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14. ABSTRACT

Mouse models suggest that chronic stress promotes ovarian tumorigenesis, but the relationship between stress and ovarian cancer has never been evaluated in humans. Over the last year of the grant, we published an analysis demonstrating that exposure to chronic stress leads to tumors that express the beta-2 adrenergic receptor, the signaling pathway identified in mouse models. We have also made progress developing a metabolomic signature of post-traumatic stress disorder (PTSD), a sentinel chronic stress condition. Overall, our continuing work on the role of stress in ovarian cancer development indicates that chronic stress may increase risk of developing ovarian cancer.

15. SUBJECT TERMS
ovarian cancer, psychosocial stress, depression, anxiety, social support, metabolomics

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INTRODUCTION

The objective of this Ovarian Cancer Academy award was to evaluate the role of psychosocial stress in ovarian cancer risk through multiple measures of stress. This study was conducted in the Nurses' Health Studies (NHS and NHSII), two large prospective cohorts with about 1200 ovarian cancer cases between the two studies. In these two study populations, we have repeated questionnaires in which we have queried psychosocial stress, as well as pre-diagnostic blood specimens on 350 cases, and tissue blocks on 250 cases. The first specific aim of this application was to examine whether self-reported stress exposures (depressive symptoms, phobic anxiety, social support, job strain, care-giving stress) are associated with ovarian cancer risk. Also, in this aim, we evaluated whether any associations are stronger for tumors which express the β_2 adrenergic receptors, as studies in mouse models have suggested that β_2 adrenergic receptor activation drives ovarian tumorigenesis. In the second aim, we used metabolomic profiling of women with and without post-traumatic stress disorder (PTSD) to derive a signature of chronic stress. We will apply that metabolomic stress signature to study women with and without ovarian cancer. As secondary aims, we evaluated whether stress is more strongly associated with more aggressive tumors (defined by how quickly fatal the tumors are, and by likely tubal vs. ovarian origin) and will leverage the metabolomics data to query other potential pathways of interest, including lipid dysregulation.

KEYWORDS

Ovarian cancer, psychosocial stress, anxiety, depression, social support, metabolomics

ACCOMPLISHMENTS

The major goals of this project were 1) to evaluate whether self-reported psychosocial stress is associated with ovarian cancer, particularly for tumors that express the β2-adrenergic receptor (SOW task 1); 2) to develop a metabolomic signature of chronic stress (SOW task 2); 3) to evaluate whether the PTSD metabolomic signature described in Task 2 is associated with risk of ovarian cancer (SOW task 3); to evaluate whether metabolomic biomarkers of lipid dysregulation are associated with ovarian cancer risk (SOW task 4); and 5) career development (SOW task 5).

Dr. Poole, the PI of this mentored career award, has accepted a non-academic job and will not complete the project. The remaining money awarded for this project will be returned to the Department of Defense and the aims will be completed by Dr. Tworoger, Dr. Poole's mentor on this award, and Dr. Oana Zeleznik, a post-doctoral fellow on the project. More details are provided in the transition plan (see Appendix, p. XX). Below is a description of status of each task in the SOW.

Task 1 has been completed. All manuscripts relating to task 1 have been submitted and most have been published (see section 6 below for the references). In general, we observed that measures of distress (e.g., depression) were associated with a modest increased risk of ovarian cancer, but that specific stressors (e.g., caregiver burden) were not. Of particularly interest, our analyses showed that many stress exposures (e.g., depression, phobic anxiety) seem to only be associated with increased risk of tumors that express the β2-adrenergic receptor, although the numbers are small (see Table 1).

Table 1. Differences in stress exposure associations (RR; 95% CI) by tumor β2-adrenergic receptor expression

	Tumor β2-adrenergic receptor expression		
	Positive	Negative	P-het
Depression	2.33 (0.85, 6.35)	1.14 (0.65, 2.01)	0.23
Phobic anxiety	2.60 (1.16, 5.87)	1.16 (0.81, 1.66)	0.07
Caregiver stress	0.95 (0.84, 1.08)	0.99 (0.95, 1.03)	0.49
High job demand	0.81 (0.26, 2.57)	0.70 (0.44, 1.13)	0.82
High job control	2.01 (0.63, 6.39)	1.13 (0.70, 1.81)	0.36

Relative risk (RR) and 95% confidence interval (CI) adjusting for age, known ovarian cancer risk factors, and cohort (NHS vs. NHSII)

