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

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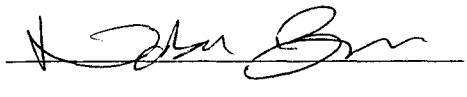

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**13. ABSTRACT** ***(Maximum 200 Words)***

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 vitamin D receptor (VDR) genes. We also planned to explore a possible functional mechanism to explain this association. Our target was to recruit 200 new breast cancer cases and 200 controls, ages 40-85, with equal numbers of African-Americans and whites. Bone mineral density (BMD) measurements of the forearm were to be obtained. We have enrolled 231 cases and 198 controls, with an age range of 39-84 years. There is an equitable distribution of the two ethnic groups, with 50.3% of the cohort being white and 49.7% African-American. We enrolled more than 400 subjects because some individuals changed their minds about giving blood, some blood samples were not analyzable, and some subjects have no bone density data due to instrument malfunctions. Genotype frequencies of the VDR and ER gene segments that we investigated were not significantly different in cases or controls. However, BMD (expressed as a z-score) in the proximal radius of the cases is significantly higher than controls, as hypothesized. Furthermore, the odds ratio associated with having higher than average bone density was 1.98 (95% C.I. 1.32-2.97). This is our most significant and clinically relevant finding. Adjustment for potential confounders did not appreciably change this odds ratio. We are in the process of obtaining tumor tissue samples from subjects who are homozygous and who have given consent to our using their tissue for our final objective--to investigate the responsivity to estrogen of the polymorphic ER genotypes.

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## Table of Contents

## 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[1-3]. 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

*Final report for each technical objective:*

**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.**

**TECHNICAL OBJECTIVES 1, 3, AND 4**: To recruit 200 white and 200 black new breast cancer patients and controls (100 each within ethnic groups) and obtain blood samples (for later analysis). Also, to perform bone density measurements and enter the results into the study database.

Recruitment was completed in July 2001. We enrolled 231 cases and 198 controls. Our final sample size (n=429) is greater than our target of 400 because some subjects were unwilling to

provide blood samples or they were not successfully analyzed for genotypes. A second reason is that the pDEXA instrument was out of service for 4-6 weeks over the 2.5 years of the study, and bone density measurements are missing for some subjects. The total number of subjects with pDEXA data as well as genotype data is 418.

Subjects ranged in age from 39 to 84 years, with a mean of 56.3 ± 10.8 years. There is an equitable distribution of the two ethnic groups, with 50.3% of the cohort being white and 49.7% African-American. Descriptive statistics are provided in Table 1.

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

| | **Breast Cancer Cases** | | **Controls** | |
|---|---|---|---|---|
| Variables | White N=120 | African-American N=111 | White N=96 | African-American N=102 |
| Age (yrs) | 57.7±10.9 | 57.0±11.3 | 55.0±10.6 | 54.9±10.2 |
| Height (cm) | 163.0±6.6 | 162.7±7.6 | 164.3±7.5 | 164.7±6.7 |
| Weight (kg)* | 73.0±16.5 | 83.4±21.0 | 70.3±14.5 | 82.1±21.1 |
| Body Mass Index ($kg/m^2$)* | 27.5±6.3 | 31.6±8.1 | 26.1±5.3 | 30.2±7.3 |
| Age at Menarche (yrs) | 12.5±1.4 | 12.6±1.8 | 12.5±1.5 | 12.8±1.6 |
| Age at Menopause (yrs)# | 49.7±5.6 | 47.8±7.2 | 49.0±4.9 | 45.9±6.9** |

**Significant ethnic differences in cases and controls, $p<0.0001$*
***Significant ethnic difference in controls, $p=0.0038$*
*#Note: 284 of the 429 subjects were postmenopausal*

DISCUSSION: The data in Table 1 indicate that the cases and controls are very similar in mean age, height, and age at menarche. There are significant ethnic differences in BMI within both the case and control groups ($p<.0001$). Among the controls, African-Americans had a significantly lower age at menopause ($p=0.0038$). However, there are no significant differences between cases and controls within ethnic group. That is, the African-American cases do not differ significantly from the African-American controls, and likewise for the white cases and controls ($p>0.05$).

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Bone density data, expressed as Z-scores, are given in Table 2 below for cases and controls. It is well-known that bone mineral density (BMD) is affected by age and ethnicity, as well as by menopause status, body size, and hormone replacement therapy. Therefore, we utilized age- and ethnicity-specific scores (Z-scores), and adjusted for the other major potential confounders in regression models. Z-scores are calculated by the pDEXA instrument based on reference data provided by the manufacturer, and are reported in standard deviation units (where "0" is the average bone density for one's age and ethnicity).

