Research Article

Cancer Epidemiology, Biomarkers & Prevention

Association of Ovarian Tumor β_2-Adrenergic Receptor Status with Ovarian Cancer Risk Factors and Survival

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Abstract

Background: The β_2-adrenergic signaling pathway mediates the effects of chronic stress on ovarian cancer progression in mouse models. The relevance of this pathway to human ovarian cancer remains unknown.

Methods: We assessed tumor expression of β_2-adrenergic receptor (ADRB2) using tissue microarrays in 237 ovarian cancer cases from the Nurses' Health Studies (NHS/NHSII). Competing risks Cox regression was used to evaluate whether associations of reproductive, hormonal, and psychosocial factors with ovarian cancer risk differed by ADRB2. We also examined the association between tumor ADRB2 expression and ovarian cancer survival.

Results: Forty-five (19%) cases were positive for ADRB2 staining. High levels of anxiety symptoms were positively associated with ADRB2-positive tumors (HR, 2.59; 95% confidence interval [CI], 1.15–5.84) but not with ADRB2-negative tumors (HR, 1.16; 95% CI, 0.81–1.66; $P_{\text{heterogeneity}} = 0.07$). We observed similar results for depression. No associations were observed for job strain, caregiving stress, or widowhood for either positive or negative ADRB2 status. Lifetime ovulatory years were more strongly associated with ADRB2-positive tumors (HR per 5 years, 1.60; 95% CI, 1.15–2.21) compared with ADRB2-negative tumors (HR, 1.11; 95% CI, 0.96–1.27; $P_{\text{heterogeneity}} = 0.04$). Significant heterogeneity by ADRB2 was also observed for parity ($P_{\text{heterogeneity}} = 0.01$), oral contraceptive use ($P_{\text{heterogeneity}} = 0.03$), and age at menopause ($P_{\text{heterogeneity}} = 0.04$). Tumor expression of ADRB2 was not associated with ovarian cancer mortality (HR, 1.05; 95% CI, 0.69–1.59).

Conclusions: Several stress- and ovulation-related factors were differentially associated with ovarian tumors responsive to β_2-adrenergic signaling.

Impact: Replication in larger studies is warranted to confirm the role of β_2-adrenergic signaling in ovarian cancer etiology. *Cancer Epidemiol Biomarkers Prev; 25(12); 1587–94. ©2016 AACR.*

Introduction

β-Adrenergic receptors are a group of G-protein–coupled receptors that mediate cellular signaling from the sympathetic nervous system (SNS). These receptors are expressed in various human tissues, including the ovary (1). In response to stress, the SNS releases catecholamines (e.g., epinephrine and nonrepinephrine) that bind to β-adrenergic receptors, activate downstream biologic processes, and prepare the body for fight-or-flight responses. β-adrenergic receptors are also expressed in precancerous lesions and malignant cells (2); downstream cellular responses to β-adrenergic signaling, such as inflammation, angiogenesis, tissue invasion, and impaired anticancer immunity, are important pathways in tumorigenesis and progression (2). A growing body of evidence suggests that chronic activation of β-adrenergic signaling pathways promotes tumor growth and accelerates metastasis in several cancer types, including ovarian cancer (3–6).

One class of β-adrenergic receptors, the β_2-adrenergic receptor (ADRB2), appears to be a key promoter of the effects of chronic stress on ovarian cancer. In mice, chronic restraint stress led to larger and more aggressive ovarian tumors; blockade of β_2-adrenergic signaling by inhibiting tumor ADRB2s abrogated the effect of chronic stress on tumor progression (6). In particular, norepinephrine has been identified as the neuroendocrine hormone responsible for β_2-adrenergic signal transduction in ovarian cancer (6–8). Among patients with ovarian cancer, use of β-blockers, particularly nonselective versus β_1-selective, has been associated with longer survival (9, 10), although a beneficial effect has not been consistently observed (11, 12). These animal and clinical studies support the role of β_2-adrenergic signaling pathway in ovarian cancer progression, but little is known about the carcinogenic potential of this pathway in ovarian cancer development.

