

AD _____

Award Number: DAMD17-98-1-8354

TITLE: How Do Genetic Determinants of Bone Mass Relate to Breast
Cancer Risk?

PRINCIPAL INVESTIGATOR: Dorothy Nelson, Ph.D.

CONTRACTING ORGANIZATION: Wayne State University
Detroit, Michigan 48202

REPORT DATE: September 2000

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

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE September 2000	3. REPORT TYPE AND DATES COVERED Annual (1 Aug 99 - 1 Aug 00)	
4. TITLE AND SUBTITLE How Do Genetic Determinants of Bone Mass Relate to Breast Cancer Risk?		5. FUNDING NUMBERS DAMD17-98-1-8354	
6. AUTHOR(S) Dorothy Nelson, Ph.D.			
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Wayne State University Detroit, Michigan 48202 E-MAIL: dnelson@intmed.wayne.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) The purpose of this study is to investigate the relationship between breast cancer risk, bone mass, and two polymorphic hormone receptor genes—the Estrogen Receptor (ER) and the Vitamin D Receptor (VDR) genes. We will explore a possible functional mechanism in the third and final year to help explain the association between ER variants and breast cancer risk. Our target sample size is 200 new breast cancer cases and 200 controls (both African-American and white), ages 40-85. To date, we have recruited 280 cases and controls and expect to reach our target halfway through the third year (February 2001). Preliminary results show that age-, weight-, and ethnicity-adjusted bone mineral density in the forearm is higher in the breast cancer cases than controls (0.345 vs 0.332 g/cm ² for distal site, 0.796 vs 0.789 g/cm ² for the proximal site). The VDR frequencies appear to be different in the cases and controls such that the "bb" genotype (associated with higher bone mass) is more prevalent in the cases than in the controls. There are no definitive findings with the ERG polymorphism at this time. In summary, our positive findings thus far support our hypotheses that higher bone mass is found in breast cancer cases compared with controls, and that variations in the VDR gene may contribute to this association.			
14. SUBJECT TERMS Breast Cancer, Bone Mass, Estrogen Receptor Gene, Vitamin D Receptor Gene, Ethnicity			15. NUMBER OF PAGES 11
			16. PRICE CODE
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited

Table of Contents

Cover.....	1
SF 298.....	2
Table of Contents.....	3
Introduction.....	4
Body.....	4-8
Key Research Accomplishments.....	8
Reportable Outcomes.....	8-9
Conclusions.....	9
References.....	9-10
Appendices.....	11

INTRODUCTION

The **objective** of this study is to investigate the relationship between two polymorphic genes that are potential determinants of bone mass, and breast cancer risk, in African-American and white women, and to explore a possible functional mechanism to explain this association. Our hypothesis is that variations in these receptor genes affect the responsivity of bone and breast tissue to a given level of steroid exposure, and therefore correspond to variations in bone mass and the risk of breast cancer. That is, there may be genetically-determined individual variation in responsivity to identical stimuli that could explain the reported relationship between a higher bone mass and a higher breast cancer risk. Our **specific aims** and **hypotheses** are as follows:

1. To compare bone mass and the distribution of genotypes of the VDRG and ERG among 200 new breast cancer cases and 200 controls. Half of each sample will be white ethnicity, the other half African-American. Our **hypothesis** is that: The breast cancer cases will have a higher bone mass and a higher prevalence of the genotypes that are associated with high bone mass; the two ethnic groups will also differ but there are insufficient data to predict in what way they will differ.
2. To identify variations within the DNA sequence encoding the structural elements of the ERG; we **hypothesize** that these will correspond with the recognized polymorphic allotypes.
3. Our ultimate aim, which follows logically from Aim 2, is to determine the significance of the variations in estrogen receptor (identified in Aim 2) to the stimulation of an estrogen responsive reporter gene regulated by promoters of diverse complexity. Our **hypothesis** is that the variants associated with elevated responsivity of the cell to estrogen will be more prevalent in the breast cancer cases compared with controls

BODY

Progress report for each task relevant to Year 2:

Approved Statement of Work

General: Recruitment and data collection for Specific Aim 1 will take place over the first 2.5 years of the study. New breast cancer patients will be recruited and then matched controls will be recruited in either a concurrent fashion, if practicable, or in a staggered design in which patients are recruited over several weeks and then matched controls are recruited, and the cycle is repeated. This may be necessary because although the bone densitometer is "portable" it is not practical to move it frequently. Analyses of genotypes will be ongoing and will finish 3 months after recruitment ends. Laboratory analyses for Specific Aim 2 will take place in Year 3 after most of the genotype data are available.

