

Relationship between Epidemiologic Risk Factors and Hormone Receptor Expression in Ovarian Cancer: Results from the Nurses' Health Study

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Abstract

Hormone receptor expression in tumors may offer etiologic information for ovarian cancer, particularly in light of known associations with hormonal and reproductive risk factors. Tissue microarrays constructed from 157 paraffin-embedded blocks of epithelial ovarian tumors collected from participants in the Nurses' Health Study were stained for estrogen receptor- α (ER α) and progesterone receptor (PR). We examined receptor expression by invasion, grade, and histologic subtype. Multivariate unconditional logistic regression was used to evaluate whether hormonal, reproductive, and anthropometric risk factors were differentially associated with the risk of developing receptor-positive or receptor-negative ovarian tumors compared with controls. PR-expressing tumors were less likely to be invasive ($P = 0.05$) and more likely to be of a lower grade ($P < 0.001$) and stage ($P = 0.007$)

compared with PR- tumors. ER α status was not associated with any pathologic features of the tumor ($P > 0.34$). Increasing age, being postmenopausal, and postmenopausal hormone use were associated with an increased risk of developing ER α +, but not ER α - ($P_{\text{heterogeneity}} = 0.001, 0.06, \text{ and } 0.06$, respectively) and PR-, but not PR+, tumors ($P_{\text{heterogeneity}} = 0.08, 0.003, \text{ and } 0.40$, respectively), whereas height was only associated with the risk of developing PR- disease ($P_{\text{heterogeneity}} = 0.08$). There were no clear risk differentials with OC use, parity, body mass index, or physical activity. Reproductive and hormonal risk factors are associated with subgroups of ovarian cancer defined by histologic subtype or ER α and PR status. These findings support specific models of hormone mediated triggers of ovarian cancer. (Cancer Epidemiol Biomarkers Prev 2009;18(5):1624-30)

Introduction

The role of estrogen and progesterone in the development of epithelial ovarian cancer is poorly understood. Several reproductive-hormonal factors [oral contraceptive use (OC), parity, and breastfeeding] are associated with decreased risk of ovarian cancer (1), whereas prolonged postmenopausal hormone (PMH) use, particularly estrogen only, increases risk (2-5). The effect of life-style factors, including diet and physical activity are unclear (6).

The risk of developing ovarian cancer due to hormone exposures may depend on hormonal receptor expression in the tumor. For breast cancer, reproductive factors and postmenopausal obesity are associated with only the risk of hormone receptor-positive tumors (7). Such a relationship has not been investigated for ovarian cancer. In addition, hormonal effects may vary by histologic classification (8, 9) or the pathway of carcinogenesis. Ovarian cancers may arise directly from a flat fallopian

tube or ovarian cortical precursor lesion, as well as through a benign epithelial proliferation such as endometriosis or cystadenoma (10). It is plausible that sex steroids play different roles in these processes (11, 12).

We used immunohistochemical staining of a tissue microarray (TMA) to quantify estrogen receptor (ER) α and progesterone receptor (PR) expression in 157 epithelial ovarian tumor samples obtained from the Nurses' Health Study (NHS), a large prospective cohort study. We evaluated hormone receptor staining by the presence of invasion, histologic subtype, and grade. We also investigated whether various hormonal, reproductive, and anthropometric risk factors differed by ER α and PR tumor expression, and whether these exposures are associated with the development of receptor positive or negative ovarian tumors compared with controls.

Materials and Methods

Study Population. The NHS was initiated in 1976, when 121,700 U.S. female registered nurses age 30 to 55 y completed a self-administered questionnaire about various risk factors for disease (13-15). Study participants have been followed biennially by questionnaire to update exposure status and disease diagnoses. Follow-up for this cohort of women was >95% through 2002.