Chronic stress/distress (e.g., depression/anxiety) has been linked with elevated norepinephrine (13–18), as well as modestly increased ovarian cancer risk (19, 20). Chronic stress/distress in

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doi: 10.1158/1055-9965.EPI-16-0534

humans represents a multidimensional construct integrating diverse factors at the biological, behavioral, and social level; it is unclear whether the observed association between psychosocial stress and ovarian cancer development is mediated by β_2-adrenergic signaling, as in mice. Furthermore, evidence is limited regarding the relationships between established reproductive and hormonal risk factors for ovarian cancer and tumor ADRB2 status. Therefore, we conducted the present study to examine whether the associations of ovarian cancer with reproductive, hormonal, and psychosocial factors differed by tumor ADRB2 expression. We hypothesized that psychosocial factors would have stronger positive associations with ADRB2-positive tumors, assuming that β_2-adrenergic signaling is the underlying biologic pathway through which stress influences ovarian cancer development. We also explored whether tumor ADRB2 expression was associated with ovarian cancer survival.

Materials and Methods

Study population

The Nurses' Health Studies (NHS and NHSII) are 2 large, ongoing, prospective cohort studies of U.S. female registered nurses. At baseline (NHS: 1976; NHSII: 1989), 121,700 NHS participants aged 30 to 55 years and 116,430 NHSII participants aged 25 to 42 years completed an initial questionnaire regarding their lifestyle factors and medical history. Information is updated biennially by follow-up questionnaires. The current analysis excluded participants who had a bilateral oophorectomy, menopause due to pelvic irradiation, or a prior diagnosis of cancer (other than non-melanoma skin cancer) at baseline. The study was approved by the institutional review board at Brigham and Women's Hospital.

Ascertainment of ovarian cancer and death

Participants reported ovarian cancer diagnoses on each biennial questionnaire. Deaths were identified by family members, the National Death Index, or the U.S. Postal Service. For all incident cases, we obtained relevant pathology reports (or death certificates) to confirm the diagnosis. A gynecologic pathologist blinded to exposure status abstracted tumor characteristics from pathology reports, including stage, grade, histology, and invasiveness. In a subset of 215 cases, the percent agreement between the pathology reports and the pathologist's review of tumor slides was 98% for invasiveness and 83% for histology (21).

Assessment of ovarian tumor ADRB2 status

Paraffin-embedded tissue blocks of representative samples of ovarian tumors were collected for 314 cases from 1,082 confirmed NHS cases diagnosed between 1976–2006 and 59 cases from 201 confirmed NHSII cases diagnosed between 1989–2005. Of these, 217 NHS and 46 NHSII cases were included on tissue microarrays. The present study included 237 cases with assessment of tumor ADRB2 status. Five-micrometer sections of the tissue microarrays were processed and stained within 2 weeks of cutting. Sections were soaked in xylene overnight to remove adhesive from the tape transfer system. Slides were deparaffinized and stained using standard heat epitope retrieval using a pressure cooker at 90°C for 30 minutes in a citrate pH 6.0 solution (Bond Epitope Retrieval Solution 1, Leica catalog# AR9961). Slides were then stained with anti-ADRB2 antibody (Sigma-Aldrich, polyclonal rabbit, dilution 1:100) on a Leica Bond III autostaining platform. Staining was first scored on the basis of intensity using a 3-tiered scale from 0 to 2. Three cores were scored for each case, with excellent reproducibility across cores [intraclass correlation coefficient = 0.79; 95% confidence interval (CI), 0.75–0.82]. As scores 0 and 1 were almost indistinguishable due to high background staining, we further used a 2-tier scoring to define ADRB2 status by combining scores 0 and 1 and considering them as negative. Score 2 was assigned for either diffuse and dark cytoplasmic staining or intense staining of luminal border of glands. The tumor was considered ADRB2-positive if $\geq$1 cores had a score of 2.

Assessment of reproductive and hormonal factors

Reproductive and hormonal factors were assessed from baseline in NHS/NHSII. In NHS, participants reported parity (defined as pregnancies lasting longer than 6 months) biennially until 1984; this information was updated in 1996. Duration of oral contraceptive use was collected biennially until 1982, when use was uncommon. History of tubal ligation was assessed biennially from 1976 to 1982 and updated in 1994. Age at menarche was asked at baseline, and duration of breastfeeding was assessed in 1986. Information on menopausal status, age at menopause, type and duration of postmenopausal hormone therapy use, and history of hysterectomy was collected biennially throughout follow-up. In the younger NHSII cohort, these reproductive and hormonal factors were updated in biennial follow-up questionnaires from baseline (except age at menarche). Lifetime ovulatory years (LOYs) were calculated as the difference between age at menopause (or current age for premenopausal women) and age at menarche, with subtraction of duration of oral contraceptive use and 1 year per pregnancy.

