgR 636; Dako; dilution 1:150). ER α was detected using the Leica Bond III autostainer (Leica Biosystems) and PR was detected using biotin-free horseradish peroxidase enzyme-labeled polymer conjugated to anti-mouse secondary antibodies (Envision+ Systems; Dako). A pathologist (JH) assessed the number of reactive vs. total cells (0%, 1-10%, 11-50%, 51-90%, >90%). The three cores for each case were scored independently. Cores were designated non-interpretable if tissue was missing from the slide or only a few cell clusters (<20 cells) were present.

Assessment of exposure and covariate information

Exposure assessment details have been described previously.[13, 19] Exposure and covariate information was obtained from the biennial questionnaire cycle prior to case diagnosis for NHS and NHSII cases and their matched controls and from at least one year prior to case diagnosis/control interview in the NECC. We centrally harmonized the data into the following categories: age at diagnosis (years), age at menarche (years), oral contraceptive (OC) use (ever vs. never; never, >0-<1 year, 1-<5 years, 5-<10 years, 10+ years; continuous), tubal ligation (ever vs. never), parity (parous vs. nulliparous; nulliparous, 1-2 children, 3-4 children, 5+

children; continuous), menopausal status (premenopausal, postmenopausal), age at natural menopause (years), hormone therapy (HT) (ever vs. never; never, >0-<5 years, 5+ years, continuous), estrogen-only HT (ever vs. never; never, >0-<5 years, 5+ years, continuous), and family history of breast/ovarian cancer (yes vs. no). Age at natural menopause excluded women reporting a hysterectomy before menopause. Ovulatory years were calculated as age at natural menopause (current age for premenopausal women) minus age at menarche, 1 year for each pregnancy and OC duration. Duration of premenopause was calculated as current age, for premenopausal women, or age at natural menopause minus age at menarche, while duration of postmenopause was calculated as current age minus age at natural menopause (premenopausal women were coded as zero).

Statistical Analysis

As the ER α and PR distribution was similar across the studies, we pooled the data for all primary analyses with 197 NHS, 42 NHSII and 75 NECC cases included in the primary analyses. For case exclusion details in the primary analyses see Supplemental Figure S1. In primary analyses, ER α and PR status was considered positive if $\geq 1\%$ of cells stained positive and negative if 0% of cells stained positive based on the maximum score of the cores. The same cut point is used for breast cancer in clinical practice.[20] The intraclass correlation coefficient (ICC) across the three cores was 0.92 for ER α and 0.93 for PR. As in breast cancer, cases scored as ER α -/PR+ were excluded from analyses as ER α status is most likely misspecified among these cases.[21] The distribution of ER α and PR staining positivity by histologic subtype and stage were assessed using Chi-square test and grade was assessed using Chi-square test for trend. We calculated the Spearman's correlation between the maximum ER α and PR scores.

We assessed the relationship between reproductive and hormonal factors and ovarian cancer risk by receptor staining positivity using polytomous logistic regression (PLR) with a three category outcome (ER α +, ER α - and controls or PR+, PR- and controls). Analyses were also conducted using joint ER α /PR status (ER α +/PR+, ER α +/PR-, ER α -/PR-, and controls). For each exposure, we assessed the heterogeneity in the odds ratios (ORs) by ER α or PR expression status versus controls using a likelihood ratio test comparing a model where the association for the exposure of interest and ovarian cancer risk was allowed to vary by hormone receptor expression (i.e. unconstrained model) to another model where the association was not allowed to vary (i.e. constrained model).[22] We adjusted for age at diagnosis and cohort, allowing the estimates to vary by receptor status, as well as family history of breast and/or ovarian cancer, OC duration, number of pregnancies, and menopausal/HT status. Women missing OC duration were set to the median and a missing indicator was created.

To determine whether differential risk factor associations by ER α or PR status were explained by histology, we used unconditional logistic regression in case-case analyses, where receptor positive tumors were considered “cases” and receptor negative tumors were considered “controls”. We controlled for the same covariates above as well as histology (serous, endometrioid, clear cell, other) and used the methods of Jun *et al* (2010) to determine if histology explained a portion of the differential associations.[23] Briefly, a model without adjustment for histology and a model with adjustment for histology were used to calculate the mediation proportion defined as the proportion of excess ER α +/PR+ cases relative to ER α - or PR-cases that can be attributed to histology.

