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PRINCIPAL INVESTIGATOR: Dorothy A. Nelson, Ph.D.

CONTRACTING ORGANIZATION: Wayne State University
Detroit, Michigan 48202

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Dorothy A. Nelson, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Wayne State University
Detroit, Michigan 48202

E*Mail: dnelson@intmed.wayne.edu

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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 will also explore a possible functional mechanism to explain this association. We will 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 will be obtained. We have enrolled 121 women (53 white and 20 African-American breast cancer, and 27 white and 21 African-American controls), with an age range of 40-81. We have increased monthly recruitment from 7 per month in the first 3 months to an average of 19 subjects per month in the past 3 months, which should allow accrual of 400 subjects as planned. Preliminary results show that age-, weight-, and ethnicity-adjusted BMD is higher in the cases than controls (0.805 vs 0.789 g/cm^2 for the radial shaft, 0.351 vs 0.348 g/cm^2 for distal site), as hypothesized. The ER genotype frequencies appear to be different in the two arms (although the cell sizes are small), but there is no apparent difference yet in the VDR genotype frequencies. The absent or low-frequency ER genotypes observed in our pilot study of breast cancer cases are also absent or low frequency in the new sample, lending support to our hypotheses.

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FOREWORD

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Dorothy Ackerman 8/18/99

PI - Signature Date

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. 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 1:

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 73 cases and 48 controls to date. Analyses of genotypes are being done in batches, so that approximately 75% of the subjects in the database also have genotype information available.

Year 1: October 1998-September 1999

TECHNICAL OBJECTIVE 1: To recruit 80 white and 80 black new breast cancer patients and controls (40 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.

Progress: As of July 30 1999 we have recruited 53 white and 20 African-American breast cancer patients, and 27 white and 21 African-American controls (total N=121). We began enrollment the last week of September, 1998, so our average rate of accrual is 12 subjects per month. However, our monthly accrual rate has increased from 7 subjects per month in the first three months of the study to 19 per month in the past 3 months (see Figure in Appendix). At the current rate, we expect to enroll 38 more subjects by the end of September 1999, for a total of 159 out of the target sample of 160.

Task 1: **Month 1:** **Database will be set up. Forms will be copied. Clinic personnel will be contacted to coordinate recruitment of subjects (schedule days and times, work out flow of data collection, etc.)**

Progress: Completed

Task 2: **Months 2-12:** **Study coordinator (full-time research assistant) will attend breast cancer or general medicine clinics at least 3 days per week to recruit 2 subjects per clinic 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 database.**

Progress: We are proceeding with the protocol as planned, but have had two interruptions. Our first study coordinator stayed only 3 months (through November) and then left for an academic position at a nearby university. We hired a replacement, Linda Darga, Ph.D., in January 1999. The bone densitometer needed repairs in February, and this stopped enrollment for approximately 10 days. The effects of these interruptions on recruitment are reflected in the Figure (see Appendix) for the months of November through February.

The study coordinator, Dr. Darga, is in clinic 4-5 days per week, enrolling an average of 15.7 subjects per month since February. The specimens are transported to Dr. Wooley's lab and/or stored in a freezer the same day they are obtained. The database is updated weekly, and the genotype data are imported into the study database after several batches are analyzed in the lab.

Task 3: **Months 3-12:** **Research assistant will generate interim data reports to be presented at monthly study staff meetings.**

Progress: Reports are generated as requested. The study coordinator meets with the P.I. at least once per week, and the study investigators have met as a group twice since the study was initiated. Data summaries of selected variables are presented in the Tables below. No tests of significance are reported at this time because only one-third of our target sample has been accrued, as planned, and they are not deemed to be representative of the final group.

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

Variables	Breast Cancer Cases		Controls	
	White N=53	African-American N=20	White N=27	African-American N=21
Age (yrs)	55.2 ± 10.1	53.3 ± 8.9	48.9 ± 7.4	52.5 ±9.6
Body Mass Index (kg/m^2)	27.3 ± 6.3	33.4 ± 9.9	25.8 ± 5.8	31.4 ± 10.7
Age at Menarche (yrs)	12.5 ± 1.4	12.3 ± 2.0	12.9 ± 1.2	12.7 ± 1.4
Age at Menopause (yrs)	48.1 ± 6.0	44.8 ± 12.1	46.2 ± 6.4	42.9 ± 8.1
Distal forearm BMD (g/cm^2)	.334 ± .06	.369 ± .06	.338 ± .09	.381 ± .08
Proximal forearm BMD (g/cm^2)	.780 ± .10	.830 ± .09	.778 ± .14	.839 ± .07

DISCUSSION: Statistical tests for significant differences between cases and controls are not done at this time because we do not expect the current sample (N=121) to be representative of the final sample (N=400). This is especially true for the controls, where the sample size and age distribution are issues that will be resolved as we focus on recruitment of controls in the next few months that "match" the cases by age and ethnic group, as well as sample size. We will also try to enhance recruitment of African-American women with breast cancer.