Assessment of psychosocial factors

The psychosocial factors evaluated in this analysis included depression, anxiety, widowhood, job strain, and caregiving stress. Assessment of depression began in 1992 in NHS and in 1993 in NHSII and was updated every 2 to 4 years. Consistent with our prior work (19), we defined depression as the presence of one or more of the following: elevated depressive symptoms using the 5-item Mental Health Index (22), antidepressant use, or physician-diagnosed depression. Anxiety was measured by the Crown–Crisp Phobic Anxiety Index (an 8-item scale scored between 0–16; ref. 23), which was asked in 1988 and 2004 in NHS and in 1993 and 2005 in NHSII (20). This index addresses common fears and tendencies of avoidance, primarily those reflected in the modern categorizations of panic and phobic disorders (24). It has been validated to discriminate patients with anxiety disorders from healthy controls or patients with other conditions (25). A Crown–Crisp score $\geq$4 was considered evidence of anxiety. In NHS, all participants were married at baseline; marital status (married, widowed, separated/divorced) was updated in 1980, 1992, and every 4 years thereafter. In NHSII, marital status was asked every 4 years since 1989. Job strain was measured with Karasek job content questionnaire (26) in 1992 and 1996 in NHS and in 1993 and 1997 in NHSII. Two subscales, job demand and job control, were used to assess job characteristics as previously described (27). In 1992, 1996, and 2000 in NHS and 2001 in NHSII, participants reported the average hours per week they provided care (outside of their employment as nurses) to children, grandchildren, a disabled/ill spouse, a disabled/ill parent, or other disabled/ill persons. We

created a composite indicator for chronic stress/distress that was defined as having one or more of the 3 individual factors (depression, elevated anxiety symptoms, and widowhood), as these psychosocial factors have been linked with elevated norepinephrine (13–18), which binds to ADRB2 to enhance β_2-adrenergic signaling and promote ovarian cancer growth.

Statistical analyses

We compared tumor characteristics by ADRB2 status using χ^2 tests or Fisher exact tests. Since tumor tissue blocks and ADRB2 staining were only available for a subset of all incident ovarian cancer cases in NHS/NHSII, we also compared tumor characteristics between cases with versus without ADRB2 assessment to evaluate potential selection bias. Similarly, we compared the distribution of reproductive, hormonal, and psychosocial factors for 3 groups: ADRB2-positive cases, ADRB2-negative cases, and cases without tumor blocks.

Person-years of follow-up were calculated from the return of baseline questionnaire (for reproductive and hormonal factors) or from the date of the first assessment (for psychosocial factors) until death, any cancer diagnosis including ovarian cancer (except non-melanoma skin cancer), bilateral oophorectomy, pelvic irradiation, or the end of the follow-up (June 2006 for NHS and June 2007 for NHSII), whichever occurred first. Using competing risks Cox models (28), we obtained separate HRs and 95% CIs associated with each risk factor for ADRB2-positive and ADRB2-negative tumors. Likelihood ratio tests comparing the models with unconstrained (i.e., allowing the association between the risk factor and ovarian cancer risk to vary by ADRB2 status) versus constrained (i.e., forcing the association to be the same by ADRB2 status) coefficients were used to assess heterogeneity in the estimates. We evaluated risk factors as continuous variables whenever possible to increase statistical efficiency. To minimize the potential that early symptoms of ovarian cancer may cause emotional distress leading to reverse causation, we evaluated the associations with psychosocial factors assessed at least 2 to 4 years prior to ovarian cancer diagnosis. The analyses were stratified by calendar time and cohort and adjusted for age. In the multivariable model, we further adjusted for parity, duration of oral contraceptive use, estrogen-only hormone therapy use, tubal ligation, and menopausal status.

In survival analysis, proportional hazards models were used to evaluate tumor ADRB2 status and ovarian cancer mortality, adjusted for age at diagnosis, tumor histology, grade, and stage. We stratified the analysis by the composite stress indicator defined as previously described to test whether ADRB2 may influence ovarian cancer survival only when chronic stress/distress was present. Significance of the interaction was evaluated by likelihood ratio tests comparing the models with versus without the cross-product term between ADRB2 status and the indicator. All analyses were conducted in SAS version 9.3.