Assessment of Exposure and Covariate Information. Information on the exposure measures and potential

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confounding variables, including body mass index (BMI), reproductive history, and PMH use was asked at baseline and updated biennially. OC use was asked from baseline through 1982, by which time use was rare because of the cohort's age distribution. Details regarding PMH use were asked on each biennial questionnaire including current use (within last month), duration of use, and type of hormones taken. Parity was defined as the number of pregnancies lasting ≥ 6 mo, and was asked through 1984. Information on height and weight at age 18 y was obtained at baseline in 1976. Current weight was updated every 2 y. BMI was calculated as weight in kilograms divided by the square of height in meters for age 18 y and at all follow-up cycles. Tubal ligation was asked from 1976 through 1984 and in 1994. Family history of breast cancer was asked in 1982, 1988, and every 4 y thereafter, whereas family history of ovarian cancer was first asked in 1992 and updated every 4 y. Physical activity was asked every 2 to 4 y starting in 1986 and reported as metabolic equivalent hours per week (MET-h/wk). We classified women as postmenopausal from the time of natural menopause or hysterectomy with bilateral oophorectomy. Women who underwent hysterectomy without bilateral oophorectomy were considered postmenopausal when they reached the age at which natural menopause had occurred in 90% of the cohort (54 y for smokers, 56 y for nonsmokers).

Ascertainment of Cases, Controls, and Ovarian Tumor Block Collection. Incident cases of epithelial ovarian cancer were identified by biennial questionnaire from 1976 to 2002. For women reporting a new ovarian cancer or cases identified via death certificate (16), we obtained pathology reports and related medical records. A gynecologic pathologist (JH), unaware of exposure status, reviewed the records to confirm the diagnosis and to classify cancers by invasion (invasive versus borderline), histologic type (serous/poorly differentiated, mucinous, endometrioid, clear cell, or other, including Brenner, carcinosarcoma, mixed, or unknown), and grade. Paraffin-embedded tissue blocks with representative samples of the ovarian carcinoma and one or more blocks of uninvolved tissue (e.g., ovarian, uterine) were requested for each of the confirmed ovarian cancer cases. Primary tumor tissue blocks were selected by the pathologist for the TMA. The tissue blocks containing ovarian carcinoma were matched to their corresponding H&E-stained slides. The initial fixation, processing, and storage conditions of these tissues is largely unknown as blocks came from multiple hospitals across the United States but are assumed to follow standard practices. Four controls per case were randomly selected from the NHS study participants who had no prior bilateral oophorectomy and no history of cancer, other than nonmelanoma skin cancer, at the time of diagnosis of the ovarian case. Cases and controls were matched on year of birth.

TMA Construction. TMAs were assembled by taking three core biopsies from paraffin-embedded ovarian cancer tissue blocks and re-embedding them into an arrayed "master" paraffin block at the Pathology Core of the Brigham and Women's Hospital (17). Areas of well-preserved tumor were circled on the slide by a gynecologic pathologist (JH), and the corresponding area of the tissue block was cored thrice. Cores were extracted

using hollow needles with a 0.6 mm diameter, from the circled areas, and transferred to the recipient block, with a spacing of 0.8 mm from core center to core center. Slides were cut from the TMA block to create array slides.

Immunohistochemistry. TMA slides were processed and stained within 2 wk of cutting. Five-micron sections were soaked in Xylene overnight to remove adhesive from the tape transfer system. Slides were deparaffinized and antigens were retrieved [heat retrieval in $1 \times$ citrate buffer (pH 6.0; Zymed), pressure cooker] and stained with the primary antibodies: ER α (mouse monoclonal; clone 1D5; DakoCytomation; 1:200 dilution) and PR (mouse monoclonal; clone PgR 636; DakoCytomation; 1:50 dilution). The primary antibodies were detected using a biotin-free, horseradish peroxidase enzyme-labeled polymer conjugated to either goat anti-mouse or anti-rabbit secondary antibodies (EnVision+ Systems; Dako).

Scoring. Staining was graded by a gynecologic pathologist (JH). Staining was scored as the number of reactive versus total cells and was categorized as 0%, 1% to 10%, 11% to 25%, 26% to 50%, and >50%. Three spots from the same case were independently assessed. Spots where tissue was missing from the slide or where only a few cell clusters (<20 cells) were present were subsequently designated as not interpretable. Concordance with a second pathologist and among the tissue cores was verified (17).

