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TITLE: Estrogen Receptor Gene Polymorphisms and Breast Cancer Risk

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13. ABSTRACT ***(Maximum 200 Words)***

Purpose: *to test the hypothesis that individual variation in the sensitivity to estrogens whether from endogenous or exogenous sources, affects woman's risk of breast cancer.*

Scope: *to test if the variability in responses to estrogens in women may be determined by a functionally significant variation in the estrogen receptor alpha (ER) though which estrogens exert biologic effects. The ER polymorphisms under study are: Xba-I, Pvu-II and microsatellite polymorphisms. In an exploratory analysis we will study the interaction between the Xba I, Pvu II and microsatellite ER polymorphisms and estrogen-related risk factors for breast cancer such as reproductive history and menopausal hormone use.*

Major findings: *not yet available (biological sample collection and laboratory analysis still ongoing.*

Up-to-date report of the progress in terms of results and significance: *no results are yet available – we are still collecting biological samples, extracting DNA from the biological materials (leukocytes and pathological slides) and processing the laboratory analysis. These activities are following the time schedule as stated in the Proposal to the Army. We expect to have preliminary results by the spring of year 2001.*

14. SUBJECT TERMS
Breast Cancer, estrogen receptor alpha gene polymorphisms, XbaI, PvuII, microsatellite polymorphisms

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(5) INTRODUCTION:

***Subject*:**

The research being carried out aims to clarify how estrogens interact with the estrogen receptor alpha in the causation of breast cancer and to define women at high risk of developing this malignancy.

Purpose:

The hypotheses to be tested in this study are:

1) There is an association between the Xba I and Pvu II polymorphisms in the estrogen receptor alpha gene and the risk of breast cancer;
2) There is an association between microsatellite polymorphisms in the estrogen receptor gene and the risk of breast cancer.

An exploratory hypothesis to be considered in our research is:

3) There will be an interaction between the Xba I, Pvu II and microsatellite ER alpha polymorphisms and estrogen-related risk factors for breast cancer such as reproductive history and menopausal hormone use.

Scope *(i.e.* ***Specific Tasks,*** as outlined in the approved Statement of Work approved by the US Army):

1) To obtain blood samples from 600 breast cancer cases and 600 population based controls. Please note: The sample size has been complemented with an addition of 1200 breast cancer cases and 1200 population controls, sponsored by the NIH, as agreed with the US Army. In the study population at least 300 cases and 300 controls have not been exposed to postmenopausal hormone use. 300 cases and 300 controls, or more, have been regular users of postmenopausal hormones for a minimum of four years.

2) To extract DNA from white blood cells or from normal tissue cells obtained from freshly cut surgical blocks.

3) To assess microsatellite, Xba I and Pvu II germline polymorphisms in the estrogen receptor gene in cases and controls

4) To assess relative risk for breast cancer associated with the microsatellite, Xba I and Pvu II polymorphisms, and to explore interactions between the polymorphisms and estrogen related breast cancer risk factors.

(6) BODY:

In this section we describe research accomplishments associated with each specific task outlined in the approved Statement of Work. We will present the available data, that is the results from the recruitment period.

Since enrolment into the study has just been closed, we still have no findings to report. However, analysis of collected samples, blood and tissue, has begun and reports on results are planned to be written in the spring of 2001.

This study has been funded simultaneously by the NIH and the US Army. An agreement with the US Army was reached at the beginning of 1999 (contact person at the Army is Cheryl R Miles), to avoid overlapping of resources. In the agreement we committed to increase the number of breast cancer cases and control subjects in the study.

Description of accomplishments associated with each task:

Scope 1 and 2 (specific tasks 1 and 2):

Table 1 shows the results of recruitment of subjects into the study. The initial selection of cases and controls was funded by the NIH.

The final selection of subjects and their inclusion in the study was somewhat delayed due to negotiations with the US Army (i.e. our contract with the Army actually started in March 1999, and not in 1997). Information on these negotiations is available through contact person Cheryl R. Miles, US Army.

As the table shows, inclusion of patients and collection of samples, blood and tissue, is now virtually complete.