Results

We stained cores from 237 ovarian tumor blocks for ADRB2; 45 (19%) cases were positive (Table 1). Compared with ADRB2-negative tumors, ADRB2-positive tumors were more likely to be of serous/poorly differentiated histology ($P = 0.002$) and high stage ($P = 0.01$). Tumor grade, morphology, and aggressiveness did not differ significantly by ADRB2 status ($P > 0.16$). Compared with cases without ADRB2 assessment, those assessed for ADRB2 were less likely to be fatal within

Table 1. Tumor characteristics by ADRB2 status and assessment availability

	ADRB2			ADRB2 assessment available		
	Positive	Negative	P^a	Yes	No	P^a
N	45	192		237	840	
Age at diagnosis, y	59.7 (8.4)	58.6 (10.5)	0.55	58.8 (10.1)	57.3 (11.1)	0.05
Histologic subtype						
Serous/Poorly differentiated	38 (84)	101 (53)	0.002	139 (59)	507 (60)	0.29
Mucinous	0 (0)	16 (8)		16 (7)	82 (10)	
Endometrioid	5 (11)	34 (18)		39 (16)	121 (14)	
Clear cell	1 (2)	16 (8)		17 (7)	38 (5)	
Other	1 (2)	25 (13)		26 (11)	92 (11)	
Stage						
I/II	10 (22)	82 (43)	0.01	92 (39)	300 (36)	0.005
III/IV	35 (78)	110 (57)		145 (61)	504 (60)	
Unknown/Missing	0 (0)	0 (0)		0 (0)	36 (4)	
Grade						
Grade 1	0 (0)	12 (6)	0.24	12 (5)	60 (7)	0.08
Grade 2	9 (20)	32 (17)		41 (17)	107 (13)	
Grade 3	16 (36)	80 (42)		96 (41)	306 (36)	
Unknown/Missing	20 (44)	68 (35)		88 (37)	367 (44)	
Morphology						
Invasive	38 (84)	168 (88)	0.53	206 (87)	718 (85)	0.84
Borderline	7 (16)	20 (10)		27 (11)	105 (13)	
Unknown/Missing	0 (0)	4 (2)		4 (2)	17 (2)	
Tumor aggressiveness						
Fatal within 3 years	18 (40)	56 (29)	0.16	74 (31)	346 (41)	0.008
Alive after 3 years	27 (60)	136 (71)		163 (69)	489 (58)	
Death from other causes	0 (0)	0 (0)		0 (0)	5 (1)	

NOTE: Values are mean (SD) for age at diagnosis and number (percent) for other variables.

[a]On the basis of *t* tests for continuous variables and χ^2 or Fisher exact tests for categorical variables.

Table 2. Reproductive, hormonal, and psychosocial factors by ADRB2 status and assessment availability

	ADRB2			ADRB2 assessment available		
	Positive	Negative	P[a]	Yes	No	P[a]
N	45	192		237	840	
Reproductive/Hormonal factors						
History of tubal ligation	4 (9)	27 (14)	0.35	31 (13)	95 (11)	0.44
History of hysterectomy	10 (22)	41 (21)	0.90	51 (22)	140 (17)	0.08
Parous	42 (93)	173 (90)	0.78	215 (91)	718 (85)	0.03
Number of children[b]	3.6 (1.6)	2.8 (1.6)	0.004	3.0 (1.6)	2.9 (1.4)	0.42
Duration of breastfeeding,[b] mo	6.3 (11.5)	5.9 (10.0)	0.83	6.0 (10.3)	5.4 (8.7)	0.40
Ever OC use	22 (49)	99 (52)	0.75	121 (51)	408 (48)	0.47
Duration of OC use,[c] mo	24.8 (24.3)	53.1 (46.4)	0.007	47.9 (44.6)	47.4 (43.7)	0.90
Age at menarche, y	12.5 (1.3)	12.7 (1.5)	0.44	12.6 (1.4)	12.5 (1.4)	0.20
Age at menopause,[d] y	52.1 (2.5)	50.7 (4.5)	0.07	51.0 (4.2)	50.9 (3.8)	0.75
Lifetime ovulatory years	35.2 (4.0)	32.7 (6.5)	0.02	33.2 (6.2)	32.7 (6.2)	0.29
Postmenopausal	34 (76)	131 (68)	0.34	165 (70)	532 (63)	0.06
Ever estrogen-only HT use[d]	13 (38)	51 (39)	0.94	64 (39)	176 (33)	0.18
Duration of estrogen-only HT use,[c,d] mo	80.3 (90.1)	82.4 (82.6)	0.94	82.0 (83.4)	82.6 (83.7)	0.96
Ever estrogen+progestin HT use[d]	10 (29)	35 (27)	0.75	45 (27)	132 (25)	0.53
Duration of estrogen+progestin HT use,[c,d] mo	61.4 (38.3)	64.9 (43.7)	0.82	64.1 (42.2)	60.2 (48.7)	0.63
Psychosocial factors						
Depression[e]	5 (22)	14 (13)	0.33	19 (15)	58 (16)	0.82
Elevated anxiety symptoms[e]	14 (58)	48 (38)	0.05	62 (42)	155 (36)	0.24
Marital status						
Married	38 (84)	159 (83)		197 (83)	687 (82)	
Divorced/Separated	2 (4)	19 (10)	0.40	21 (9)	76 (9)	0.78
Widowed	5 (11)	14 (7)		19 (8)	80 (9)	
No caregiving[e]	14 (78)	39 (49)	0.03	53 (55)	148 (50)	0.44
Total caregiving time,[e,f] h/wk	7.1 (9.2)	5.2 (7.2)	0.63	5.4 (7.3)	5.1 (6.9)	0.85
High job demand[e]	6 (50)	34 (48)	0.89	40 (48)	143 (55)	0.26
High job control[e]	7 (58)	32 (45)	0.39	39 (47)	121 (47)	0.97