Statistical Analysis. The cores were dichotomized into positive (+) if >10% of cells stained positive and negative (-) if $\leq 10\%$ stained positive, based on the maximum value of the 3 cores. This cut point is used for breast cancer in our clinical practice (18). Staining intensity is a less reproducible parameter given the variability in the age and preservation of the samples and thus, was not evaluated. The Fisher's exact test was used to examine the distribution of the stains by histologic type, invasion, grade, and stage. The Pearson's correlation coefficient between continuous values for ER α and PR expression were calculated.

For the case-case analysis, tumors staining positive for ER α or PR were considered "cases" and those staining negative were considered "controls." Unconditional logistic regression adjusting for potential confounders was used to evaluate whether hormonal (OC and PMH use), reproductive (age, parity, menopausal status), and anthropometric (height, BMI, physical activity) exposures were associated with ER α or PR staining positivity. In the case-control analysis, we used polytomous logistic regression with three outcome categories (ER α + tumors, ER α - tumors, and control or PR+ tumors, PR- tumors, and control) to evaluate whether any of exposures mentioned above were differently associated with receptor status of the tumor compared with controls (19). To determine if the odds ratios (OR) across case groups differed, we compared a model holding the association of the exposure variable and ovarian cancer constant across case groups to one allowing the association to vary, using the likelihood ratio test (19). We evaluated all ovarian cancers combined and invasive cases only.

The ORs and 95% confidence intervals (CIs) are reported. We selected cut-points for the exposures using

the distribution in the control subjects and *a priori* hypotheses based on previous publications (5, 20-22). To maximize power, some categories were collapsed into binary variables. The analyses were adjusted for age, duration of OC use, number of pregnancies, and menopausal status, as applicable (see Table legends). We also considered other potential confounders including age at menarche, tubal ligation, and family history of breast and/or ovarian cancer; however, due to our small sample size, we were not able to obtain stable results when these variables were included in the models. All the covariates and exposures were assessed one cycle before the diagnosis of cases and the comparable cycle for matched controls. Trend tests were conducted by modeling the continuous variable and calculating the Wald statistic (23). All tests of statistical significance were two sided. SAS version 9.1 (SAS Institute, INC.) was used for the statistical analyses.

Results

Study Population. A total of 157 epithelial ovarian cancer cases and 649 controls were available for analysis. Characteristics of this study population have been described elsewhere (17). Fifty percent of the tumors expressed ER α and 28% expressed PR (Table 1). Of the 157 ovarian tumors, 14% expressed both ER α and PR, 36% were expression negative for ER α and PR, 14% expressed PR but not ER α , and 36% expressed ER α but not PR (Table 1), with minimal correlation between expression of these 2 receptors ($\rho = 0.07$; $P = 0.42$). PR and ER α expression varied by histologic subtype; however, the difference only achieved statistical significance for PR ($P = 0.003$ and 0.07 for PR and ER α , respectively; Table 1). Expression of PR in the serous, endometrioid, mucinous, and clear cell subtypes was 24%, 50%, 33%, and 0%, respectively, whereas expression

of ER α was 54%, 56%, 33%, and 20%, respectively. Lack of PR expression was associated with invasive ($P = 0.05$), higher grade ($P < 0.001$), and stage disease ($P = 0.007$), whereas there was no relationship between ER α expression, tumor invasion ($P = 1.0$), grade ($P = 0.76$), and stage ($P = 0.34$).