In sensitivity analyses, we used a 10% cutpoint for ER α and PR staining positivity, restricted to invasive cases, and accounted for grade (grade 1/2, grade 3, unknown grade) in case-case analyses. Between-cohort heterogeneity was tested using a likelihood ratio test including interaction terms between the exposure of interest and indicators for cohort.

To assess for selection bias, we compared the distribution of reproductive/hormonal factors and tumor characteristics between confirmed ovarian cancer cases, cases eligible for tumor block collection, cases with collected tumor blocks and cases included on the TMA.

Analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA.) or Stata version 11.0 (StataCorp LP, College Station, TX, USA). P-values were 2-sided and considered statistically significant if less than 0.05.

Results

Tumor characteristics

We had 315 cases (NHS=197, NHSII=42, NECC=76) and 1,679 controls (NHS=786, NHSII=168, NECC=725). 78% of ovarian tumors expressed ER α while 45% expressed PR (Table 1); 45% were ER α +/PR+, 32% were ER α +/PR- and 22% were ER α -/PR-. The modest correlation between ER α and PR expression (ρ =0.42; p <0.0001) differed by histology (0.19 for serous to 0.64 for mucinous tumors). The distribution of ER α and PR expression varied significantly by histology (p <0.0001). A greater proportion of serous and endometrioid tumors expressed ER α and PR versus clear cell and mucinous tumors. The distribution of joint ER α and

PR expression differed by histology; the majority of serous tumors were either ER α + /PR+ (50%) or ER α + /PR- (39%) while the majority of clear cell tumors were ER α - /PR- (84%) (Supplemental Table S1). PR expression also differed by grade ($p < 0.0001$), with lower grade tumors more likely to be PR+ and Grade 3 tumors more likely to be PR-. High stage tumors were more likely to be ER α + compared to ER α - ($p < 0.0001$).

Comparison to full sample

Confirmed NHS and NHSII cases, cases eligible for tumor block collection, cases with tumor blocks and TMA cases were similar with respect to reproductive/hormonal factors and tumor characteristics except that TMA cases were older at diagnosis, had lower stage cancer, and a shorter lapse time from diagnosis date to tissue request date (Supplemental Tables S2 and S3). A greater proportion of NHSII TMA cases were parous compared to confirmed cases (76% vs. 70%, respectively); however, the mean number of children among parous women was similar. All confirmed invasive NECC cases, cases eligible for block collection, cases with tumor blocks, and TMA cases were similar with respect to reproductive/hormonal factors and tumor characteristics, except that TMA cases were slightly older and had higher stage tumors (Supplemental Table S4).

Reproductive and hormonal factors by ER α expression

We observed significant heterogeneity between ER α + and ER α - tumors for number of children and tubal ligation, with stronger protective associations for ER α - versus ER α + tumors (Table 2). Each additional child was associated with a 28% decreased risk of ER α - (95%CI=0.60–0.87) compared to a non-significant 3% decreased risk of ER α + tumors (95%CI=0.89–1.06; p -

heterogeneity=0.002). Histology explained approximately 82% of the differential association between number of children and ER α status (p=0.03) (Supplemental Table S5). For tubal ligation, there was a stronger protective effect for ER α - (OR=0.25; 95%CI=0.09–0.69) versus ER α + tumors (OR=0.65; 95%CI=0.43–0.97; p-het=0.05). Histology did not explain this differential association. ER α status was not associated with age at diagnosis (p=0.37). No significant heterogeneity by ER α status was observed for OC use, HT use, age at menarche, or menopause-related variables (p-het $\geq$ 0.07; Table 2).

Reproductive and hormonal factors by PR expression

Factors relating to menopausal status were differentially associated by PR status (Table 2). Postmenopausal (versus premenopausal) women had a 2-fold increased risk of PR- tumors (OR=2.07; 95%CI=1.15–3.75) and a non-significant decreased risk of PR+ tumors (OR=0.77; 95%CI=0.43–1.39; p-het=0.01). Each five year increase in age at natural menopause was associated with a non-significant increased risk of PR+ tumors (OR=1.23; 95%CI=0.89–1.72), but a significant decreased risk of PR- tumors (OR=0.77; 95%CI=0.61–0.96; p-het=0.01). Duration of postmenopause was associated with a borderline decreased risk of PR+ tumors and increased risk of PR- tumors (p-het=0.0009). Duration of premenopause was not differentially associated by PR status (p-het=0.19). A stronger positive association was observed for ovulatory years with PR+ versus PR- tumors (p-het=0.05). Accounting for histology did not significantly alter the risk estimates (Supplemental Table S5). In case-case analyses, age at diagnosis was differentially associated by PR status (p=0.04). Women aged $\geq$ 55 years versus <55 were 73% (95%CI=1.03–2.93) more likely to develop PR- versus PR+ tumors. No significant

heterogeneity by PR status was observed for parity, OC use, HT use, tubal ligation, or age at menarche ($p\text{-het}>0.16$).