Task 2 (the development of a metabolomics score of PTSD) is completed and the manuscript is in preparation. In this task, Dr. Poole mentored post-doctoral fellow, Oana Zeleznik to the subtasks. Dr. Zeleznik has completed analyses of the association of metabolites with PTSD. We assayed 239 known and 1996 unknown metabolites in 100 women with PTSD, 100 women with no trauma exposure, and 25 women exposed to trauma, but who did not develop PTSD. We identified 78 metabolites associated with PTSD at a false

discovery rate (FDR) <0.20. Known metabolites associated with PTSD included glutamate, serotonin, tryptophan, C16:0 ceramide, C34:2 diacylglycerol (DAG), C34:3 DAG, C36:4 DAG, and C38:3 PE plasmalogen. The manuscript is now in preparation and we expect to submit it by May, 2017.

For task 3; we completed 3a (selection of samples) in prior grant periods. In this period, we completed the metabolomics assays (3b) on 300 ovarian cancer cases and 300 matched non-cancer controls. Drs. Poole and Zeleznik began working on data analyses during year 3 of this project. Drs. Zeleznik and Tworoger will continue the data analyes (Tasks 3c-d); we anticipate that manuscript preparation will begin in September 2017

Task 4 (the analysis of lipid metabolites) is ongoing. We anticipate that this task will be completed in conjunction with Task 3 and that the manuscript will be drafted in September, 2017.

For task 5 (career development), Dr. Poole had many opportunities for career development over the course of this project. She met weekly with Dr. Tworoger (her Academy mentor) and monthly with Dr. Kubzansky (a co-mentor on this project). Dr. Poole led bi-weekly meetings of our internal Ovarian Cancer Analysis Group (OCAG) with her mentor, Dr. Tworoger, as well as with Dr. Katie Terry, a fellow Ovarian Cancer Academy Early Career Investigator. Dr. Poole also attended the regular bi-monthly meetings of the stress and cancer working group. Regarding scientific conferences, Dr. Poole attended the AACR/Rivkin Special Conference on Ovarian Cancer in October 2015, the Dana Farber/Harvard Cancer Center (DF/HCC) annual breast and gynecologic cancer retreat in March, 2016, the annual meeting of the Ovarian Cancer Association Consortium in April, 2016, the Society for Epidemiologic Research (SER) annual meeting in June, 2016, the DoD Ovarian Cancer Academy meeting in September 2016, and the Rivkin Center's Ovarian Cancer symposium in September 2016 (held in conjunction with the DoD academy meeting).

In addition to the career development tasks outlined in the statement of work, Dr. Poole had a leadership role as the Associate Director for omics data for the Harvard Cohorts (including the Nurses' Health Studies) and as director of the Channing Division of Network Medicine (CDNM)'s Junior Faculty group, which meets bi-weekly to discuss career challenges, present grant aims and receive feedback, and invites outside speakers to provide career advice. Dr. Poole was also a peer mentor to three post-doctoral fellows working in the CDNM.

Results from the ongoing research in the role of stress in ovarian cancer have been communicated to the scientific community in various ways. Dr. Poole presented on her work at local meetings (the DF/HCC annual breast and gynecologic cancer retreat), the Society for Epidemiologic Research, and the Rivkin Symposium on ovarian cancer. Her students and post-docs have also presented posters at local and international meetings. For example, Dr. Zeleznik will present a poster on the PTSD metabolomics signature in November 2016 at a metabolomics meeting.

IMPACT

The major impact of this project to date is the evidence that self-reported psychosocial stress seems to be related to developing ovarian cancer, the first demonstration of this in humans. While this adds to the evidence that stress management is important for long-term health, validation in other studies is required. The upcoming work on metabolomics will help elucidate the biologic underpinnings linking stress to ovarian cancer risk.

CHANGES/PROBLEMS

Nothing to Report

PRODUCTS

Publications, conference papers, and presentations

Journal publications.

1. Huang T, Poole EM, Okereke OI, Kubzansky LD, Eliassen AH, Sood AK, Wang M, Tworoger SS. Depression and risk of epithelial ovarian cancer: Results from two large prospective cohort studies. Gynecol Oncol. 2015 Dec;139(3):481-6.

