AD_____

Award Number: DAMD17-99-1-9376

TITLE: Genetic Determinants of Aromatase Expression and Susceptibility to Postmenopausal Breast Cancer

PRINCIPAL INVESTIGATOR: Sue Ann Ingles, Ph.D.

CONTRACTING ORGANIZATION: University of Southern California
Los Angeles, California 90033

REPORT DATE: June 2002

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	June 2002	Annual (1 Jun 01 - 31 May 02)

4. TITLE AND SUBTITLE
Genetic Determinants of Aromatase Expression and Susceptibility to Postmenopausal Breast Cancer

5. FUNDING NUMBERS
DAMD17-99-1-9376

6. AUTHOR(S)
Sue Ann Ingles, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Southern California
Los Angeles, California 90033

E*Mail: ingles@hsc.usc.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. Abstract ***(Maximum 200 Words)*** ***(abstract should contain no proprietary or confidential information)***

The major source of estrogen in postmenopausal women is conversion of androstenedione in adipose tissue to estrone by the enzyme aromatase. In adipose tissue, the aromatase gene, CYP19, is regulated by the cytokines TNF-α and IL-6, which act on a regulatory region of the CYP19 gene called the I.4 promoter. The objective of this study is to determine whether polymorphism in the TNF-α gene, in the IL-6 gene, or in the CYP19 gene I.4 promoter is associated with the plasma estrone (E1) to androstenedione (A) ratio, or with risk of post-menopausal breast cancer. This investigation is being carried out using DNA and serum samples from 1360 women enrolled in the Hawaii-Los Angeles Multiethnic cohort study.

We have identified five new polymorphisms that may have an impact on aromatase expression. Three of the polymorphisms lie in the CYP19 I.4 promoter; the other two lie in the TNF-α gene. The functionality and frequency of these five new polymorphisms are still unknown. If they are found to significantly impact aromatase expression in adipose tissue, these polymorphisms could, either singly or in combination, significantly contribute to breast cancer susceptibility in post-menopausal women.

14. SUBJECT TERMS	15. NUMBER OF PAGES
breast cancer	7
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

INTRODUCTION

The major source of estrogen in postmenopausal women is conversion of androstenedione in adipose tissue to estrone by the enzyme aromatase. In adipose tissue, the aromatase gene, CYP19, is regulated by the cytokines TNF-α and IL-6, which act on a regulatory region of the CYP19 gene called the I.4 promoter. The objective of this study is to determine whether polymorphism in the TNF-α gene, in the IL-6 gene, or in the CYP19 gene I.4 promoter is associated with (1) the plasma estrone (E1) to androstenedione (A) ratio, a phenotypic measure of aromatase expression, or with (2) risk of post-menopausal breast cancer. This investigation is being carried out using DNA and serum samples from 1360 women enrolled in the Hawaii-Los Angeles Multiethnic cohort study.

BODY

At the end of year 3, we are awaiting receipt of DNA samples from the parent study so that we can complete tasks 3, 4, and 8-10 (see below). We have taken a one-year no-cost extension so that we may complete our aims.

Task 1

Task 1 has been completed. PCR amplification conditions were optimized for the genomic region containing the TNFα polymorphism at nucleotide position -308. By cutting PCR products with the NcoI restriction enzyme, control samples were identified for each of the three genotypes, GG, GA, and AA. Sequencing of PCR products for these control subjects unexpectedly revealed two additional, previously unidentified polymorphisms. A -244G→A polymorphism was observed in two African-American subjects, and a -241A→T in one Latina subject. These new polymorphisms are adjacent to a previously recognized, but relatively rare polymorphism at position -238. All three of these polymorphisms, -238G→A, -241A→T, and -244G→A, lie in a Y-box regulatory motif. Because alteration of the Y-box motif by the -238G→A polymorphism is known to influence TNF-α inducibility, it is likely that the newly identified polymorphisms will also have functional effects.
Because new polymorphisms were identified, the TNF-α genotyping (Task 3) will be carried out by sequencing rather than by restriction enzyme cutting, as previously planned. This will allow us to estimate allele frequencies of the new polymorphisms in each of the four ethnic groups (white, Hispanic, African-American, Asian). We will also evaluate the new polymorphisms for association with plasma E1/A ratio (Task 9) and for association with breast cancer risk (Task 10), as described in the original hypothesis for the TNF-α -308G→A polymorphism.