**TABLE 2**: COMPARISON OF MEAN Z-SCORES (S.D. UNITS) FOR BONE DENSITY IN THE CASES VERSUS CONTROLS

| Variables | Breast Cancer Cases N=220 | Controls N=196 | p |
|---|---|---|---|
| Distal Forearm Z-Score | 0.22 ± 0.97 | 0.19 ± 1.01 | 0.832 |
| Proximal Forearm Z-Score | 0.45 ± 1.07 | 0.19 ± 1.08 | 0.015 |

Using Student's t tests, we found that there was a significant difference between cases and controls at the proximal site (radial shaft) but not at the distal site. We also tested whether there were ethnic differences in mean Z-scores within the case and control groups, and found no significant ethnic differences ($p>0.05$).

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

Genetic analyses were done in batches and entered at intervals into the database. All subjects' data have been analyzed and entered. Summaries of the genotype frequencies for the 3 loci are provided in Tables 3-6 below. Tables 5a and 5b show the frequencies for XbaI and PvuII combined.

**TABLE 3**: FREQUENCIES OF ERG PVUII HAPLOTYPES IN **CASES** (N=220) AND **CONTROLS** (N=191)

| | PVUII HAPLOTYPES | | |
|---|---|---|---|
| | pp | Pp | PP |
| CASES | 53 | 11 | 56 |
| CONTROLS | 41 | 102 | 48 |

**P-value=0.79 for differences in odds ratios across genotypes. Adjusted for ethnicity, p=0.74.*

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**TABLE 4:** FREQUENCIES OF ERG XBAI HAPLOTYPES IN **CASES** (N=220) AND **CONTROLS** (N=190)

| | XBAI HAPLOTYPES | | |
|---|---|---|---|
| | xx | Xx | XX |
| CASES | 100 | 92 | 28 |
| CONTROLS | 87 | 86 | 17 |

**P-value=0.45 for differences in odds ratios across genotypes. Adjusted for ethnicity, p=0.49 .*

**TABLE 5A**: FREQUENCIES (ACTUAL AND PERCENT) OF ESTROGEN RECEPTOR GENE HAPLOTYPES AT BOTH LOCI (PVUII AND XBAI) IN **BREAST CANCER CASES** (N=220)

| | XBAI HAPLOTYPE | | |
|---|---|---|---|
| PVUII HAPLOTYPE | | | |
| | XX | Xx | Xx |
| PP | 24 (10.9%) | 20 (9.1%) | 12 (5.5%) |
| Pp | 3 (1.4%) | 69 (31.4%) | 39 (17.7%) |
| pp | 1 (0.5%) | 3 (1.4%) | 49 (22.3%) |

**TABLE 5B**: FREQUENCIES (ACTUAL AND PERCENT) OF ESTROGEN RECEPTOR GENE HAPLOTYPES AT BOTH LOCI (PVUII AND XBAI) IN **CONTROLS** (N=190)

| | XBAI HAPLOTYPE | | |
|---|---|---|---|
| PVUII HAPLOTYPE | | | |
| | XX | Xx | xx |
| PP | 17 (8.9%) | 14 (7.4%) | 17 (8.9%) |
| Pp | 0 | 69 (36.3%) | 33 (17.4%) |
| Pp | 0 | 3 (1.6%) | 37 (19.5%) |

**Tables 5A and 5B: P-value for differences in odds ratios across genotypes>0.05.*

**TABLE 6**: FREQUENCIES (ACTUAL AND PERCENT) OF VITAMIN D RECEPTOR GENE BSM1 HAPLOTYPES IN **CASES** (N=220) AND **CONTROLS** (N=192)

| | BSM1 HAPLOTYPES | | |
|---|---|---|---|
| GROUP | | | |
| | BB | Bb | bb |
| CASES | 36 (16.4%) | 88 (40.0%) | 96 (43.6%) |
| CONTROLS | 40 (20.8%) | 66 (34.4%) | 86 (44.8%) |

**P-value=0.37 for differences in odds ratios across genotypes. Adjusted for ethnicity, p=0.35 .*

**TECHNICAL OBJECTIVE 6**: To test hypotheses and report study results.

**Hypothesis 1**: The 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.

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Our data suggest that radial shaft ("proximal") bone density (expressed as a Z-score) is a significant predictor of breast cancer risk. First, we used the proximal bone density Z-score as a continuous variable in a logistic regression model to determine the risk of breast cancer in relation to the Z-score. The odds ratio was 1.25 ($p=0.02$). Thus, for a one-standard deviation unit increase in bone density in the radial shaft, the increased risk of breast cancer is 25%. We then assessed the effect modification and confounding effects of menopause status, age group (<50 years, <60 years, ≥60 years), body mass index, ever/never use of HRT, and duration of HRT. There were no significant interactions and no appreciable confounding effects of these variables on the relationship between bone density and breast cancer risk ($p>0.05$).

