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TITLE: Finding Metabolomic Signatures in Pregnancy that Predict Breast Cancer: 60-Year Prospective Study in the Child Health and Development Studies Pregnancy Cohort

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CONTRACTING ORGANIZATION: Emory University, Atlanta, GA

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14. ABSTRACT

In this project, we have proposed to identify predictive gestational biomarkers which could lead to new approaches to prevent breast cancer. For this, we are applying ultrahigh-resolution mass spectrometry-based high-resolution metabolomics (HRM) to analyze 2nd and 3rd trimester archival serum samples of women in the Child Health and Development Studies (CHDS) pregnancy cohort who subsequently developed breast cancer or did not develop breast cancer. HRM data of these two groups will be compared to identify environmental chemicals and metabolites, and biochemical metabolic networks in pregnancy which are linked to subsequent breast cancer occurrence. There were minor delays caused by staff and offices transitioning to remote work due to the COVID-19 pandemic in CHDS (Site 1). CHDS still accomplished all major tasks specified in the first year (obtaining all human subjects' approvals, sample selection, preparation and order of archived pregnancy serum) and will deliver these samples to Emory (Site 2) in this month. Emory has not yet received samples. While waiting for samples, we have expanded our list of chemicals with confirmed identity which we can measure and have proceeded with methods to optimize workflow. A key workflow change has been to establish cloud computing through Emory-sponsored Amazon Web Services (AWS) to obtain more efficient and expandable service to extend chemical coverage.

15. SUBJECT TERMS

Breast cancer, environmental chemicals, high-resolution metabolomics, metabolic pathways, metabolome-wide association study

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1. Introduction

This project is to use high-resolution metabolomics (HRM) to address the overarching challenges, to identify determinants of breast cancer initiation risk or susceptibility in order to prevent breast cancer. The objective is to establish proof of concept that Metabolome-Wide Association Studies **(**MWAS) patterns in pregnant women during the 2nd and 3rd trimesters predict subsequent breast cancer case status for individual women. Emory team will perform HRM on serum samples that are randomly selected and provided by CHDS. These samples are from women with already measured levels of 3rd trimester steroid hormones, available placental morphology and clinical pregnancy measures.

2. Keywords

Breast cancer, environmental chemicals, high-resolution metabolomics, metabolic pathways, metabolome-wide association study.

3. Accomplishments

Major goals: This project is to to study 2nd and 3rd trimester archival serum samples of women in the Child Health and Development Studies (CHDS) pregnancy cohort who subsequently developed breast cancer, compared to 2nd and 3rd trimester archival serum samples from women who did not develop breast cancer, to identify predictive gestational biomarkers which could lead to new approaches to prevent breast cancer. For this, we will apply powerful high-resolution metabolomics (HRM) with advanced computational tools to identify chemicals and biochemical metabolic networks in pregnancy which are linked to subsequent breast cancer occurrence in two Specific Aims.

1) Aim 1 is to conduct MWAS to describe pathways in the 2nd and 3rd trimester that distinguish women who go on to develop breast cancer.
2) Aim 2 is to compare MWAS pathways by time to diagnosis after pregnancy, tumor characteristics (stage at diagnosis, receptor status), age at pregnancy, placenta morphology, and third trimester pregnancy estrogens (estradiol, estrone, estriol).

Accomplishment under these goals: Emory expects to receive 1,179 serum samples from CHDS in next month (April, 2021). CHDS has submitted documents to NICHD to request for serum samples. The Emory team has analyzed the Spectrum library of 2400 chemicals with a minor but important modification of our mass spectrometry analysis which expands ability to detect and readily confirm metabolites in the archival serum samples from the CHDS pregnancy cohort. We changed columns for the liquid chromatography to have smaller particle size and narrower column diameter. This improves the chromatographic resolution so that some chemicals are better separated from others with nearly identical properties. We are in the process of data analysis for the 2400 chemicals in the library to determine which can be reliably measured and ranges of concentration which can be measured. We also have proceeded to optimize workflow through the Emory University Information Technology office to establish cloud computing for data analysis and storage. The Emory-sponsored Amazon Web Services (AWS) gives more efficient and expandable service which will allow us to have more flexibility in threshold selection for data extraction. Specifically, by having greater computing power, we can set thresholds to detect chemicals found in only a small percentage of samples. In the past, we have only extracted data for chemicals found in at least 50% of the samples. With the new capabilities, we expect to be able to measure chemicals present in 10% of the samples. This extended chemical coverage may reveal whether chemicals with infrequent exposure contribute to breast cancer outcome.