Relationship between Hormonal, Reproductive, and Anthropometric Factors and ER α Expression. In the case-control analysis, the relationships for age, menopausal status, and PMH use varied by ER α status ($P_{\text{heterogeneity}} = 0.001, 0.06, \text{ and } 0.06$, respectively; Table 2). There was a nonsignificant increased risk of developing ER α + (OR, 1.45; 95% CI, 0.76-2.75; $P_{\text{trend}} = 0.07$) and a nonsignificant decreased risk of developing ER α - (OR, 0.74; 95% CI, 0.44-1.24; $P_{\text{trend}} = 0.05$) ovarian tumors compared with controls for women age >60 years versus ≤ 60 years. Likewise, postmenopausal women had a modestly increased risk of developing ER α + tumors compared with premenopausal women (OR, 2.09; 95% CI, 0.74-5.88); however, menopausal status was not associated with developing ER α - tumors (OR, 0.81; 95% CI, 0.40-1.62). Overall, there was no association between PMH use for >5 years and ER α - tumors, but a significant positive association with ER α + tumors was observed ($P_{\text{heterogeneity}} = 0.06$). There was a 3-fold increase in risk of developing ER α + tumors with long-term PMH use (OR, 3.05; 95% CI, 1.52-6.12; $P_{\text{trend}} = 0.001$). There was no significant heterogeneity for OC use or parity and risk by ER α tumor status ($P_{\text{heterogeneity}} \geq 0.30$), although the data were suggestive of decreased risk of ER α + disease among parous women (OR, 0.50; 95% CI, 0.18-1.34).

None of the anthropometric variables, including height, BMI at diagnosis, BMI at age 18 years, and physical activity, were associated with ER α expression status of the tumors or disease risk (Table 3). The results were similar in the case-case analysis and when limited to invasive cancers (data not shown).

Table 1. PR and ER α expression by histologic subtype, morphology, grade, and stage

	All cases	PR+	PR-	ER α +	ER α -	ER α + / PR+	ER α - / PR-	ER α + / PR-	ER α - / PR+
Total, <i>n</i> (%) [*]	157 (100%)	44 (28%)	113 (72%)	78 (50%)	79 (50%)	22 (14%)	57 (36%)	56 (36%)	22 (14%)
Histology, <i>n</i> (%)									
Serous	80 (51%)	19 (43%)	61 (54%)	43 (55%)	37 (47%)	7 (32%)	25 (44%)	36 (64%)	12 (55%)
Endometrioid	34 (22%)	17 (39%)	17 (15%)	19 (24%)	15 (19%)	10 (45%)	8 (14%)	9 (16%)	7 (32%)
Mucinous	15 (10%)	5 (11%)	10 (9%)	5 (6%)	10 (13%)	3 (14%)	8 (14%)	2 (4%)	2 (9%)
Clear cell	15 (10%)	0 (0%)	15 (13%)	3 (4%)	12 (15%)	0 (0%)	12 (21%)	3 (5%)	0 (0%)
Other	13 (8%)	3 (7%)	10 (9%)	8 (10%)	5 (6%)	2 (9%)	4 (7%)	6 (11%)	1 (5%)
<i>P</i> [‡]			0.003		0.07				
Morphology, <i>n</i> (%)									
Borderline	18 (11%)	9 (20%)	9 (8%)	9 (12%)	9 (11%)	5 (23%)	5 (9%)	4 (7%)	4 (18%)
Invasive	139 (89%)	35 (78%)	104 (92%)	69 (88%)	70 (89%)	17 (77%)	52 (91%)	52 (93%)	18 (82%)
<i>P</i>			0.05		1.00				
Grade, <i>n</i> (%)									
I	14 (11%)	10 (30%)	4 (5%)	8 (13%)	6 (9%)	4 (25%)	0 (0%)	4 (9%)	6 (35%)
II	27 (21%)	9 (27%)	18 (19%)	12 (20%)	15 (23%)	6 (38%)	12 (24%)	6 (13%)	3 (18%)
III	86 (68%)	14 (42%)	72 (77%)	41 (67%)	45 (68%)	6 (38%)	37 (76%)	35 (78%)	8 (47%)
<i>P</i>			<0.001		0.76				
Stage, <i>n</i> (%)									
I/II	69 (44%)	27 (61%)	42 (37%)	31 (40%)	38 (48%)	17 (77%)	28 (49%)	14 (25%)	10 (45%)
III/IV	88 (56%)	17 (39%)	71 (63%)	47 (60%)	41 (52%)	5 (23%)	29 (51%)	42 (75%)	12 (55%)
<i>P</i>			0.007		0.34				

^{*}Percentages may not add up to 100% due to rounding.