Task 2

Task 2 has been completed. A genotyping assay was designed for the TaqMan system. Two oligonucleotide probes, one specific for the G allele and one for the C allele, were designed and labeled with two different fluorophores. PCR primers were designed to amplify a 100 bp region surrounding the polymorphism. When the labeled probes are included in the PCR reaction mixture, hybridization of the reporter probe to its allele-specific target sequence leads to nucleolytic cleavage of the probe during the DNA synthesis step as a result of the 5'>3' nuclease activity of Taq polymerase. Fluorescence is detected using an ABI 7700 Sequence Detection System and the alleles are scored using Sequence Detector Software (ABI). A series of control samples, to be included in each TaqMan run, were identified by direct sequencing.

Task 3

Task 3 is partially completed. TNF-α genotyping has been completed on 295 samples from women without breast cancer. We have found that the frequency of the -308 A allele varies by ethnicity: 16% in African-Americans, 14% in whites, 8% in Latinas, and 3% in Japanese-Americans.

We are awaiting receipt of DNA from the parent study (The Hawaii-Los Angeles Multiethnic Cohort Study) to finish this aim, and have taken a one year no-cost extension. Personel from the Multi-ethnic cohort study are re-extracting DNA using a new method that will provide DNA of sufficient purity to be used with new automated genotyping methods. In addition, DNA samples will be selected in a manner suitable for case-cohort analysis. (Previously, DNA samples were obtained sequentially.) In the next few months we expect to receive the newly extracted DNA arranged in "case-cohort" plates. Genotyping will be completed at that time.

Task 4

Task 4 is partially completed. IL-6 genotyping has been completed on 315 samples from women without breast cancer. We have found that the frequency of the -174 C allele also varies by ethnicity: 34% in whites, 19% in Latinas, 11% in African-Americans, and 1% in Japanese-Americans.

We are awaiting receipt of DNA from the parent study (The Hawaii-Los Angeles Multiethnic Cohort Study) to finish this aim, and have taken a one year no-cost extension (see Task 3 above).

Task 5
Task 5 has been completed. Five overlapping primer pairs were designed to amplify 1148 basepairs of the CYP19 I.4 promoter region (from position 18 to 1166, according to Genbank L21982). Positions of the primers are given below. Anealing temperature for all primer pairs is 51°C.

Forward primer concentration	Reverse primer	PCR product length	$MgCl_2$
18-39	283-302	285 bp	2.5 mM
197-223	582-600	404 bp	1.5 mM
542-562	882-901	360 bp	2.0 mM
793-811	996-1016	224 bp	2.0 mM
882-901	1145-1166	285 bp	2.5 mM

Task 6
Task 6 has been completed. Serum androstenedione (A) and estrone (E1) levels were available for 235 postmenopausal women who were not on hormone therapy. Serum E1/A ratios were calculated as a phenotypic measure of aromatase activity. Three subjects with low and three with high E1/A ratios were identified in each ethnic group (totally, 12 subjects with low and 12 with high ratios).

Task 7
Task 7 has been completed. For the 24 women identified in task 6, the genomic region containing the CYP19 promoter I.4 was amplified in five overlapping fragments, and these PCR fragments were sequenced. We identified three additional polymorphisms: -303G→A and -302C→A in two African-American women, and -616G→A in two Latina women. The first two of these three polymorphisms lie approximately 20 bp upstream of a GAS regulatory element, which is a target of IL-6 signalling. Polymorphisms in this region may potentially impact regulation of aromatase expression by IL-6. The third polymorphism lies in a regulatory region which appears to silence glucacorticoid-inducible aromatase activity. The funtion and frequency of these polymorphisms are not yet known.

Tasks 8
We are awaiting receipt of DNA from the parent study (The Hawaii-Los Angeles Multiethnic Cohort Study) to finish this aim, and have taken a one year no-cost extension (see Task 3 above).

Tasks 9 and 10
Tasks 9 and 10 will be completed after completion of genotyping (tasks 3 & 4). We have taken a one-year no cost extension to complete these tasks.

KEY RESEARCH ACCOMPLISHMENTS

1. Three new polymorphisms were identified in the CYP19 I.4 promoter region.
2. Two new polymorphisms were identified in the promoter region of the TNF-α gene.

REPORTABLE OUTCOMES

There have been no reportable outcomes to date.

CONCLUSIONS

To date, at the end of year 3, we have identified five new polymorphisms that may have an impact on aromatase expression. Three of the polymorphisms lie in the CYP19 I.4 promoter, the region of the aromatase gene that regulates aromatase expression in adipose tissue. The other two polymorphisms lie in the regulatory region of the TNF-α gene. These polymorphisms, by influencing TNF-α inducibility, might potentially inpact aromatase expression. The functionality and frequency of these five new polymorphisms are still unknown. If they are found to impact aromatase expression in adipose tissue, these polymorphisms could, either singly or in combination, significantly contribute to breast cancer susceptibility in postmenopausal women.

REFERENCES

None.

APPENDICES

None.