ER/PR joint analyses

There was significant heterogeneity between number of children and joint ER α and PR expression ($p\text{-het}=0.002$; Table 3). Increasing number of children was associated with a significantly protective effect for ER α -/PR- tumors (OR=0.72; 95%CI=0.60–0.86), but not for ER α +/PR+ or ER α +/PR- tumors. Significant heterogeneity was observed for menopausal status and age at natural menopause with joint ER α and PR expression ($p\text{-het}=0.03$ and 0.05, respectively). Postmenopausal status was positively associated with both ER α +/PR- tumors (OR=2.51; 95%CI=1.06–5.96) and ER α -/PR- tumors (OR=1.91; 95%CI=0.84–4.33) but with a non-significant decreased risk of ER α +/PR+ tumors. Each five year increase in duration of postmenopause was associated with a borderline decreased risk of ER α +/PR+ tumors (OR=0.85; 95%CI=0.73–1.00), but a borderline increased risk of ER α +/PR- tumors (OR=1.14; 95%CI=0.99–1.32; $p\text{-het}=0.003$).

Sensitivity analyses

Results obtained using the 10% cutpoint were similar to those using the 1% cutpoint for staining positivity (data not shown). Significant heterogeneity was observed between cohorts for HT ($p\text{-het}=0.005$ for ER α and 0.001 for PR), estrogen-only HT ($p\text{-het}=0.02$ for ER α and 0.003 for PR), and duration of postmenopause ($p\text{-het}=0.01$ for ER α). All other p -heterogeneity values were >0.07 . The heterogeneity was driven by NECC and when considering NHS/NHSII only, the associations were similar to the main findings (data not shown). Adjustment for grade did not

substantially alter the main findings (data not shown). Generally results were similar for invasive cases, except that age at menarche was differentially associated with ovarian cancer by ER α status ($p=0.04$), with an increased risk of ER α - tumors (OR=1.20; 95%CI=1.03–1.40), and there was significant heterogeneity for ovulatory years by PR status, with a stronger positive association for PR+ versus PR- tumors (OR=1.40; 95%CI=1.16-1.69 for PR+ vs. OR=1.06; 95%CI=0.91-1.24 for PR-; p -het=0.02) (data not shown).

Discussion

In the current study, we observed a modest correlation between ER α and PR expression for ovarian tumors, with 45% of the ovarian tumors expressing both ER α and PR; however the proportion greatly varied by histology. Factors relating to menopause timing were differentially associated with ovarian cancer by PR expression and appeared to be independent of ER α status. The results suggested that postmenopausal women had an increased risk of PR- ovarian tumors compared to premenopausal women. Tubal ligation and number of children were differentially associated by ER α expression although the association for number of children appeared to be explained by histology, specifically variations in ER α expression by endometrioid and clear cell tumors.

The one previous study on this topic was conducted among 157 NHS ovarian cancer cases (also included in this analysis), using a 10% cutpoint to define positivity.[13] The results were similar for menopausal status, including the increased risk of PR- tumors among postmenopausal versus premenopausal women. However, the prior study observed a differential association of age by ER α expression; possibly due to the increased sensitivity of the ER α antibody used in the current

study. The majority (94%) of cores stained positive for ER α using the previous and the new antibodies; however, 48% that stained negative with the previous antibody were classified as ER α + with the new antibody.

The ER α and PR distributions within our study are similar to other studies, although the proportion of ER α + / PR+ ovarian tumors was slightly higher in our study,[24, 25] possibly due to differences in the cutpoint used to define positivity and the antibodies used. One consistent finding across studies is the small proportion of clear cell ovarian tumors that express either ER α or PR.[13, 26-28] Additionally, we observed that a higher proportion of PR- ovarian tumors were high grade compared to PR+ tumors; consistent with the finding that women with PR- tumors have worse survival compared to women with PR+ tumors.[28] In comparison to breast cancer, combined ER α + / PR+ expression is lower among ovarian tumors; approximately 30-45% of ovarian tumors and 50-65% of breast tumors express both hormone receptors.[13, 24, 25, 29, 30] PR expression is a downstream marker of ER activation by estrogen[31] and given that many ovarian tumors are PR- suggests that a large proportion of these tumors are not estrogen sensitive. Additionally, while basal-like breast tumors are molecularly similar to high-grade serous ovarian tumors (i.e. both characterized by *p53* mutations and low frequencies of *PTEN* mutations), hormone receptor expression patterns differ between the two.[32, 33] Approximately 55-85% of basal-like breast tumors are ER α - / PR-,[34] while only 11% of high-grade serous tumors were ER α - / PR- in our study. Overall, this suggests that the underlying biology of tumor development through hormone receptor pathways may differ between breast and ovarian cancers. In ovarian tumors, the loss of PR function may be an important marker of tumor progression.