Table 1. Final recruitment (collection will be finished in December 2000)

	Cases	**Controls**
Study population	1798	1562
Number of blood samples collected	1321 (73,5%)	1156 (74%)
Number of tissue samples (still being collected, includes samples from deceased cases)	392 (21,8%)	-
Number of study population that declined to participate	67 (3,7%)	384 (24,6%)
Number of study population that has not been possible to contact (due to lack of response)	16	14
Total participation rate	**1713 (95,3%)**	**1156 (74%)**

N.B.: collection of tissue samples is in its final stage. Contact has been established with all relevant pathology departments and we expect to complete this task by the end of December 2000.

Current work:

We have recently finished locating and contacting subjects. Those who initially failed to respond, or were hesitant to participate, have all been contacted and interviewed by phone. This was done in order to explain the study and if possible to change the women's mind about participation in the study. It also gave us a possibility to collect data on reasons for declination among those in the population who eventually choose not to join.

Among cases, some women preferred to allow us access to stored tissue samples, taken at their initial cancer operation, rather than to draw blood. All relevant pathology departments have been contacted, and we are now receiving freshly cut tissue in paraffin blocks. From these we will extract DNA and process the analyses proposed in this study.

Scope 3 (specific task 3):

- Samples are continuously being transferred to the laboratories for DNA isolation and analysis of the polymorphisms.

- DNA from nearly all blood samples has been isolated.

- We expect to finalise all the laboratory analyses by the end of year 2000.

Scope 4 (specific task 4):

- Not yet performed.

- The statistical analysis will be performed when all samples have been collected and analysed in the laboratory.

Problems in accomplishing any of the tasks:

- The DNA originating from tissue samples (surgical material) was too fragmented for the XbaI and PvuII RFLP analyses to be performed.

- The restriction sites have therefore been *successfully sequenced* in order to be able to analyse the polymorphisms using the minisequencing single nucleotide primer extension assay.

- The method has been tested and validated and the genotyping is ongoing.

Findings (positive or negative): Not yet available.

Publications based on this study: Not yet available.

Presentations based on this study: Era of Hope, US Army Breast Cancer Meeting, Atlanta (Georgia, year 2000). We presented the methodology used in the study (see Appendix).

Statistical tests of significance shall be applied to all data whenever possible: Does not apply (yet).

Figures and graphs: Not available yet.

Discussion (including relevance of the original hypothesis): Does not apply yet.

Recommended changes or future work to better address the research topic: None available yet.

(7) KEY RESEARCH ACCOMPLISHMENTS

- Completion of organisational prerequisites.
- Construction of a detailed administrative database.
- Results from this research are not yet available.
- We are still collecting biological material for a few remaining subjects and performing laboratory work.
- Preliminary results are expected to be available by the end of year 2000 (or beginning of year 2001).

(8) REPORTABLE OUTCOMES:

- ***manuscripts, abstracts, presentations***

  A poster containing the methodology of the research project was presented at the 'Era of Hope' conference in Atlanta, Georgia, June 8-11, 2000, by the post-doctoral student working in the project (Elisabete Weiderpass, Karolinska Institutet, Stockholm, Sweden).

- ***patents and licenses applied for and/or issued:***

  Not applicable.

- ***degrees obtained that are supported by this award:***

  Two cancer epidemiologists in training are working on this project, namely:

  As post-doctoral fellow: Elisabete Weiderpass, M.D., Ph.D.
  As Ph.D. student: Sara Wedrén, M.D.

  The graduate student in cancer epidemiology, Dr. Sara Wedrén (Karolinska Institutet, Stockholm, Sweden), is in part supported by this project. Her thesis

work will be partially based on this project (two scientific articles out of a minimum of five required scientific articles for a Ph.D. thesis at Karolinska Institutet). She is expected to defend her thesis, thus obtaining her Ph.D. degree at the end of year 2001 or – at latest – at the beginning of year 2002.

The post-doctoral student in cancer epidemiology, Elisabete Weiderpass, M.D., Ph.D. (Karolinska Institutet, Stockholm, Sweden), is working in this project. She is being supervised by the PI of this study (professor Hans-Olov Adami) and by professor John Baron, Dartmouth Medical School, and is receiving further training in molecular epidemiology and cancer epidemiology.

- ***Development of cell lines, tissue or serum repositories:***

  DNA from breast cancer patients and control subjects are being extracted from white blood cells and stored in the collaborating laboratories at Uppsala University.

  A biological bank containing DNA samples from breast cancer patients and control women is being created and will be maintained using financial resources obtained elsewhere. The creation and maintenance of the biological bank follows the Swedish law for storage of biological samples for scientific purposes. The use of these biological materials follows the Swedish laws of safeguarding of privacy, and has been approved by the local Ethics Committees.