2. Huang T, Poole EM, Eliassen AH, Okereke OI, Kubzansky LD, Sood AK, Forman JP, Tworoger SS. Hypertension, use of antihypertensive medications, and risk of epithelial ovarian cancer. International journal of cancer. 2016; 139(2):291-9.
3. Poole EM, Kubzansky LD, Sood AK, Okereke OI, Tworoger SS. A prospective study of phobic anxiety, risk of ovarian cancer, and survival among patients. Cancer causes & control : CCC. 2016; 27(5):661-8.
4. Huang T, Tworoger SS, Hecht JL, Rice MS, Sood AK, Kubzansky LD, Poole EM. Association of ovarian tumor β2-adrenergic receptor status with ovarian cancer risk factors and survival. Cancer Epidemiol Biomarkers Prev. 2016; 25(12):1587-94.
5. Trudel-Fitzgerald C, Poole EM, Idahl A, Lundin E, Sood AK, Kawachi I, Kubzansky LD, Tworoger SS. The association of work characteristics with ovarian cancer risk and mortality. Psychosom Med. 2017, epub.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name:	Elizabeth Poole
Project role:	PI
Research Identifier	ORCID: 0000-0002-4680-4587
Nearest person month worked	7
Contribution to Project	PI – coordinated all analyses and assays
Funding Support	

Name:	Shelley Tworoger
Project role:	Mentor/co-investigator
Research Identifier	ORCID: 0000-0002-6986-7046
Nearest person month worked	1
Contribution to Project	Project mentor – provided guidance and feedback on all tasks; met weekly with Dr. Poole
Funding Support	

Name:	Tianyi Huang
Project role:	Post-doctoral fellow
Research Identifier	ORCID: 0000-0001-8420-9167
Nearest person month worked	1
Contribution to Project	Performed analyses of β2-adrenergic receptor
Funding Support	US National Cancer Institute

Name:	Claudia Trudel-Fitzgerald
Project role:	Post-doctoral fellow
Research Identifier	ORCID: 0000-0001-9989-4259
Nearest person month worked	1
Contribution to Project	Performed analyses of job strain and risk of ovarian cancer
Funding Support	Canadian National Cancer Institute

Name:	Mollie Barnard
Project role:	Doctoral student
Research Identifier	N/A
Nearest person month worked	1
Contribution to Project	Performed analyses of caregiver burden and risk of ovarian cancer
Funding Support	US National Cancer Institute
Name:	Oana Zeleznik
Project role:	Post-doctoral fellow

Research Identifier	ORCID: 0000-0002-8705-1163
Nearest person month worked	6
Contribution to Project	Developed metabolomics signature of PTSD
Funding Support	US National Cancer Institute

No changes in active support to be reported.

No other organizations were involved as partners.

APPENDICES

Transition plan for Grant W81XWH-13-1-0493

Dr. Poole has accepted a non-academic job and is giving up her DoD Academy grant (W81XWH-13-1-0493). However, as the metabolomics assays have already been completed, she has worked with her mentor to make a plan to complete the aims in this grant. Below is the full statement of work, with the plan (or completed results) detailed in **bold**. In brief, Dr. Tworoger, Dr. Poole's mentor on this project, will oversee a post-doctoral fellow, Dr. Oana Zeleznik, in completing these projects. Dr. Poole will be available for questions that may arise during the completion of the aims and will be a co-author on the projects.

This grant was in its fourth year. There is $295,023 in total costs remaining in the budget. This money will be returned to the DoD. Although the work will continue to complete the aims of this grant, both Drs. Tworoger and Zeleznik have other funds that will allow them to complete this work. No further funding from the OCRP will be requested for this project.

Task 1. Evaluate whether self-reported psychosocial stress is associated with ovarian cancer, particularly for tumors that express the β_2-adrenergic receptor (Aims 1 and 3). **ALL SUBTASKS ASSOCIATED WITH TASK 1 HAVE BEEN COMPLETED.**

a. Obtain IRB approval (Month 1)
b. Perform statistical analyses of stress metrics in relation to overall ovarian cancer risk (Months 2-8).
c. Complete construction of tumor tissue microarrays (TMAs), funded by P01 CA87969 (PI: Stampfer, Project Leader: Tworoger) (Months 3-4)
   Dr. Poole will work with Dr. Jonathan Hecht to identify representative sections of tumors for inclusion in TMAs. TMAs will be constructed at the Pathology Core Laboratory at the Brigham and Women's Hospital. Dr. Poole will work with the Core Laboratory to answer any questions and to coordinate TMA construction.
d. Stain tumor tissue microarrays (TMAs) for expression of the β_2-adrenergic receptor (Month 5-6).
   The TMAs will be stained in the Pathology Core laboratory at Brigham and Women's Hospital.
e. Pathologist evaluates β_2-adrenergic receptor staining (Months 7-8).
f. Perform statistical analysis of psychosocial measures and ovarian cancer risk stratified by expression of the β_2-adrenergic receptor as well as for aggressive tumors using competing risks Cox proportional hazard modeling (Months 9-15).
g. Manuscript preparation (Months 16-18).