These results underline the importance of further investigating ER α and PR regulation in ovarian cancer.

Factors relating to menopause timing had differential associations by PR expression. Postmenopausal women were more likely to develop PR- and less likely to develop PR+ tumors compared to premenopausal women and later age at natural menopause was inversely associated with PR- tumors, irrespective of ER expression. Increasing duration of postmenopause was associated with a greater risk of developing PR- versus PR+ tumors, particularly ER+/PR- tumors, suggesting that ER signaling dysregulation and/or PR loss may be important drivers in ovarian cancer development among postmenopausal women. Conversely, in breast cancer premenopausal women tend to develop PR- tumors while postmenopausal women tend to develop PR+ tumors[11, 29] suggesting that the mechanism leading to PR loss or ER dysregulation differs between ovarian and breast cancer.

The biologic mechanism underlying the increase in PR- ovarian tumors among postmenopausal women is not clear. Ovarian cancer cell lines and primary tissue cultures tend not to express PR.[35] One hypothesis is that lower hormone levels among postmenopausal women may lead to ER α dysregulation leading to PR loss; however, results are conflicting on PR regulation by estrogen in ovarian cancer cells. In mouse xenografts with PE04 (ER α +/PR+) ovarian cancer cells, administration of estrogen up-regulated PR expression,[36] but estradiol led to the downregulation of PR expression in two other ER+/PR+ ovarian cancer cell lines.[37] A second hypothesis is that lower hormone levels among postmenopausal women leads to less ER α +/PR+ tumors promotion. In ovariectomized mice implanted with ER α +/PR+ PE04 ovarian cancer cells,

administration of estradiol versus vector led to increased tumor growth,[36] suggesting that decreases in estradiol may slow the progression of ER α + /PR+ tumors among postmenopausal women. Finally, other changes occurring after menopause may affect PR expression of ovarian tumors including higher gonadotropin levels and lower progesterone levels. Future research should investigate the role of ER α dysregulation and PR loss in ovarian tumor development after menopause and the role of estrogen in promoting tumors in both the pre- and post-menopausal periods.

Number of children and tubal ligation were differentially associated with ovarian cancer by ER α status. For both, we observed a stronger protective association for ER α - tumors compared to ER α + tumors. However, the differential association observed for parity was likely due to the differential distribution of ER α expression by histology. The protective association for parity appears to be strongest for endometrioid and clear cell ovarian tumors,[7] however, clear cell tumors are predominantly ER α -, whereas endometrioid tumors are predominantly ER α +. The results for number of children and ER α expression were the opposite as for breast cancer, where parity is inversely associated with ER α + /PR+ tumors but either positively or not associated with ER α - /PR- tumors.[11, 29]

While tubal ligation has been most strongly associated with endometrioid and clear cell tumors,[38] accounting for histology did not explain the stronger association of tubal ligation with preventing ER α - tumors. Endometrioid and clear cell cancers may develop from endometriosis precursors and tubal ligation may prevent ovarian cancer by blocking endometriosis from reaching the ovary.[1, 39] Akahane *et al* 2005 observed that endometriosis

and atypical endometriosis that developed into clear cell cancer appeared to undergo a gradual reduction in ER α expression with malignant transformation.[40] The opposite was observed for endometrioid tumors in which there was up-regulation of ER α with malignant transformation. This suggests that ER α loss may be an early step in the clear cell tumor developmental process. Future research should investigate factors that may influence ER α loss and the significance of such loss in clear cell tumor development.

Our study was based on a modest sample size, especially for the histology and joint ER α /PR analyses, limiting our power to detect associations and our results should be replicated in a larger study. We assessed several exposures and may have observed significant results by chance. If we had used Bonferroni correction for the 12 exposures we investigated, the significance threshold would be 0.004 at which the differential association for duration of postmenopause and PR status would still be significant. As ER α and PR expression are continuous, the use of a dichotomous score may have been an oversimplification. However, we observed similar results with a 10% cutpoint. One major strength of our study is that exposure information in both the NHS and NHSII studies were collected prospectively. The NECC study could be subject to recall bias as cases and controls were asked to recall past exposures, although results were similar when considering the NHS/NHSII alone. A further strength was the use of TMAs allowing for the simultaneous staining of many cases, reducing variability in results due to assay variation over time.