[†]Other includes Brenner, carcinosarcoma, mixed, other, or unknown.

[‡]*P* value was calculated using Fisher's exact test comparing PR+ versus PR- and ER α + versus ER α -.

Table 2. Relationship between hormonal and reproductive factors and ER α or PR expression in ovarian tumors

	OR (95%CI)		$P_{\text{heterogeneity}}$	OR (95%CI)		$P_{\text{heterogeneity}}$
	ER α +	ER α -		PR+	PR-	
Age*						
≤60 y	1.00 (Reference)	1.00 (Reference)	0.001	1.00 (Reference)	1.00 (Reference)	0.08
>60 y	1.45 (0.76-2.75)	0.74 (0.44-1.24)		0.95 (0.48-1.87)	1.17 (0.74-1.85)	
$P_{\text{trend}}^{\dagger}$	0.07	0.05		0.34	0.25	
OCs						
Never	1.00 (Reference)	1.00 (Reference)	0.89	1.00 (Reference)	1.00 (Reference)	0.81
Ever	0.83 (0.45-1.52)	0.83 (0.50-1.35)		0.89 (0.46-1.73)	0.84 (0.55-1.29)	
$P_{\text{trend}}^{\ddagger}$	0.23	0.19		0.55	0.16	
Parity						
Never	1.00 (Reference)	1.00 (Reference)	0.30	1.00 (Reference)	1.00 (Reference)	0.59
Ever	0.37 (0.14-1.03)	0.80 (0.27-2.36)		0.76 (0.30-1.89)	0.30 (0.11-0.82)	
No children	1.00 (Reference)	1.00 (Reference)		1.00 (Reference)	1.00 (Reference)	
1-2 children	0.49 (0.17-1.42)	1.29 (0.43-3.89)		0.49 (0.15-1.61)	1.11 (0.46-2.65)	
3-4 children	0.39 (0.14-1.14)	0.87 (0.29-2.62)		0.49 (0.16-1.55)	0.61 (0.25-1.49)	
5+ children	0.90 (0.29-2.79)	1.13 (0.33-3.79)		0.60 (0.16-2.28)	1.04 (0.40-2.71)	
P_{trend}^{\S}	0.80	0.22		0.25	0.20	
Menopausal status at diagnosis [§]						
Premenopausal	1.00 (Reference)	1.00 (Reference)	0.06	1.00 (Reference)	1.00 (Reference)	0.003
Postmenopausal	2.09 (0.74-5.88)	0.81 (0.40-1.62)		0.41 (0.19-0.91)	1.53 (0.74-3.16)	
PMH						
Never	1.00 (Reference)	1.00 (Reference)	0.06	1.00 (Reference)	1.00 (Reference)	0.40
>0-≤5 y	1.37 (0.64-2.95)	1.25 (0.71-2.17)		1.05 (0.48-2.27)	1.44 (0.86-2.40)	
>5 y	3.05 (1.52-6.12)	1.00 (0.51-1.98)		0.97 (0.40-2.36)	2.57 (1.55-4.27)	
$P_{\text{trend}}^{\parallel}$	0.001	0.47		0.19	0.0003	

*Adjusted for duration of OC use (months), number of pregnancies (continuous), and menopausal status at diagnosis (premenopausal/postmenopausal).

†Adjusted for age (continuous), number of pregnancies, and menopausal status at diagnosis.

‡Adjusted for age, duration of OC use, and menopausal status at diagnosis.

§Adjusted for age, duration of OC use, and number of pregnancies.

||Among postmenopausal women.