Progress: We have been recruiting both new breast cancer patients and controls in a concurrent fashion, although we have concentrated on maximizing the rate of accrual of cases. We have enrolled 185 cases and 95 controls to date. Analyses of genotypes are being done in batches, so that 85-90% of the subjects in the database also have genotype information available at any given time.

Year 2: October 1999-September 2000

TECHNICAL OBJECTIVE 3: To recruit an additional 80 white and 80 black new breast cancer patients and controls (40 each within ethnic groups).

Progress: As of July 27, 2000 we have recruited 117 white and 68 African-American breast cancer patients, and 58 white and 37 African-American controls (total N=280, compared with 121 at the time of last year's progress report). We began enrollment the last week of September, 1998, so our average rate of accrual is 12 subjects per month. We need 15 more cases, which we expect to recruit within 2-3 months (concentrating on enhancing the African-American subsample). We need 105 controls, which we anticipate we can accrue much faster than the recruitment rate of cases. Based on our experience with media announcements and the distribution of flyers for our study, we have found an excellent response from healthy women who are interested in having a bone density test. Thus, we believe that we will finish recruitment on time by the end of February 2000.

Interim data reports are generated approximately twice yearly. The study coordinator meets with the P.I. at least once per week, and the study investigators have met as a group four times since the study was initiated. Data summaries of demographic variables are presented in Table 1. No tests of significance are reported at this time because we have not finished recruitment.

TABLE 1: DESCRIPTIVE STATISTICS (MEAN \pm S.D.) OF DEMOGRAPHIC DATA FOR CASES AND CONTROLS, BY ETHNIC GROUP

Variables	Breast Cancer Cases		Controls	
	White N=117	African- American N=68	White N=58	African- American N=37
Age (yrs)	57.5 \pm 11.1	57.3 \pm 11.1	50.2 \pm 7.8	53.2 \pm 10.4
Body Mass Index (kg/m ²)	27.1 \pm 6.7	31.8 \pm 7.6	26.4 \pm 5.8	31.2 \pm 9.0
Age at Menarche (yrs)	12.5 \pm 1.4	12.6 \pm 1.8	12.6 \pm 1.3	13.0 \pm 1.4
Age at Menopause (yrs)	49.5 \pm 5.6	47.9 \pm 7.6	47.6 \pm 4.8	42.8 \pm 9.6

DISCUSSION: The data in Table 1 indicate that we need to concentrate our efforts on recruiting African-American cases and both groups of controls over the next 6 months. We also need to fill in the older age groups in the controls. Within ethnic groups, cases and controls do not differ notably on body mass index; however, the African-Americans are heavier, on average. The menarche and menopause data suggest a longer period of estrogen exposure (i.e. number of years between menarche and menopause) in the breast cancer cases compared with controls.

Table 2 provides bone density data on cases and controls, showing both unadjusted means and adjusted means for the distal and proximal forearm sites. The distal site was adjusted for age, ethnicity, weight, and ever-use of hormone replacement therapy. The covariates for the proximal site are slightly different: age, ethnicity and weight, but not hormone use, were important in the model.

TABLE 2: COMPARISON OF UNADJUSTED AND ADJUSTED MEANS FOR BONE DENSITY IN THE CASES VERSUS CONTROLS

Variables	Breast Cancer Cases N=177	Controls N=94
Unadjusted Distal Forearm BMD (g/cm ²)	0.338 ± 0.16	0.346 ± 0.08
Adjusted* Distal Forearm BMD (g/cm ²)	0.345 ± 0.11	0.332 ± 0.13
Unadjusted Proximal Forearm BMD (g/cm ²)	0.785 ± 0.11	0.810 ± 0.08
Adjusted** Proximal Forearm BMD (g/cm ²)	0.796 ± 0.08	0.789 ± 0.09

* Adjusted for age, weight, ethnicity, and use of HRT (ever use); N=213

**Adjusted for age, weight, and ethnicity

DISCUSSION: Since the controls are 4-7 years younger than the cases, at a critical time for bone loss, the unadjusted means for bone density are higher in controls. However, once adjusted for age (as well as ethnicity and body size), the cases have a higher bone density, which is consistent with our hypothesis.^{1,2} The bone density difference is 4% for the distal site and 1% for the proximal site. We reported these findings in 193 subjects in our poster presentation³ to the ERA of HOPE Breast Cancer Program Meeting in June, 2000 (see Appendix). Interestingly, hormone use affected the distal site but not the proximal, possibly due to the greater trabecular bone composition of the distal site.