NOTE: Values are mean (SD) for continuous variables and number (percent) for categorical variables.

Abbreviations: HT, hormone therapy; OC, oral contraceptive.

[a]On the basis of *t* tests for continuous variables and χ^2 or Fisher exact tests for categorical variables.

[b]Among parous women.

[c]Among ever users.

[d]Among postmenopausal women.

[e]Among a subset of participants with psychosocial assessment.

[f]Among women who provided caregiving.

3 years of diagnosis ($P = 0.008$) or missing stage information ($P = 0.005$); no significant differences were observed for tumor histology, grade, or morphology ($P > 0.08$).

The prevalence of depression, anxiety, widowhood, and caregiving was higher in ADRB2-positive than in ADRB2-negative cases (Table 2). ADRB2-positive cases were more likely to be postmenopausal, have shorter duration of oral contraceptive or hormone therapy use, provide more caregiving, and report high job control compared with ADRB2-negative cases. Compared with the cases without ADRB2 assessment, cases assessed for ADRB2 were more likely to be postmenopausal, be parous, have ever used oral contraceptive or estrogen-only hormone therapy, report elevated anxiety symptoms, and have a history of hysterectomy.

The associations of ovarian cancer with most hormonal and reproductive factors did not differ significantly by tumor ADRB2 status; the results were similar between age-adjusted and multivariable models ($P_{\text{heterogeneity}} > 0.29$; Table 3). However, we observed significant differences for several ovulation-related factors. The multivariable-adjusted HRs (95% CIs) for ADRB2-positive and ADRB2-negative ovarian tumors, respectively, were 1.15 (0.98–1.34) and 0.91 (0.83–0.99) per each child ($P_{\text{heterogeneity}} = 0.01$), 0.47 (0.23–0.99) and 0.97 (0.79–1.20) per 5 years of oral contraceptive use ($P_{\text{heterogeneity}} = 0.03$), and 1.11 (1.00–1.24) and 0.99 (0.95–1.04) per 1 year increase in age at menopause ($P_{\text{heterogeneity}} = 0.04$). These factors contributed to significant heterogeneity by ADRB2 for LOYs ($P_{\text{heterogeneity}} = 0.04$). Increased LOYs had a stronger positive association with ADRB2-positive tumors (HR per 5 LOYs, 1.60; 95% CI, 1.15–2.21) versus ADRB2-negative tumors (comparable HR, 1.11; 95% CI, 0.96–1.27).

As assessment of psychosocial factors (except widowhood) was initiated later in follow-up and included on different questionnaires, the number of cases eligible for analysis ranged from 83 (job strain) to 153 (anxiety). In multivariable analyses (Table 4), there was a significant positive association between anxiety and ADRB2-positive tumors (HR, 2.59; 95% CI, 1.15–5.84); no significant association was observed for ADRB2-negative tumors (HR, 1.16; 95% CI, 0.81–1.66; $P_{\text{heterogeneity}} = 0.07$). Similarly, the HRs (95% CIs) associated with depression were 2.33 (0.85–6.35) for ADRB2-positive tumors and 1.10 (0.62–1.94) for ADRB2-negative tumors ($P_{\text{heterogeneity}} = 0.22$). The composite stress indicator suggested a significantly increased risk for ADRB2-positive tumors (HR, 2.52; 95% CI, 1.28–4.95) and a slightly increased risk for ADRB2-negative tumors (HR, 1.31; 95% CI, 0.95–1.80; $P_{\text{heterogeneity}} = 0.09$). No association was observed for job strain or caregiving; nor was any significant heterogeneity by ADRB2 status.