Task 2. Develop a metabolomic signature of chronic stress (Aim 2) **THIS AIM IS IN PROGRESS; DR. OANA ZELEZNIK IS LEADING THIS ANALYSIS AND THE MANUSCRIPT IS IN**

PREPARATION. DR. TWOROGER WILL LEAD COMPLETION OF THIS MANUSCRIPT AND DR. JESSICA LASKY-SU, AN EXPERT IN METABOLOMICS AT THE CHANNING DIVISION OF NETWORK MEDICINE, WILL PROVIDE ADDITIONAL MENTORING WHERE NEEDED. WE EXPECT THAT THE MANUSCRIPT WILL BE SUBMITTED BY 4/30/2017. DR. POOLE WILL BE A CO-AUTHOR ON THIS MANUSCRIPT.

a. Select 75 Nurses' Health Study II (NHSII) women with no trauma exposure (little chronic stress), 75 women with trauma who did not develop PTSD (acute stress without long-term chronic stress), and 75 women who have PTSD (high long-term chronic stress), pull samples from the Biorepository, randomly sort samples, and add blinded QCs (Months 19-21).
b. Perform metabolomics assays on these 225 women plus 10% QC samples (Months 22-24).
   Metabolomics assays will be performed at the Broad Institute in Dr. Clary Clish's laboratory (see letter of collaboration from Dr. Clish). Dr. Poole will work closely with Dr. Clish to coordinate these assays and to address any problems that arise.
c. Develop a signature that distinguishes women with PTSD from the 2 non-PTSD groups in 2/3 of the 225 women (Months 25-28).
d. Validate the PTSD metabolomic signature in the remaining women (Months 29-30).
e. Manuscript preparation (Months 31-32).

Task 3. Evaluate whether the PTSD metabolomic signature described in Task 2 is associated with risk of ovarian cancer (Aims 2 and 3). **WE HAVE RECEIVED THE METABOLOMICS ASSAY RESULTS (TASKS 3A-3C) FOR THIS TASK AND ANALYSES ARE ONGOING (TASK 3D). DR. TWOROGER WILL OVERSEE COMPLETION OF THIS ANALYSIS, WITH DR. ZELEZNIK TAKING THE LEAD ON THE ANALYSIS. WE EXPECT TO BEGIN MANUSCRIPT PREPARATION IN SEPTEMBER 2017. DR. POOLE WILL BE A CO-AUTHOR ON THIS MANUSCRIPT.**

a. Select appropriate ovarian cancer cases and matched controls from the NHS and NHSII, pull samples from the Biorepository, sort samples into matched case-control order, add QCs, and blind samples (Months 31-35).
b. Perform metabolomics assays on these 626 women plus 10% QC samples (Months 36-41).
c. Calculate the metabolomic signature of stress in the ovarian cancer nested case-control study (Month 42).
d. Perform statistical analyses of the stress signature in relation to ovarian cancer risk (Months 43-45).
e. Perform statistical analyses to determine whether the associations with the metabolomic stress signature are stronger for more aggressive disease or for women at high risk of ovarian cancer (Months 46-48).
f. Manuscript preparation (Months 49-51).

Task 4. Evaluate whether metabolomic biomarkers of lipid disregulation are associated with ovarian cancer risk (Aim 4). **SEE TASK 3 – ANALYSES OF LIPID BIOMARKERS ARE ONGOING AND DRS. ZELEZNIK AND TWOROGER ARE LEADING THIS ANALYSIS. DR. POOLE WILL SERVE AS A CO-AUTHOR ON THIS MANUSCRIPT.**

a. Perform a statistical analysis of lipid-related biomarkers and risk of ovarian cancer in the 626 cases and controls from the ovarian cancer nested case-control study described in Task 3 (Months 52-54).
b. Perform statistical analyses to determine whether the associations with lipid biomarkers are stronger for more aggressive disease or for women at high risk of ovarian cancer (Months 55-57).
c. Manuscript preparation (Months 58-60).