This study's results highlight that the development of ovarian and breast cancers through ER α and PR pathways is potentially different and future research should be directed at understanding

how ER α and PR expression influence ovarian carcinogenesis. Our results suggest that loss of PR function may be important in ovarian tumor development particularly among older, postmenopausal women. Future research should focus on the factor(s) driving loss of PR expression among postmenopausal women, the possibility of ER α dysregulation in the ovarian tumor development , and the possibility that ER β as well as ER α may play a role in ovarian carcinogenesis.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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Table Legends

Table 1. Distribution of ovarian cancer cases by estrogen- α (ER α) and progesterone (PR) receptor status in the Nurses' Health Study, Nurses' Health Study II and New England Case-Control Study

Table 2. Multivariate association between reproductive and hormonal factors and ovarian cancer by estrogen- α and progesterone receptor status in the NHS, NHSII and NECC using 1% as the cutpoint for staining positivity

Table 3. Association between reproductive and hormonal factors and ovarian cancer by joint ER α and PR status in the NHS, NHSII and NECC using 1% as the cutpoint for staining positivity

Table 1. Distribution of ovarian cancer cases by estrogen- α (ER α) and progesterone (PR) receptor status in the Nurses' Health Study, Nurses' Health Study II and New England Case-Control Study

	All cases	ER α + ^a	ER α -	PR+ ^a	PR-
Total, n (%) ^b	315 (100%)	245 (78%)	70 (22%)	143 (45%)	172 (55%)
Histology, n (%)					
Serous	195 (62%)	174 (71%)	21 (30%)	98 (69%)	97 (56%)
Endometrioid	51 (16%)	43 (18%)	8 (11%)	30 (21%)	21 (12%)
Mucinous	16 (5%)	8 (3%)	8 (11%)	5 (4%)	11 (6%)
Clear cell	25 (8%)	4 (2%)	21 (30%)	1 (1%)	24 (14%)
Other	28 (9%)	16 (7%)	12 (17%)	9 (6%)	19 (11%)
<i>P</i> ^c			<0.0001		<0.0001
Grade, n (%) ^d					
I	26 (10%)	24 (12%)	2 (3%)	20 (17%)	6 (4%)
II	54 (20%)	40 (19%)	14 (22%)	32 (27%)	22 (14%)
III	190 (70%)	143 (69%)	47 (75%)	66 (56%)	124 (82%)
<i>P</i> ^e			0.14		<0.0001
Stage, n (%)					
I/II	117 (37%)	77 (31%)	40 (57%)	57 (40%)	60 (35%)
III/IV	198 (63%)	168 (69%)	30 (43%)	86 (60%)	112 (65%)
<i>P</i>			<0.0001		0.36

a - Ovarian tumors were classified as ER α + and PR+ if 1% or greater of cells stained positive

b - Total N excludes 16 cases with unreadable ER α and PR staining and 10 cases scored as ER α -/PR+

c - P-value calculated using Chi-square test comparing ER α + vs. ER α - and PR+ vs. PR-

d - 270 cases had data on grade

e - P-value calculated using Chi-square test for trend

Table 2. Multivariate association between reproductive and hormonal factors and ovarian cancer by estrogen- α and progesterone receptor status in the NHS, NHSII and NECC using 1% as the cutpoint for staining positivity^a