Opportunities for training and professional development: The Emory team has supported training of two Emory University PhD students and one MD/MPH student in mass spectrometry methods used for this project. This has included extensive training in computational tools and data analysis using in-laboratory and online resources. Hands-on instrument training in mass spectrometry has been limited because this is better handled by skilled technicians than by trainees.

Dissemination to communities of interest: Nothing to report

Plans during the next funding period: The Emory team expects to receive 1,179 serum samples from CHDS in April, 2021 and plans to analyze 1000 of these pregnancy serum samples within the next funding period. We will work with the CHDS team for best planning to ensure completion of the mass spectral analyses in time to complete the biostatistics and bioinformatics analyses. For mass spectrometry, samples will be processed by our usual extraction procedures and analyzed with the most current methods for liquid chromatography-high resolution mass spectrometry. We will share data with the CHDS team and work with them to begin to test for associations of metabolites and environmental chemicals with breast cancer outcome.

4. Impact

Development of the principal disciplines, technology transfer, society beyond science and technology

The team is working toward development of a functional class designation for environmental chemicals along with other chemicals, such as personal use products, food-derived metabolites, microbiome metabolites and drugs. Many such classification systems are available in specific research areas, but none are suitable for use with the global metabolomics methods used in this project. Thus, we expect to be able to improve impact of this project by also testing whether other chemical exposures, in addition to environmental chemicals, contribute to breast cancer outcome.

5. Changes/Problems

With ongoing return to normal laboratory operations, no changes or problems are anticipated.

6. Products

None to report

7. Participants & Other Collaborating Organizations

Dean P Jones, PhD no change, and Young-Mi Go, PhD no change. Xin Hu, PhD (20%) replaces Ravneet Kaur, MS for biostatistics and bioinformatics support.

8. Special Reporting Requirements

See the award chart in Appendix

9. Appendices

The award chart has been provided in Appendix.

W81XWH2010103: Finding Metabolomic Signatures in Pregnancy that Predict Breast Cancer: 60-Year Prospective Study in the Child Health and Development Studies Pregnancy Cohort

PI: Dean P. Jones, Emory University, Georgia **Budget** $470,190.00

Topic Area: DoD Breast Cancer Research Program **Mechanism:** FY19, BCRP, Breakthrough Award Levels 1 and 2 (Funding Level 1, Partnering PI Option)

Research Area(s): Breast Cancer **Award Status:** March 1, 2020 – February 28, 2023

Study Goals:

We propose to study 2nd and 3rd trimester archival serum samples of women in the Child Health and Development Studies (CHDS) pregnancy cohort who subsequently developed breast cancer, compared to 2nd and 3rd trimester archival serum samples from women who did not develop breast cancer, to identify predictive gestational biomarkers which could lead to new approaches to prevent breast cancer. For this, we will apply powerful high-resolution metabolomics (HRM) with advanced computational tools to identify chemicals and biochemical metabolic networks in pregnancy which are linked to subsequent breast cancer occurrence.

Specific Aims:

Aim 1: Conduct MWAS to describe pathways in the 2nd and 3rd trimester that distinguish women who go on to develop breast cancer.
Aim 2: Compare MWAS pathways by time to diagnosis after pregnancy, tumor characteristics (stage at diagnosis, receptor status), age at pregnancy, placenta morphology, and third trimester pregnancy estrogens (estradiol, estrone, estriol).

Key Accomplishments and Outcomes:

Publications: none to date

Patents: none to date

Funding Obtained: none to date