Relationship between Hormonal, Reproductive and Anthropometric Factors, and PR Expression. Among cases, menopausal status was a strong predictor of having a PR- versus PR+ tumor (OR for PR+, 0.24; 95% CI, 0.07-0.80; Table 3). Furthermore, there was significant heterogeneity for this relationship in the case-control analysis ($P_{\text{heterogeneity}} = 0.003$) such that postmenopausal women had a 59% decreased risk of developing PR+ tumors (95% CI, 0.19-0.91) and a 53% increased risk of developing PR- tumors (95% CI, 0.74-3.16). Although not significant, older age was associated with developing PR- but not PR+ tumors ($P_{\text{heterogeneity}} = 0.08$). PMH use for >5 years was significantly associated with an increased risk of developing PR- (OR, 2.57; 95% CI, 1.55-4.27; $P_{\text{trend}} = 0.0003$) but not PR+ tumors (OR, 0.97; 95% CI, 0.40-2.36; $P_{\text{trend}} = 0.19$). There was no significant heterogeneity for the relationships with OC use and parity ($P_{\text{heterogeneity}} \geq 0.30$); however, there was a decreased risk of developing PR+ tumors among parous women (OR, 0.30; 95% CI, 0.11-0.82).

Taller women were more likely to develop PR- tumors ($P_{\text{trend}} = 0.08$), and although not significant, the association seemed to differ by the PR status of the tumor ($P_{\text{heterogeneity}} = 0.08$). Increasing height was associated with increased risk of developing PR- tumors versus controls (OR >66 versus ≤62 inches, 1.88; 95% CI, 1.01-3.50). Height was not associated with PR-expressing tumors (OR >66 versus ≤62 inches, 0.59; 95% CI, 0.20-1.79; $P_{\text{trend}} = 0.40$). BMI at diagnosis, BMI at age 18 years, and physical activity did not influence PR expression or risk. The results did not change substantially when we limited the analysis to invasive cancers (data not shown).

Furthermore, these associations were similar in the case-case analysis (data not shown).

Discussion

We have described the relationship of hormonal, reproductive, and anthropometric variables and the risk of ER α - and PR-expressing ovarian tumors. Overall, ER α and PR expression and staining by histologic subtype was comparable with what has previously been reported in the literature (12, 24-26). Women who were age >60 years, postmenopausal at diagnosis, or who used PMH for >5 years were at an increased risk of developing ER α + and PR- but not ER α - or PR+ ovarian tumors. Among the anthropometric variables, height was associated with developing PR- tumors.

We found that PR- tumors were more likely to be invasive and of higher grade and stage, a finding consistent with reports of improved patient survival among patients with PR+ tumors (12, 25, 27-29). ER α status was not associated with tumor invasion, grade or stage, although the predictive value of ER α on survival remains unresolved (12, 28, 29). We found coexpression of both receptors in only 14% of the tumors, whereas others have reported coexpression in up to 36% of their samples (28, 30-32). These data suggest that unlike breast cancer, PR may play a more important role in ovarian tumor biology than ER α . The lower prevalence of PR expression in the higher stage and grade tumors, as seen in our study, suggests a tumor suppressor function of this receptor that may be lost with tumor progression or

hormone independent growth, along with reflecting a potential disruption of ER α downstream signaling pathways (33).

Ovarian cancer may be divided into two broad categories: type I and type II tumors (34). Type I tumors, including low-grade serous and endometrioid carcinomas, are slow growing and develop from precursor lesions such as adenofibromas, borderline tumors, or endometriosis (34). On the other hand, type II tumors, including high-grade serous and undifferentiated carcinomas, present at an advanced stage and often lack an identifiable precursor likely due to their rapid growth (34). A third category of tumors might include clear cell and mucinous carcinomas.

Receptor expression differs between these groups. Type I ovarian tumors, those of endometrioid histology, seem to be similar to cancers arising in the breast and endometrium because they are more likely to coexpress both ER α and PR compared with the other histologic subtypes and are also associated with a hormone responsive precursor (i.e., endometriosis; ref. 35). In the present study, 29% of endometrioid tumors coexpressed both ER α and PR compared with 9% of the serous tumors. The role of estrogen in type I ovarian carcinogenesis may be to drive growth in ER α + / PR+ -positive cells. Thus, similar to breast cancer, estrogen exposure may act as a continual growth stimulus to promote cellular proliferation whereas progesterone may cause tumor regression in type I cancers. This is supported by a suggestively stronger association of PMH use with endometrioid tumors compared with other subtypes (5). Clear cell and mucinous are genetically and epidemiologically distinct cancers and are among the least likely to express ER α and PR (36, 37).