		OR (95% CI)				OR (95% CI)			
	Controls	Cases	ERα+	ERα-	<i>P</i> -hetero	PR+	PR-	<i>P</i> -hetero	
Parity									
Nulliparous	174	45	1.00 (Ref)	1.00 (Ref)	0.23	1.00 (Ref)	1.00 (Ref)	0.72	
Parous	1504	269	0.59 (0.38, 0.91)	0.37 (0.19, 0.71)		0.56 (0.33, 0.94)	0.49 (0.30, 0.80)		
Nulliparous	174	45	1.00 (Ref)	1.00 (Ref)	0.01	1.00 (Ref)	1.00 (Ref)	0.73	
1-2 children	732	136	0.62 (0.39, 0.98)	0.55 (0.29, 1.06)		0.60 (0.35, 1.04)	0.60 (0.36, 1.00)		
3-4 children	625	93	0.50 (0.31, 0.81)	0.16 (0.07, 0.38)		0.47 (0.26, 0.84)	0.34 (0.20, 0.60)		
5+ children	147	40	0.81 (0.46, 1.44)	0.26 (0.09, 0.78)		0.70 (0.34, 1.46)	0.59 (0.31, 1.15)		
<i>Per child</i>	1678	314	0.97 (0.89, 1.06)	0.72 (0.60, 0.87)	0.002	0.91 (0.81, 1.02)	0.93 (0.84, 1.03)	0.77	
Oral Contraceptive (OC) use									
Never	650	154	1.00 (Ref)	1.00 (Ref)	0.28	1.00 (Ref)	1.00 (Ref)	0.60	
Ever	1029	161	0.75 (0.56, 1.01)	0.55 (0.32, 0.93)		0.75 (0.52, 1.10)	0.66 (0.47, 0.94)		
Never ^b	650	154	1.00 (Ref)	1.00 (Ref)	0.41	1.00 (Ref)	1.00 (Ref)	0.81	
>0-<1 year	187	34	0.79 (0.50, 1.26)	0.59 (0.25, 1.39)		0.86 (0.49, 1.53)	0.65 (0.37, 1.15)		
1-<5 years	372	72	0.97 (0.67, 1.39)	0.55 (0.27, 1.10)		0.99 (0.63, 1.56)	0.76 (0.49, 1.18)		
5-<10 years	246	32	0.57 (0.34, 0.93)	0.62 (0.28, 1.36)		0.55 (0.29, 1.03)	0.62 (0.35, 1.07)		
10+ years	192	14	0.30 (0.15, 0.61)	0.47 (0.18, 1.26)		0.33 (0.14, 0.79)	0.36 (0.17, 0.77)		
<i>Per 5 years^b</i>	1647	306	0.68 (0.55, 0.85)	0.78 (0.56, 1.09)	0.51	0.72 (0.55, 0.92)	0.70 (0.55, 0.90)	0.91	
Tubal ligation									
Never	1345	279	1.00 (Ref)	1.00 (Ref)	0.05	1.00 (Ref)	1.00 (Ref)	0.51	
Ever	334	36	0.65 (0.43, 0.97)	0.25 (0.09, 0.69)		0.62 (0.37, 1.03)	0.49 (0.29, 0.83)		
Menopausal status at diagnosis ^b									
Premeno	480	69	1.00 (Ref)	1.00 (Ref)	0.23	1.00 (Ref)	1.00 (Ref)	0.01	
Postmeno	1134	230	1.10 (0.68, 1.77)	1.93 (0.85, 4.37)		0.77 (0.43, 1.39)	2.07 (1.15, 3.75)		
Hormone therapy (HT) use ^{b,c}									
Never	574	83	1.00 (Ref)	1.00 (Ref)	0.78	1.00 (Ref)	1.00 (Ref)	0.35	
Ever	511	130	1.62 (1.14, 2.31)	1.78 (0.98, 3.26)		1.39 (0.86, 2.25)	1.84 (1.25, 2.73)		