Of 237 ovarian cancer cases, 147 died during follow-up, and all deaths were due to ovarian cancer. In age-adjusted models,

Table 3. Associations of ovarian cancer with hormonal and reproductive factors by ADRB2

	Age-adjusted model[a]			Multivariable model[b]		
	ADRB2 status			ADRB2 status		
	Positive	Negative	$P_{heterogeneity}$	Positive	Negative	$P_{heterogeneity}$
N	45	192		45	192	
Parity						
Per child	1.14 (0.98–1.34)	0.90 (0.82–0.99)	0.01	1.15 (0.98–1.34)	0.91 (0.83–0.99)	0.01
OC use						
Per 5 years of use	0.47 (0.23–0.99)	0.98 (0.79–1.20)	0.03	0.47 (0.23–0.99)	0.97 (0.79–1.20)	0.03
Breastfeeding						
Per month	1.00 (0.97–1.03)	0.99 (0.97–1.00)	0.38	0.99 (0.96–1.03)	0.99 (0.97–1.01)	0.80
Age at menarche						
Per 1 year	0.94 (0.77–1.16)	1.04 (0.95–1.15)	0.39	0.94 (0.76–1.16)	1.05 (0.95–1.15)	0.37
Age at menopause[c]						
Per 1 year	1.14 (1.02–1.27)	1.00 (0.95–1.04)	0.02	1.11 (1.00–1.24)	0.99 (0.95–1.04)	0.04
Lifetime ovulatory years[d]						
Per 5 year	1.63 (1.18–2.25)	1.12 (0.98–1.29)	0.03	1.60 (1.15–2.21)	1.11 (0.96–1.27)	0.04
Tubal ligation						
No	1.00 (ref)	1.00 (ref)	0.38	1.00 (ref)	1.00 (ref)	0.29
Yes	0.50 (0.18–1.43)	0.81 (0.53–1.22)		0.49 (0.17–1.39)	0.86 (0.57–1.31)	
Hysterectomy						
No	1.00 (ref)	1.00 (ref)	0.93	1.00 (ref)	1.00 (ref)	0.83
Yes	1.14 (0.56–2.33)	1.19 (0.84–1.69)		0.88 (0.42–1.84)	0.96 (0.64–1.42)	
Postmenopausal						
No	1.00 (ref)	1.00 (ref)	0.63	1.00 (ref)	1.00 (ref)	0.62
Yes	1.42 (0.52–3.86)	1.08 (0.66–1.79)		1.47 (0.54–4.02)	1.11 (0.67–1.83)	
Estrogen HT use						
Per 5 years of use	1.27 (0.92–1.73)	1.28 (1.10–1.49)	0.94	1.22 (0.89–1.68)	1.27 (1.09–1.48)	0.83
Estrogen + progestin HT use						
Per 5 years of use	1.30 (0.76–2.25)	1.19 (0.90–1.59)	0.79	1.52 (0.87–2.67)	1.23 (0.92–1.64)	0.51

Abbreviations: HT, hormone therapy; OC, oral contraceptive.

[a]Stratified by calendar year and cohort and adjusted for age.

[b]Stratified by calendar year and cohort and adjusted for age, menopausal status, tubal ligation, duration of OC use, parity, and estrogen-only HT use.

[c]Conducted among postmenopausal women.

[d]The multivariable model for lifetime ovulatory years did not adjust for duration of OC use and parity.

ADRB2 expression was suggestively associated with higher mortality (HR, 1.34; 95% CI, 0.91–1.99; Table 5). Adjustment for age at diagnosis and tumor characteristics, particularly stage, substantially attenuated this association (HR, 1.05; 95% CI, 0.69–1.59). However, the association was potentially modified by the composite stress/distress indicator ($P_{interaction} = 0.09$). The HRs (95% CIs) for mortality comparing ADRB2-positive versus ADRB2-negative tumors were 1.85 (0.89–3.86) for chronically distressed cases and 0.73 (0.41–1.28) for cases without chronic distress.

Discussion

Our analysis suggests that psychosocial factors, including anxiety and depression, were positively and more strongly associated with ADRB2-positive ovarian tumors. These findings support our hypothesis that chronic distress may promote ovarian cancer development through SNS activation and the β_2-adrenergic signaling pathway. We also observed that LOYs and several contributing factors (parity, oral contraceptive use, and age at menopause) were differentially associated with ovarian cancer risk by ADRB2. In addition, while based on small numbers, ADRB2 expression may be associated with increased mortality in the presence of chronic distress. To our knowledge, this is the first study in humans that explores the interplay of ADRB2 with ovarian cancer risk factors and its potential impact on survival.

