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TITLE: Urinary Level of Prostaglandin E2 Metabolite and Risk of Incident Breast Cancer

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14. ABSTRACT
This ongoing case-cohort analysis examines how urinary levels of prostaglandin E_2 (PGE_2) metabolite interacts with estrogen biosynthesis and influences breast cancer risk in postmenopausal women. The study includes 301 breast cancer cases and 308, a subsample of the cohort, who were aged 50 years or older, were postmenopausal and did not report current use of hormones. This case-cohort set mostly comprises white women with mean (standard deviation [SD]) age of 61.4 years (6.0). Approximately 70% of women underwent menopause naturally, and mean age at menopause was older in women reporting a natural menopause compared to those reporting a surgical menopause (50.8 years [SD=4.5] vs. 42.8 years [SD=8.0]). Prevalence of overweight and obesity was 66%. Few were current smokers (8%). About 60% of women reported ever use of nonsteroidal anti-inflammatory drugs (NSAIDs), with median pill-years of 24.5 (interquartile range: 7.5-55.1) among ever users. Approximately 40% of women also reported taking NSAIDs within 24 hours of urine collection; 75% of these women were chronic users of NSAIDs who reported at least 5 years of NSAID use. Urinary levels of PGE-M are being measured using liquid chromatography/tandem mass spectrometry (LC/MS/MS), which is generally accepted as the most accurate index of endogenous PGE_2 formation. A recently-developed high-performance liquid chromatography/mass spectrometry method is being used to determine concentrations of 15 estrogens/estrogen metabolites with creatinine correction for urine dilution. It is expected that the present study will contribute to understanding the role of inflammation in estrogen biosynthesis and breast cancer risk in postmenopausal women.

15. SUBJECT TERMS
Breast Cancer; Inflammation; Prostaglandin; Estrogen

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Introduction

This training grant is a Postdoctoral Fellowship Award in breast cancer research. The project involves focused mentorship and training in breast cancer research, with a particular focus on molecular epidemiology of breast cancer. The training program outlined in the grant emphasizes three main areas to further develop skills and knowledge that are required to independently carry out innovative multi-disciplinary breast cancer research as follows: 1) advancing skills in epidemiologic methods and quantitative analysis; 2) broadening knowledge in breast cancer biology; and 3) acquiring working knowledge of molecular biology techniques. The research segment of this grant is conducting a case-cohort study within the Sister Study, a large prospective cohort study of women with family history of breast cancer, to investigate the major determinants of urinary prostaglandin E_2-metabolite (PGE-M) and how the urinary level of PGE-M interacts with estrogen biosynthesis in relation to breast cancer risk in postmenopausal women. This study includes a total of 609 women (301 cases and 308 subcohort members) who were aged 50 or older, were postmenopausal and did not report current use of hormones at the time of enrollment. First morning urine samples collected from these women are being analyzed for PGE-M, 15 estrogens/estrogen metabolites and creatinine. This project was added to the Sister Study protocol, and was reviewed and approved by the NIEHS IRB on December 22, 2009.

Body

This project received notice of award on October 12, 2009 and notification of final approval on November 29, 2009 stating that the project was scheduled to begin on January 2, 2010. Training and preparation for the proposed research project began immediately after the final notification. However, there was substantial delay in searching for the best way to obligate funds and to transfer them to a contract laboratory. During this time period, the PI became aware of a new method to quantify urinary estrogens developed at NCI. Compared to the radioimmunoassay orignally proposed, this new method (high-performance liquid chromatography (HLPC)-electrospray ionization (ESI)-mass spectrometry) has higher sensitivity and specificity, measuring 15 estrogen and estrogen metabolites using a single 0.5 ml urine sample. Because of the scientific merit of this new assay, the PI and her mentors took several steps over the past few months to adopt this method in the proposed research project. Unfortunately, at that time, only the NCI laboratory had the assay up and running and they were not able to take on the additional work required for this new research. The PI identified a laboratory that began planning to adapt the assay and provide information on quality assuarnce. It was not until the PI submitted a proposal for a quality control experiment to the NCI to compare results between the selected laboratory and the NCI lab that it became clear that the assay had recently been licensed to a different laboratory. Arrangments have now been made to have the work done in the only licensed laboratory after completing several quality assurance steps to assess the performance of this laboratory. Collaboration with the NCI has been secured for an inter-laboratory comparison of urinary estrogen measurements using the new technique. This collaborative work as well as additional quality control experiments for short-term reproducibility of PGE-M measurements was reported in detail in the annual scientific report reviewed on June, 2010. While these new changes will enhance the quality of the proposed research project, they did lead to unavoidable delay in the proposed timeline. However, the PI has taken advantage of this time by working on other breast cancer research projects to further her training. She has presented her research in seminars at the NIH and other academic institutions as a part of search for a tenure-track faculty position in breast cancer research and has also presented at departmental seminars at NIEHS. Key research accomplishments and major activities are outlined in the sections that follow.

Key Research Accomplishments

<u>Progress in proposed research project</u>

- Sample selection and evaluation of data quality of the selected samples were initiated immediately after the project approval. The PI works closely with Cynthia Kleeberger, the contract data manager who oversees biospecimens collection for the Sister Study.