- ***Informatics such as databases and animal models, etc:***

  An administrative database containing information about study subjects has been created at Karolinska Institutet;

  A database containing questionnaire information from breast cancer case patients and control women has been created at Karolinska Institutet, and the questionnaire information has already been checked out and corrected for typing errors and logical inconsistencies.

  Programs (using the SAS programme) allowing calculation of time of use of different sorts of postmenopausal hormones have been developed at Karolinska Institutet and they work properly.

  Databases containing results from the laboratory analyses have been created in Uppsala, Sweden, where the laboratory analyses are being performed. These files will be transferred to Karolinska Institutet at the completion of the laboratory analyses (expected date: end of January 2001).

- ***Funding applied for based on work supported by this award:***

  A post-doctoral fellowship (cancer epidemiology) was granted from the Swedish Cancer Society to Dr. Elisabete Weiderpass (Karolinska Institutet, Stockholm, Sweden) to work in this project. This fellowship partially covers her salary from January to December 2000.

- ***Employment or research opportunities applied for and/or received on experiences/training supported by this award:***

The graduate student working in the project, Dr Sara Wedrén, obtained a training fellowship from the Karolinska Hospital - campus South - to complete her medical training. She qualified to this fellowship/training program in part due to her working experience with the project supported by the US Army.

The post-doctoral student, Dr. Elisabete Weiderpass, has been appointed Associate Professor in Cancer Epidemiology at Karolinska Institutet, Sweden, in September 2000. She qualified for this position partially due to the experience she acquired working with the project supported by the US Army.

(9) CONCLUSIONS:

Currently we do not have any laboratory results available for the whole material (we are still collecting biological material and performing laboratory analyses).

Laboratory personnel are blinded to the case-control status of each sample. The results from the laboratory work have not yet been linked to the database containing information on case-control status. Therefore we can not present any conclusions or preliminary conclusions.

Results will be available by the end of year 2000, or at the beginning of year 2001 at the latest (an extension of the period to submit the final report has been agreed with Cheryl R. Miles, US Army).

The collection of biological material is progressing according to the plans approved by the US Army.

(10) REFERENCES:

None.

(11) APPENDICES:

Abstract: Hormone receptor gene polymorphism and breast cancer. Era of Hope, Proceedings Volume II, page 477.

(13) FINAL REPORT:

Due in December 2000. **We intend to submit another report with results of the study in the spring of 2001.**

HORMONE RECEPTOR GENE POLYMORPHISM AND BREAST CANCER

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Much of the variation in breast cancer occurrence is not explained by known risk factors for the disease. Estrogen-, androgen-, and vitamin D receptors (ER, AR and VDR) have important effects in tissues responsive to sex hormones, and therefore may be of etiologic importance. Variation between individuals in the receptor genes may cause varying sensitivity to steroid hormones and explain why some women develop breast cancer and some do not. Steroid hormone receptor gene variants have been associated with breast cancer in some studies but these studies have been relatively small and attention to covarying factors that may modify or confound the associations has been limited. Our objective is to test the hypothesis that individual variation in the sensitivity to steroid hormones affects a woman's risk of breast cancer.

We are collecting germline DNA from 1800 women with breast cancer and 1700 women without the disease. All subjects are postmenopausal and have been randomly chosen among the participants in an earlier population-based case-control study where information about known and suspected risk factors was collected via an extensive questionnaire. We are analyzing the XbaI, PvuII and TA_n polymorphisms of the ER gene, the CAG polymorphism of the AR gene, and the poly A polymorphism in the VDR gene. The association of the genetic variants with breast cancer risk will be analyzed using logistic regression models, taking into account possible confounders. In stratified analyses particular attention will be paid to the putative role of postmenopausal hormone replacement therapy as an effect measure modifier.

We are currently concluding the collection of biological material from the study subjects. To date the participation rate is 90 % and 80% among cases and controls, respectively. Approximately half of the samples have been analyzed. All analyses are expected to be complete by the end of year 2000. Our research aims to clarify the importance of the steroid hormone receptors in the etiology of breast cancer.

The U.S. Army Material and Medical Command under DAMD17-97-1-7322 and DAMD17-98-1-8301 supports this work.

(Era of Hope, Proceedings Volume II, page 477)