Never	574	83	1.00 (Ref)	1.00 (Ref)	0.74	1.00 (Ref)	1.00 (Ref)	0.44
>0-<5 years	272	54	1.16 (0.75, 1.81)	1.53 (0.76, 3.08)		1.18 (0.66, 2.10)	1.30 (0.81, 2.11)	
5+ years	238	76	2.20 (1.47, 3.31)	2.16 (1.05, 4.44)		1.68 (0.95, 2.96)	2.57 (1.64, 4.02)	
<i>Per 5 years</i>	1084	213	1.43 (1.24, 1.64)	1.17 (0.88, 1.54)	0.16	1.40 (1.17, 1.68)	1.36 (1.17, 1.58)	0.80
Estrogen-only HT use^{b,c,d}								
Never	835	132	1.00 (Ref)	1.00 (Ref)	0.35	1.00 (Ref)	1.00 (Ref)	0.83
Ever	215	72	2.24 (1.53, 3.27)	1.59 (0.82, 3.11)		2.17 (1.29, 3.65)	2.02 (1.34, 3.06)	
Never	835	132	1.00 (Ref)	1.00 (Ref)	0.77	1.00 (Ref)	1.00 (Ref)	0.95
>0-<5 years	115	32	1.75 (1.06, 2.89)	1.41 (0.60, 3.32)		1.76 (0.89, 3.49)	1.61 (0.93, 2.77)	
5+ years	98	37	2.66 (1.64, 4.33)	1.96 (0.81, 4.71)		2.68 (1.38, 5.21)	2.38 (1.39, 4.07)	
<i>Per 5 years</i>	1048	201	1.48 (1.25, 1.74)	1.21 (0.86, 1.71)	0.25	1.49 (1.20, 1.85)	1.39 (1.16, 1.67)	0.57
Age at menarche								
<i>Per year</i>	1669	312	0.99 (0.90, 1.09)	1.16 (0.99, 1.35)	0.07	0.97 (0.86, 1.09)	1.08 (0.97, 1.20)	0.16
Age at natural menopause^{b,c}								
<i>Per 5 years</i>	996	169	0.96 (0.77, 1.20)	0.74 (0.52, 1.04)	0.20	1.23 (0.89, 1.72)	0.77 (0.61, 0.96)	0.01
Duration of premenopause^{b,e}								
<i>Per 5 year</i>	1466	236	1.08 (0.92, 1.27)	0.93 (0.72, 1.19)	0.29	1.14 (0.93, 1.41)	0.97 (0.81, 1.16)	0.19
Duration of postmenopause^{b,e}								
<i>Per 5 year</i>	1466	236	0.99 (0.90, 1.12)	1.07 (0.90, 1.27)	0.40	0.85 (0.73, 1.00)	1.11 (0.98, 1.26)	0.0009
Ovulatory years in quartiles^{b,f}								
Quartile 1	359	31	1.00 (Ref)	1.00 (Ref)	0.28	1.00 (Ref)	1.00 (Ref)	0.05
Quartile 2	365	71	2.34 (1.39, 3.95)	2.10 (0.89, 4.96)		1.60 (0.85, 3.02)	3.19 (1.68, 6.08)	
Quartile 3	334	57	2.04 (1.17, 3.55)	2.01 (0.82, 4.91)		1.85 (0.95, 3.59)	2.41 (1.22, 4.76)	
Quartile 4	381	70	2.57 (1.50, 4.39)	1.19 (0.45, 3.12)		2.59 (1.37, 4.90)	2.03 (1.02, 4.03)	
<i>Per 5 years</i>	1439	229	1.19 (1.05, 1.36)	1.05 (0.84, 1.31)	0.33	1.28 (1.08, 1.52)	1.07 (0.92, 1.25)	0.10

a - Analyses were adjusted for age at diagnosis (per year), cohort (NHS/NHSII/NECC), family history of breast/ovarian cancer (yes/no), duration of OC use (per month), menopausal status (premenopausal/postmenopausal ever HT/postmenopausal never HT), and number of pregnancies (continuous)

b - Total N does not add up to 1994 due to missingness in the exposure (parity missing=2; age at menarche missing=13; OC missing=41;

menopause missing=81; ever HT use missing=66; duration HT use miss=67; E-only HT use missing=110; duration E-only HT use missing=115; age at natural menopause missing=199; duration of post/pre-menopause missing=292; ovulatory years missing=326)

c - Among postmenopausal women

d - In ever/never analyses additionally adjusted for ever estrogen plus progestin HT use (yes/no), ever other HT use (yes/no) and in duration analyses additionally adjusted for duration of estrogen plus progestin HT use and duration of other HT use in duration analyses

e – Not adjusted for age at diagnosis

f - Ovulatory year was calculated as age at natural menopause (or age at diagnosis for premenopausal women) minus age at menarche with additional subtraction of one year of each pregnancy and OC duration

Table 3. Association between reproductive and hormonal factors and ovarian cancer by joint ERα and PR status in the NHS, NHSII and NECC using 1% as the cutpoint for staining positivity^a