- Protocols for sample preparation and batch arrangement for each of analytes in the project have been documented and distributed to the sample coordinator, and the PI has met with the data management team (staff at data repository, biospecimens collection) to address questions regarding this project.
- Design of the funded study and characteristics of the study subjects have been summarized and submitted as an abstract for presentation at the 2011 Era of Hope meeting
- Urine samples have been pulled for PGE-M and creatinine analyses and are currently being pulled for estrogen analyses
- Laboratory analyses are expected to be completed by the second quarter of this year; data analyses will begin immediately after the completion of the laboratory analyses

Training

- Regular meetings with mentors (Drs. Dale Sandler and Jack Taylor) have been held to discuss the research project, ongoing training, other related projects and strategies for progress of the project and future work
- Consulted with Dr. Grace Kissling, a biostatistician at NIEHS for guidance in the design of quality control experiments that were added to the funded project at no cost to the granting agency
- Attend Friday Cancer Epidemiology Journal Club at UNC-Chapel Hill
- Frequently attend seminars in the Laboratory of Molecular Carcinogenesis; met individually with invited speaker, Dr. Andrew Dannenberg, who is a MD/molecular biologist with expertise in the connection between chronic inflammation and cancer with an emphasis on prostaglandin biology

Other breast cancer research projects

- Collaborated with Dr. Jack Taylor and postdoctoral fellow Dr. Sophie Bolick on short-term reproducibility experiments of DNA methylation and mitochondrial DNA measurements, which are being examined as a potential biomarker of breast cancer in the Sister Study
- Submitted a manuscript on telomere length in blood and breast cancer risk in the Sister Study. In this prospective cohort of women aged 35-74 years, no association was observed between relative telomere length in blood and breast cancer risk. Subgroup analyses by menopausal status, invasiveness or estrogen-receptor status of breast cancer did not reveal evidence of association between telomere length in blood cells and subsequent breast cancer risk. This prospective investigation, along with two recent prospective investigations, does not support telomere length in blood cells as a biomarker for breast cancer risk.
- Prepared a manuscript on short-term reproducibility of telomere length measurement in blood cells demonstrating good short-term reproducibility of telomere length measurement using blood from a single draw. However, the existence of technical variability, particularly plate effects, reinforces the need for technical replicates and balancing of case and control samples across plates.

Reportable Outcomes

Manuscripts

Kim S, Sandler DP, Carswell G, DeRoo LA, Parks CG, Cawthon RM, Weinberg CR, Taylor JA. Telomere length in peripheral blood and breast cancer risk in a prospective case-cohort analysis: results from the Sister Study *Submitted*

Kim S, Sandler DP, Carswell G, Cawthon RM, Weinberg CR, Taylor JA. Reproducibility and short-term intra-individual variability of telomere length measurement using a monochrome multiplexing quantitative PCR. *Manuscript in Preparation*

Bolick SC, White AJ, **Kim S**, Carswell G, Xu Z, Sandler DP, Taylor JA. LINE-1 Methylation Levels Remain Stable in Peripheral Blood DNA from Healthy Women. *Manuscript in Preparation*

Kim S, DeRoo LA, Sandler DP (2010). Eating Patterns and Nutritional Characteristics Associated with Sleep Duration. Public Health Nutrition. Oct 29:1-7.

Kim S, Sandler DP, Galanko J, Martin C, Sandler RS (2010). Intake of Polyunsaturated Fatty Acids and Distal Large Bowel Cancer Risk in Whites and African Americans. American Journal of Epidemiology. 171(9); 969-79.

Poster

Kim S, Sandler DP, Carswell G, Cawthon RM, Weinberg CR, Taylor JA. Reproducibility and short-term intra-individual variability of telomere length measurement using a monochrome multiplexing quantitative PCR. 9th Annual American Association for Cancer Research International Conference: Frontiers in Cancer Prevention Research November 7-10, Philadelphia, PA

Presentation

- Presented seminar on "Quantitation of Estrogens in Epidemiologic Studies" in the weekly Epidemiology Branch Meeting, which summarizes the literature and discuss the advantages and disadvantages of various estrogen quantitation methods
- Invited panelist for Molecular Epidemiology Working Group discussion on "Consortia: the Good, the Bad, and the Ugly" at 8th Annual American Association for Cancer Research International Conference: Frontiers in Cancer Prevention Research November 2-5, Houston, TX
- Presented a seminar titled "Obesity and Inflammation: from Colorectal Cancer to Breast Cancer" at NCI (November 20, 2010), NIH-Earl Stadtman Investigator Search (December 15, 2010), Memorial-Sloane Kettering Cancer Center (January 3, 2011), Albert Einstein School of Medicine (January 10, 2011), and Karmanos Cancer Center (January 20, 2011)

Conclusions

In the past year, the DOD Postdoctoral Fellowship provided the PI with invaluable experience in designing and conducting molecular epidemiologic studies. The PI learned about relevant practical issues such as legal implications of using a newly patented technique. These are intangible assets that prepare her to become an independent researcher. These experiences have proven to be an advantage in her career search to become a breast cancer epidemiologist at an academic institution. In the coming months, data from laboratory assays will be generated for the analyses, and the PI will be able to address the study questions proposed in the grant. Depending on the obtained results, the next appropriate scientific steps will follow.

References

None

Appendices

None