The psychosocial factors evaluated in this study can be broadly categorized as reflecting emotional distress (depression, anxiety) or chronic stressors (job strain, caregiver stress, widowhood; ref. 29). Interestingly, factors that were associated with risk and suggestively differed by ADRB2 were emotional factors. Emotional distress, which is often preceded by exposure to chronic stressors and represents the consequences of a negative response to stressors, is associated with dysregulated SNS activity and increased norepinephrine levels (13–17). The stronger associations with ADRB2-positive tumors suggest that these emotional factors could influence ovarian cancer risk via β_2-adrenergic signaling. In contrast, exposure to chronic stressors alone may not necessarily result in adverse biologic responses, depending on how one perceives, responds to, and copes with the stressors (30, 31). For example, 91% of NHS nurses reported caregiving as rewarding and among those who self-rated caregiving as stressful, 83% also perceived caregiving as rewarding. Therefore, caregiving, which is perceived as a chronic stressor in the general population (32), may not confer stress-induced negative impacts on nurses who are professionals trained to provide care. Considering the small sample size and the homogeneous population, these findings should be investigated in larger studies with more diverse populations.

While our initial hypothesis did not speculate that the associations with reproductive and hormonal factors would differ by ADRB2, we observed significant heterogeneity in LOYs and in 3 of its determinants—parity, oral contraceptive use, and age at menopause. It is well-documented that plasma norepinephrine levels are significantly elevated during ovulation (33, 34). Given the direct sympathetic neural innervation in

Table 4. Associations of ovarian cancer with psychosocial factors by ADRB2 status

	Age-adjusted model[a]			Multivariable model[b]		
	ADRB2			ADRB2		
	Positive	Negative	$P_{heterogeneity}$	Positive	Negative	$P_{heterogeneity}$
Depression						
No	1.00 (ref)	1.00 (ref)	0.23	1.00 (ref)	1.00 (ref)	0.22
Yes	2.36 (0.87–6.42)	1.14 (0.65–2.01)		2.33 (0.85–6.35)	1.10 (0.62–1.94)	
Elevated anxiety symptoms						
No	1.00 (ref)	1.00 (ref)	0.07	1.00 (ref)	1.00 (ref)	0.07
Yes	2.60 (1.16–5.87)	1.16 (0.81–1.66)		2.59 (1.15–5.84)	1.16 (0.81–1.66)	
Marital status						
Married	1.00 (ref)	1.00 (ref)	0.58	1.00 (ref)	1.00 (ref)	0.62
Divorced/Separated	0.70 (0.16–2.94)	1.19 (0.73–1.93)		0.73 (0.17–3.08)	1.12 (0.68–1.84)	
Widowed	1.37 (0.50–3.72)	0.91 (0.51–1.61)		1.40 (0.52–3.80)	0.90 (0.51–1.60)	
Composite stress indicator[c]						
No	1.00 (ref)	1.00 (ref)	0.09	1.00 (ref)	1.00 (ref)	0.09
Yes	2.55 (1.30–5.02)	1.33 (0.97–1.83)		2.52 (1.28–4.95)	1.31 (0.95–1.80)	
Caregiving hours						
Per 5 h/wk	0.95 (0.84–1.08)	0.99 (0.95–1.03)	0.49	0.94 (0.82–1.08)	0.99 (0.96–1.03)	0.39
Job demand						
Low	1.00 (ref)	1.00 (ref)	0.82	1.00 (ref)	1.00 (ref)	0.80
High	0.81 (0.26–2.57)	0.70 (0.44–1.13)		0.82 (0.26–2.61)	0.70 (0.44–1.13)	
Job control						
Low	1.00 (ref)	1.00 (ref)	0.36	1.00 (ref)	1.00 (ref)	0.34
High	2.01 (0.63–6.39)	1.13 (0.70–1.81)		2.05 (0.64–6.52)	1.12 (0.70–1.79)	

[a]Stratified by calendar year and cohort and adjusted for age.
[b]Stratified by calendar year and cohort and adjusted for age, menopausal status, tubal ligation, duration of oral contraceptive use, parity, and estrogen-only hormone therapy use.
[c]Defined as having one or more of the following: depression, elevated anxiety symptoms, or widowhood.