Year 2: October 1999-September 2000

TECHNICAL OBJECTIVE 2: To determine the genotypes for the VDRG and ERG in the breast cancer patients and controls.

Task 4: Months 3-33: Laboratory assistant under Dr. Wooley's direction will perform genetic analyses. Results will be entered into study database.

Progress: Genetic analyses are being done in batches and entered at intervals into the database. At present, 248 of the 280 subjects have ERG results, and 251 have VDRG results. Summaries of genotypes presently available are provided in the tables below.

TABLE 3A: FREQUENCIES (ACTUAL AND PERCENT) OF ESTROGEN RECEPTOR GENE HAPLOTYPES (PVUII AND XBAI) IN BREAST CANCER CASES (N=165)

PVUII HAPLOTYPE	XBAI HAPLOTYPE		
	XX	Xx	xx
PP	16 (10%)	14 (8.5%)	6 (4%)
Pp	2 (1%)	53 (32%)	28 (17%)
pp	1 (.5%)	3 (2%)	42 (25%)

TABLE 3B: FREQUENCIES (ACTUAL AND PERCENT) OF ESTROGEN RECEPTOR GENE HAPLOTYPES (PVUII AND XBAI) IN CONTROLS (N=83)

PVUII HAPLOTYPE	XBAI HAPLOTYPE		
	XX	Xx	xx
PP	12 (14%)	2 (2.5%)	8 (10%)
Pp	0	32 (38.5%)	12 (14.5%)
Pp	0	2 (2.5%)	15 (18%)

DISCUSSION: The genotype data in Tables 3a and 3b suggest some differences in frequencies of the estrogen receptor gene between cases and controls, but no definitive statement can be made until our target sample size has been achieved, providing adequate cell (sample) sizes. Since our last report, the distribution of subjects into the four genotypes we had identified as being low frequency in cases compared with controls (XXPp, XXpp, xxPP, Xxpp) has changed. At this time, these do not appear to differentiate the two groups. The VDR genotype frequencies are shown in Table 4.

TABLE 4: FREQUENCIES (ACTUAL AND PERCENT) OF VITAMIN D RECEPTOR GENE BSM1 HAPLOTYPES IN CASES (N=165) AND CONTROLS (N=86)

GROUP	BSM1 HAPLOTYPES		
	BB	Bb	bb
CASES	25 (15%)	68(41%)	72(44%)
CONTROLS	20(23%)	33(38%)	33(38%)

DISCUSSION: With larger sample sizes in each cell for this locus, we can begin to see a difference in genotype distribution between cases and controls. The "BB" genotype is found in lower frequency in the cases than controls, while the "bb" genotype is found in higher frequency in the cases. Based on data in the literature, the "BB" genotype is associated with a lower bone mass and the "bb" with a higher bone mass.⁴ Thus, the higher frequency of the "high bone mass" genotype in the cases is consistent with our hypotheses about genetic determinants of bone mass and their relationship to breast cancer risk. That is, the higher bone mass found in cases may have a genetic underpinning related to variations in the Vitamin D Receptor.

Task 5: Months 13-24: Study coordinator will attend breast cancer clinics and/or general medicine clinics at least 3 days per week to recruit 2 subjects per day. Blood sample and bone densitometry will be obtained. Specimens will be transported to Dr. Wooley's lab and stored at -70 degrees. Bone density and questionnaire variables will be entered into the database.

The study coordinator attends clinics 5 days per week and recruits an average of 12 subjects per month. We expect that this rate will increase when she finishes recruitment of cases and then concentrates on controls, who require less screening and are relatively easier to recruit because of widespread interest in bone density testing.

KEY RESEARCH ACCOMPLISHMENTS

- Recruitment of 12 subjects per month, on average, for a total of 280.
- Recruitment of both African-American and white cases and controls.
- Recruitment of subjects over most of the targeted age range.
- A database that is clean and up to date.
- The genotyping of specimens at a reasonable rate (85-90% of enrolled subjects at any time).
- The expectation of meeting our goal of recruiting 400 subjects by the middle of the third year of the study.

REPORTABLE OUTCOMES

- Development of serum repository for genotyping.
- Database development for all study variables.

- A poster presentation at the ERA OF HOPE Breast Cancer Research Program Meeting, Atlanta, GA, June 2000.³
 - A-6: "Bone Mass and Estrogen Receptor Gene in Breast Cancer Cases and Controls." Nelson DA, Darga LL.