		ERα+/PR+			ERα+/PR-		ERα-/PR-		<i>P-hetero</i>
Controls		N	OR (95% CI)	N	OR (95% CI)	N	OR (95% CI)		
Parity									
	Never	174	21	1.00 (Ref)	10	1.00 (Ref)	14	1.00 (Ref)	0.44
	Ever	1504	122	0.56 (0.33, 0.94)	91	0.66 (0.33, 1.33)	56	0.37 (0.19, 0.71)	
	<i>Per child</i>	1678	143	0.90 (0.80, 1.02)	101	1.05 (0.93, 1.18)	70	0.72 (0.60, 0.86)	0.002
Oral Contraceptive (OC) use									
	Never	650	63	1.00 (Ref)	55	1.00 (Ref)	36	1.00 (Ref)	0.55
	Ever	1029	80	0.75 (0.51, 1.10)	47	0.76 (0.49, 1.17)	34	0.55 (0.32, 0.93)	
	<i>Per 5 years^b</i>	1647	139	0.72 (0.55, 0.92)	98	0.63 (0.45, 0.90)	69	0.78 (0.56, 1.09)	0.69
Tubal ligation									
	Never	1345	124	1.00 (Ref)	89	1.00 (Ref)	66	1.00 (Ref)	0.14
	Ever	334	19	0.62 (0.37, 1.03)	13	0.71 (0.39, 1.30)	4	0.25 (0.09, 0.69)	
Menopausal status at diagnosis ^b									
	Premeno	480	44	1.00 (Ref)	9	1.00 (Ref)	16	1.00 (Ref)	0.03
	Postmeno	1134	89	0.77 (0.43, 1.39)	89	2.51 (1.06, 5.96)	52	1.91 (0.84, 4.33)	
Hormone therapy (HT) use ^{b,c}									
	Never	574	35	1.00 (Ref)	29	1.00 (Ref)	19	1.00 (Ref)	0.63
	Ever	511	45	1.39 (0.86, 2.25)	53	1.89 (1.16, 3.08)	32	1.78 (0.98, 3.26)	
	<i>Per 5 years</i>	1084	80	1.40 (1.17, 1.68)	82	1.45 (1.22, 1.71)	51	1.17 (0.88, 1.54)	0.36
Estrogen-only HT use ^{b,c,d}									
	Never	835	49	1.00 (Ref)	48	1.00 (Ref)	35	1.00 (Ref)	0.64
	Ever	215	27	2.17 (1.29, 3.64)	31	2.31 (1.40, 3.81)	14	1.59 (0.82, 3.11)	
	<i>Per 5 years</i>	1048	75	1.49 (1.21, 1.85)	77	1.46 (1.19, 1.79)	49	1.21 (0.86, 1.71)	0.50
Age at menarche									
	<i>Per year</i>	1669	141	0.97 (0.86, 1.09)	101	1.02 (0.89, 1.17)	70	1.16 (1.00, 1.35)	0.23
Age at natural menopause ^{b,c,e}									

<i>Per 5 years</i>	950	65	1.21 (0.87, 1.69)	64	0.78 (0.59, 1.03)	35	0.72 (0.51, 1.03)	0.05
Duration premenopause ^{b,f}								
<i>Per 5 years</i>	1466	110	1.14 (0.93, 1.41)	72	1.00 (0.79, 1.26)	54	0.93 (0.72, 1.20)	0.38
Duration postmenopause ^{b,f}								
<i>Per 5 years</i>	1466	110	0.85 (0.73, 1.00)	72	1.14 (0.99, 1.32)	54	1.06 (0.89, 1.27)	0.003
Ovulatory years in quartiles ^{b,g}								
Quartile 1	359	18	1.00 (Ref)	5	1.00 (Ref)	8	1.00 (Ref)	0.09
Quartile 2	365	26	1.60 (0.84, 3.01)	27	4.95 (1.86, 13.1)	18	2.10 (0.89, 4.95)	
Quartile 3	334	25	1.85 (0.95, 3.59)	16	3.08 (1.10, 8.67)	16	2.01 (0.82, 4.91)	
Quartile 4	381	38	2.59 (1.37, 4.89)	21	3.35 (1.22, 9.22)	11	1.19 (0.45, 3.12)	
<i>Per 5 years</i>	1439	107	1.28 (1.08, 1.52)	69	1.09 (0.89, 1.34)	53	1.05 (0.84, 1.31)	0.27

a -Analyses were adjusted for age at diagnosis (per year), cohort (NHS/NHSII/NECC), family history of breast/ovarian cancer (yes/no), duration of OC use (per month), menopausal status (premenopausal/postmenopausal ever HT/postmenopausal never HT), and number of pregnancies (continuous)

b - Total N does not add up to 1994 due to missingness in the exposure

c - Among postmenopausal women

d - In ever/never analyses additionally adjusted for ever estrogen plus progestin HT use (yes/no), ever other HT use (yes/no) and in duration analyses additionally adjusted for duration of estrogen plus progestin HT use and duration of other HT use in duration analyses

e - Excludes NHSII cases and controls due to small sample size

f - Not adjusted for age at diagnosis

g - Ovulatory year was calculated as age at natural menopause (or age at diagnosis for premenopausal women) minus age at menarche with additional subtraction of one year of each pregnancy and OC duration