We explored whether having "above average" bone density was a significant predictor of breast cancer risk. In other words, if the Z-score is >0, what is the odds ratio of having breast cancer? Using logistic regression analysis with two categories of Z-score (≤0, >0), we found that **the odds ratio is nearly two-fold for higher than average bone density: OR=1.98 (95% confidence interval 1.32-2.97). We consider this our most significant finding since the peripheral DEXA technique of measuring bone density is inexpensive, noninvasive, efficient (results available within 5-10 minutes), and portable.** Thus, we believe that this is a relatively simple, clinically relevant tool for use in the assessment of risk factors for breast cancer.

Chi-squared analyses of the genotype frequency data presented in Tables 3-6 showed no significant differences between cases and controls ($p>0.05$). We also adjusted these data by ethnicity and found no difference in the risk of breast cancer by genotypes after adjusting for ethnicity. These findings do not support our hypothesis that: "The cases will have … a higher prevalence of the genotypes that are associated with high bone mass; the two ethnic groups will also differ …".

We also explored, by logistic regression analysis, the odds ratio for proximal bone density Z-score as a continuous variable when the VDR and ER genotypes were included in the model (one at a time). The OR did not change from the value of 1.25 that was noted in the models without genotype data, and this OR remained significant ($p<0.022$). There were no significant interactions between the genotypes and the radial BMD Z-score ($p>0.05$). We repeated this process for the two categories of proximal Z-score (above or below average), and found that the OR of 1.95 did not change appreciably and remained significant ($p<0.002$) when the VDR and ER genotypes were included in the logistic regression model. There were not significant interactions ($p>0.05$).

**TECHNICAL OBJECTIVE 5: To identify exon(s) on ERG that code for structural protein and which are polymorphic, for Specific Aim 2.**

*Progress:* Work began on this task during the final year of the project. We had not budgeted to finish this objective, but we did explore and begin to develop the methods. Since we were not able to identify variations in estrogen receptor that are more prevalent in the breast cancer cases compared with controls, we selected cases who were homozygous at both ER loci. After initial investigations and review of the literature, it was determined that peripheral blood samples were not the appropriate tissue to be used in the planned experiments. Rather, tumor tissue specimens were needed for these experiments. This required obtaining further consent from subjects, for which we obtained IRB approval. We contacted several patients with homozygous genotypes at the PvuII

and XbaI sites to ask for their consent to use their tissue (stored in Pathology for clinical purposes). We received consent from four patients, and contacted Pathology for tumor tissue. We learned that, unfortunately, paraffin-embedded specimens are typically stored, not snap-frozen tissue. Dr. Wooley investigated the use of paraffin-embedded tissue for this experiment and determined that snap-frozen tissue was necessary. We are in the process of identifying and seeking the consent of more cases in order to find appropriate tissue samples. We conclude that, in future, such studies should prospectively collect tumor tissue for these analyses, which our design did not include.

## KEY RESEARCH ACCOMPLISHMENTS

- Recruitment of 231 newly diagnosed breast cancer cases and 198 controls.
- Recruitment of nearly equal numbers of both African-American and white cases and controls.
- Recruitment of subjects over most of the targeted age range.
- The genotyping of all blood samples.
- A database for 429 breast cancer cases and controls with associated data for bone density, genotypes for ER and VDR gene polymorphisms, and variables related to medical and family history.

## REPORTABLE OUTCOMES

- Development of serum repository for genotyping.
- Database with 231 breast cancer cases and 198 controls
  - Equitable distribution of ethnic groups
  - Comprehensive questionnaire data on medical and reproductive history
- A poster presentation at the ERA OF HOPE Breast Cancer Research Program Meeting, Atlanta, GA, June 2000.[4]
  - A-6: "Bone Mass and Estrogen Receptor Gene in Breast Cancer Cases and Controls." Nelson DA, Darga LL.
- The novel and clinically relevant finding that having above-average bone density in the radial shaft, regardless of black/white ethnicity, is associated with an odds ratio of nearly 2 for increased risk of breast cancer.

## CONCLUSIONS

We have amassed a relatively large data set from a case/control study of nearly equal numbers of African-American and white breast cancer cases and controls. These data should prove to be valuable for hypothesis generation and testing beyond the objectives of the current project.

Our genetic data did not support our hypothesis about differences in genotype frequency between cases and controls. The knowledge to be gained from this study is the identification of a new tool for assessing breast cancer risk (peripheral bone mass measurement). This new tool is relevant to decisions about hormone replacement therapy, and/or increased surveillance, in white or African-American women over 40 years of age who have higher-than-average radial bone mass.

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