the ovary, the ovaries and surrounding tissues are likely directly exposed to high norepinephrine level during ovulation (7, 8). One possibility is that ovulation is a physical stressor that leads to similar SNS responses as chronic distress and influences ADRB2-positive ovarian cancer risk through adrenergic signaling. However, this hypothesis does not explain why higher parity, which corresponds to fewer LOYs, was associated with a slightly increased risk of ADRB2-positive tumors. Although effects of higher parity may travel through some psychosocial pathways (e.g., higher parity leads to higher levels of distress related to childbearing or rearing; refs. 35–38), other biological mechanisms, such as the possibility of selective ADRB2 expression in specific tissues of origin of ovarian cancer that are more strongly influenced by reproductive factors, are also possible and require further investigation.

The results from the analysis of ovarian cancer survival suggest that the influence of ADRB2 on mortality may be limited to patients with ovarian cancer experiencing chronic distress. This is not surprising, given that ADRB2 is considered a mediator on the biologic pathway through which chronic stress promotes ovarian tumor growth. Our data provide additional support that β_2-adrenergic signaling is a key biologic pathway that mediates the effect of biobehavioral stress on tumor progression (2, 6–8).

Table 5. Ovarian cancer mortality according to ADRB2

Positive vs. negative	Cases/Deaths	HR (95% CI)
Age-adjusted model	237/147	1.34 (0.91–1.99)
Multivariable model[a]	237/147	1.05 (0.69–1.59)
With stress indicators[a,b]	86/55	1.85 (0.89–3.86)
Without stress indicators[a,b]	151/92	0.73 (0.41–1.28)
$P_{interaction}$		0.09

[a]Adjusted for age at diagnosis, tumor histology, stage, and grade.
[b]Defined as having one or more of the following: depression, elevated anxiety symptoms, or widowhood.

However, given the small sample size, additional studies examining these associations are warranted.

Our findings warrant cautious interpretation. First, as the analyses were based on a small sample size, the possibility of chance findings cannot be excluded. Second, we only had data for a subset of the cases, which may result in selection bias. However, we believe that such bias is minimal, as the distributions of tumor characteristics and most of the risk factors were similar comparing this subset with other cases in the cohorts. Also, the associations with reproductive, hormonal, and psychosocial factors were consistent with prior findings in the full cohorts (19, 20, 39). Third, because of the sample size, we did not adjust for stress-related behavioral factors, such as smoking, obesity, or physical inactivity, to assess potential residual confounding. Given that these factors are generally weakly associated with ovarian cancer (40–42), it is unlikely that accounting for them would alter the results materially. Adjusting for these behavioral factors in our prior analysis of depression/anxiety had little influence on the relative risk estimates (19, 20). Finally, the homogeneity of the study subjects (predominantly white registered nurses in the United States) may limit the generalizability of our results.

Psychosocial factors reflecting emotional distress as well as certain reproductive and hormonal factors related to LOYs may be more strongly associated with risk of ADRB2-positive ovarian cancer tumors. Tumor ADRB2 expression may worsen ovarian cancer survival among patients experiencing chronic distress. These findings, although they need to be confirmed in larger studies, provide further insights into the importance of β_2-adrenergic signaling pathway in stress-induced ovarian cancer development and progression.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: T. Huang, S.S. Tworoger, J.L. Hecht, A.K. Sood, E.M. Poole
Development of methodology: T. Huang
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S.S. Tworoger, J.L. Hecht, E.M. Poole
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): T. Huang, S.S. Tworoger, L.D. Kubzansky, E.M. Poole
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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): T. Huang
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Acknowledgments

We would like to thank the participants and staff of the Nurses' Health Study and Nurses' Health Study II for their valuable contributions as well as the following state cancer registries for their help: AL, AZ, AR, CA, CO, CT, DE, FL, GA, ID, IL, IN, IA, KY, LA, ME, MD, MA, MI, NE, NH, NJ, NY, NC, ND, OH, OK, OR, PA, RI, SC, TN, TX, VA, WA, WY. The authors assume full responsibility for analyses and interpretation of these data.

Grant Support

E.M. Poole was supported by a DoD OCRP award (W81XWH-13-1-0493). S.S. Tworoger was supported by a grant from NCI (R01 CA163451). A.K. Sood received a grant from NCI (R01 CA109298). The Nurses' Health Studies were supported by grants from the NIH (UM1 CA186107, P01 CA87969, UM1 CA176726).

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Received July 5, 2016; revised August 23, 2016; accepted August 24, 2016; published OnlineFirst September 1, 2016.

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