CONCLUSIONS

The preliminary data in 280 of the 400 subjects suggest that there is a trend for bone density in the proximal and distal forearm, when adjusted for age, weight, and ethnicity (and HRT use for the distal site), to be higher in the cases as predicted. There are apparent differences between cases and controls in the genotype frequencies of the Vitamin D Receptor gene. While preliminary, there is an apparent trend for the "bb" genotype, generally associated with higher bone mass, is found in higher frequency in the cases than the controls. This lends support to our hypothesis. The trends that were apparent last year for the Estrogen Receptor Gene have not been borne out with a larger sample. However, we have not yet reached our target sample size and can make no definitive statement about this variable. In the next few months, we will begin the laboratory experiments focusing on variants in the ERG and how they relate to estrogen responsivity. Our plans for the final year of this award include completion of recruitment; completion of genotyping; and the planned laboratory experiments with variants of the estrogen receptor gene.

In summary, although no definitive conclusions can be drawn at this time, the preliminary results indicate that continued recruitment and study of our target sample will provide appropriate data for the evaluation of our hypotheses. The knowledge to be gained from this study may provide new tools for assessing breast cancer risk early in life (such as bone mass measurement or genotyping) that could lead to modifications in hormone replacement therapy in postmenopausal women and/or increased surveillance for breast cancer in women with high bone mass.

REFERENCES:

1. Cauley JA, Lucas FL, Kuller LH, Vogt MT, Browner WS, Cummings SR, for the Study of Osteoporotic Fractures Research Group. Bone mineral density and risk of breast cancer in older women: the Study of Osteoporotic Fractures. *JAMA* 1996;276:1404-1408.
2. Zhang Y, Kiel DP, Kreger BE, Cupples LA, Ellison RC, Dorgan JF, Schatzin A, Levy D, Felson DT. Bone mass and the risk of breast cancer among postmenopausal women. *New Engl J Med* 1997;336:611-617.
3. Nelson DA, Darga LL. Bone mass and estrogen receptor gene in breast cancer cases and controls. DoD Breast Cancer Research Program, Era of Hope Meeting, June 2000, Atlanta, GA. (poster).

4. Morrison NA, Qi JC, Tokita A, Kelly PJ, Crofts L, Nguyen TV, Sambrook PN, Eisman JA. Prediction of bone density from vitamin D receptor alleles. Letters to *Nature* 1994;207:284-287.

APPENDIX: Abstract of poster presented at ERA of HOPE meeting in Atlanta, GA, June 2000.

BONE MASS AND ESTROGEN RECEPTOR GENE IN BREAST CANCER CASES AND CONTROLS

Dorothy A. Nelson and Linda L. Darga

Wayne State University School of Medicine,
Detroit, MI 48201

dnelson@intmed.wayne.edu

The study's objective is to investigate prospectively, in 400 women, the relationship between higher breast cancer risk and higher bone mineral density (BMD), with a focus on two polymorphic genes that may contribute to the association. BMD is measured in the radius (2 sites) by dual-energy x-ray absorption. Variants of the Vitamin D Receptor Gene (VDRG) and Estrogen Receptor Gene (ERG) are determined by polymerase chain reaction (PCR) followed by endonuclease digestion of the PCR product. The target sample is 200 new breast cancer cases and 200 controls, both African-American (Af-Amer) and white, over age 40. Recruits come mainly from cancer clinics and mammography, but also by media announcements and community outreach centers. We report on the first 200 subjects, who are described in the table below (* denotes $p < 0.05$, ethnic differences):

Variable	Cases (n=116)		Controls (n=77)	
	White (n=84)	Af-Amer (n=32)	White (n=45)	Af-Amer (n=32)
Age (yr)	57±11	55±10	51±8	52±10
Weight (kg)	73±17	83±24*	70±15	85±28*
Height (cm)	163±7	161±9	165±8	164±6
% postmenopause	62%	75%	55%	53%

BMD (adjusted for age, ethnicity, weight, menopause status) is higher in the breast cancer cases (difference is 4% distal radius, 1% proximal). Mean proximal BMD differs significantly across the ERG-*XbaI* haplotypes (n=134, $p=0.048$). When *XbaI* and *PvuII* are combined into 9 genotypes, PpXX, PPxx, and ppXX comprise 8% of cases compared with 17% of controls ($p < 0.10$), consistent with our pilot study.

We conclude from our preliminary data (50% of target sample) that BMD when adjusted for covariates is higher in the breast cancer cases. There is a difference in the distribution of the ERG haplotypes in the cases and controls. However, the age distribution and sample sizes are not yet optimal, and may confound the analyses.

The U.S. Army Medical Research and Materiel Command under DAMD17-98-1-8354 supported this work.