Conversely, estrogen may be more likely to play a role in an initiating event rather than as a growth

factor for type II cancers. The toxic effect may be mediated by local estrogen production during folliculogenesis (32), a model that is supported by the link between the number of lifetime ovulatory cycles and p53 mutations (38, 39). Growth may be facilitated by loss of PR and a defective ER α signaling pathway. ER α signaling is thought to be defective in most type II cancers because expression levels do not predict clinical response to treatment with Tamoxifen or other aromatase inhibitors (40), whereas loss of PR expression represents hormone-independent growth in cancer cell lines (41). This may be due to disruption of the ER signaling pathway or other changes in cell signaling such as increased expression of epidermal growth factor receptor and HER2 (41). ER α was expressed in more than half of type II cancers, whereas PR was expressed in only 20. Furthermore, we did not observe a correlation between ER α and PR expression in this subgroup, suggesting a defective ER α signaling pathway analogous to ovarian cancer cells line that express ER α but that are resistant to both estrogens and antiestrogens (42).

Women who were older than 60 years, postmenopausal at diagnosis, or had ever used PMH were at an increased risk of developing ER α + and PR- but not ER α - or PR+ ovarian tumors. These associations are unlike what is seen with breast cancer where older or postmenopausal women, as well as PMH users, typically develop dual positive cancers but younger cases develop more aggressive breast cancers that are frequently ER-, PR-, and Her2- cancers and more likely to have a hereditary component (7, 43). Moreover, the higher grade and stage ovarian tumors were more likely to be PR- with a fairly equal distribution of ER α + and ER α -, suggesting loss of PR expression among the more aggressive subtypes.

Table 3. Relationship between anthropometric factors and ER α or PR expression in ovarian tumors

	OR (95%CI)		<i>P</i> _{heterogeneity}	OR (95%CI)		<i>P</i> _{heterogeneity}
	ER α +	ER α -		PR+	PR-	
Height*						
≤62 inches	1.00 (Reference)	1.00 (Reference)	0.83	1.00 (Reference)	1.00 (Reference)	0.08
>62-≤64	1.46 (0.63-3.36)	1.31 (0.69-2.47)		1.14 (0.52-2.48)	1.34 (0.76-2.37)	
>64-≤66	2.24 (0.85-5.90)	0.84 (0.33-2.13)		0.73 (0.22-2.39)	1.63 (0.80-3.30)	
>66	1.34 (0.50-3.57)	1.59 (0.78-3.23)		0.59 (0.20-1.79)	1.88 (1.01-3.50)	
<i>P</i> _{trend}	0.44	0.52		0.40	0.08	
BMI*						
≤22	1.00 (Reference)	1.00 (Reference)	0.51	1.00 (Reference)	1.00 (Reference)	0.47
>22	1.29 (0.59-2.79)	0.95 (0.51-1.75)		1.53 (0.65-3.61)	0.98 (0.58-1.65)	
>25-≤28	0.59 (0.23-1.48)	0.61 (0.31-1.21)		0.78 (0.29-2.07)	0.50 (0.27-0.93)	
>28	0.88 (0.38-2.01)	0.61 (0.31-1.20)		0.78 (0.29-2.06)	0.69 (0.39-1.21)	
<i>P</i> _{trend}	0.47	0.07		0.74	0.08	
BMI at 18 y*						
≤19	1.11 (0.56-2.22)	1.40 (0.77-2.55)	0.25	1.57 (0.74-3.34)	1.02 (0.61-1.68)	0.24
>19-≤21	1.00 (Reference)	1.00 (Reference)		1.00 (Reference)	1.00 (Reference)	
>21-≤23	0.71 (0.25-1.98)	1.49 (0.71-3.11)		0.41 (0.09-1.85)	1.08 (0.57-2.05)	
>23	1.04 (0.42-2.61)	1.69 (0.82-3.49)		1.23 (0.45-3.40)	1.37 (0.75-2.51)	
<i>P</i> _{trend}	0.50	0.34		0.54	0.25	
Physical activity*						
≤3 mets/wk	1.00 (Reference)	1.00 (Reference)	0.35	1.00 (Reference)	1.00 (Reference)	0.34
>3-≤21	0.90 (0.38-2.14)	0.84 (0.42-1.70)		1.37 (0.49-3.83)	0.71 (0.40-1.28)	
>21	1.81 (0.75-4.36)	1.05 (0.48-2.28)		1.34 (0.43-4.23)	1.20 (0.65-2.22)	
<i>P</i> _{trend}	0.09	0.65	0.89	0.10		

*Adjusted for age (continuous), duration of OC use (months), number of pregnancies (continuous), and menopausal status at diagnosis (premenopausal/postmenopausal).

Although numerous hypotheses to explain ovarian carcinogenesis have been proposed, incessant or uninterrupted ovulation emerges as one of the strongest and most consistent risk factors (44). Accordingly, the protective effects of pregnancy and OC use have been linked to their ability to suppress or interrupt ovulation and decrease overall exposure to estrogen produced during folliculogenesis (45). We did not observe any clear differences in risk by hormone receptor status for OC use or parity.

In a recent pooled analysis of 12 cohort studies, height was positively associated with an increased risk of ovarian cancer, particular among premenopausal women (46). It is believed that attained height is a biomarker for other exposures that influence growth and adult height, such as genetic, hormonal, and environmental factors (47). The evidence suggests that insulin-like growth factor I, a major growth factor in determining height, and estrogen are both mitogens that act synergistically to promote epithelial cell proliferation (48). Among the anthropometric variables evaluated in our study, only height was predictive of receptor status and risk and the observed association was only seen with tumors lacking PR. The mechanism by which height enhances the risk of PR- disease requires further exploration.

Given the small sample size, we were unlikely to detect marginal differences in the association between adult or adolescent BMI, physical activity, and ovarian cancer risk by tumor subtype. Previous studies have reported that a positive association between BMI and risk seems to be limited to premenopausal women (46). Due to the small number of tumor samples, we were not able to stratify our BMI analysis by menopausal status, which may have masked any significant findings. We did not observe any association between physical activity and risk, which is in accordance with the epidemiologic evidence to date (49).

This study was limited by the sample size and precluded stratified analyses by histologic subtype or combined ER α /PR subgroups. In addition, ER α and PR are continuous variables such that our classification based on dichotomized staining score may have been an oversimplification. Despite these limitations, ours is the first population-based study that has evaluated risk factors for ovarian cancer according to ER α or PR status. The prospective design of the NHS allows for the detailed collection of unbiased risk factor information and thus accurate control for confounding. Moreover, we used TMA, an efficient, high-throughput technique that allows for the evaluation of protein expression profiles from archived tissue samples. The simultaneous staining of many cases minimized variability in the results due to differences in experimental conditions. We plan to continue to investigate these findings with additional follow-up of the NHS in addition to the inclusion of the NHSII.

In conclusion, our data indicate that some hormonal, reproductive, and anthropometric factors are associated with subsets of ovarian tumors based on ER α and PR status. Future epidemiologic studies of ovarian cancer should take into consideration hormone receptor status, as well as, common pathologic features of the tumor. Although the evidence strongly suggests that ER α + /PR+ and ER α - /PR- breast cancers are distinct disease entities with different risk factor profiles, the subtypes

important for ovarian cancer seem to be different given the lack of correlation between ER α and PR expression. Furthermore, it may be that the ER α + /PR- tumors represent the most aggressive subtype of ovarian cancer. Larger studies aimed at correlating ER α , PR, and possibly ER β and the androgen receptor tumor expression with various exposures and clinical characteristics including patient survival will provide insight into the etiology of ovarian cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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