The bone density data in Table 1 suggest an ethnic difference (African-Americans greater than whites), as expected (*1-2*). Since BMD is affected by factors such as age, body size, and the ethnic composition of the samples (*3*), we also present adjusted means for BMD at the two skeletal sites in Table 2. There is a trend for a higher BMD among the cases, which would support our hypothesis. We plan to adjust bone density by these (and possibly other) covariates as necessary in the full cohort.

TABLE 2: COMPARISON OF UNADJUSTED AND ADJUSTED (AGE, WEIGHT, ETHNICITY) MEANS FOR BONE DENSITY IN THE CASES VERSUS CONTROLS

Variables	Breast Cancer Cases N=68	Controls N=47
Unadjusted Distal Forearm BMD (g/cm^2)	0.345 ± 0.06	0.357 ± 0.08
Adjusted* Distal Forearm BMD (g/cm^2)	0.351 ± 0.07	0.348 ± 0.06
Unadjusted Proximal Forearm BMD (g/cm^2)	0.794 ± 0.10	0.805 ± 0.12
Adjusted* Proximal Forearm BMD (g/cm^2)	0.805 ± 0.10	0.789 ± 0.10

*Adjusted for age, weight, and ethnicity

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: As reported above, genetic analyses are being done in batches and entered at intervals into the database. 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=61)

	XBAI HAPLOTYPE		
PVUII HAPLOTYPE	XX	Xx	xx
PP	7 (11%)	5 (8%)	1 (2%)
Pp	1 (2%)	21 (34%)	12 (20%)
pp	0	0	14 (23%)

TABLE 3B: FREQUENCIES (ACTUAL AND PERCENT) OF ESTROGEN RECEPTOR GENE HAPLOTYPES (PVUII AND XBAI) IN CONTROLS (N=29)

	XBAI HAPLOTYPE		
PVUII HAPLOTYPE	XX	Xx	xx
PP	7 (24%)	1 (3%)	4 (14%)
Pp	0	11 (38%)	2 (7%)
Pp	0	0	4 (14%)

DISCUSSION: The genotype data presented suggest some differences in frequencies of the estrogen receptor gene between cases and controls (Tables 3a and 3b) but no definitive statement can be made using small cell (sample) sizes. The distribution of the cases' genotypes is consistent with those observed in our pilot study (*4*) preceding the current study. Specifically, the four genotypes in the cases that have either 0 or 1 subject in the cell (XXPp, XXpp, xxPP, Xxpp) are those with the smallest frequencies in the cases from the pilot study. While three of these genotypes are also low-frequency in the controls, the sample size for this group is very small (N=29 out of 200 to be studied).

The VDR genotype frequencies appear to be comparable between cases and controls at this time (Table 4). We intend to continue data collection at the current (if not higher) rate in order to test the original hypotheses stated in the proposal.

TABLE 4: FREQUENCIES (ACTUAL AND PERCENT) OF VITAMIN D RECEPTOR GENE BSM1 HAPLOTYPES IN CASES (N=48) AND CONTROLS (N=27)

	BSM1 HAPLOTYPES		
GROUP	BB	Bb	bb
CASES	9 (19%)	19 (40%)	20 (42%)
CONTROLS	4 (15%)	12 (44%)	11 (41%)

END OF FIRST YEAR'S TASKS

KEY RESEARCH ACCOMPLISHMENTS

- Recruitment of 12 subjects per month, on average, for a total of 121. An increase in the accrual rate to 19 per month since May 1999 should yield a year-end total of 159 of the targeted 160 research subjects.
- Recruitment of both African-American and white cases and controls.
- Recruitment of subjects over most of the targeted age range (40-81 compared with the target of 40-85).

- A database that is clean and up to date.
- The genotyping of specimens at a reasonable rate (75% 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.

CONCLUSIONS

We conclude that the proposed protocol for recruitment and study of 400 subjects over 3 years is realistic and productive. The preliminary data in 121 of the subjects suggest that there is a trend for bone density in the proximal and distal forearm, when adjusted for age, weight, and ethnicity, to be higher in the cases as predicted. There are apparent differences between cases and controls in the genotype frequencies for the Estrogen Receptor Gene that are consistent with the findings of our previous pilot study (*4*). No definitive conclusions can be drawn at this time, but 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.

Our plans for the immediate future include an emphasis on recruiting age-appropriate controls in order to match the current sample for cases. Thereafter, we will try to stagger recruitment in both groups to keep them comparable for interim analyses. We need to enhance recruitment of African-Americans in the breast cancer arm, although to the best of our knowledge there has been no significant recruitment bias by ethnicity. We believe that our sample of breast cancer cases is representative of the ethnic composition of the clinic population. We are making efforts to reach other potential subjects through community cancer outreach centers, where volunteers are being trained to identify possible recruits and provide the necessary information and help to determine their interest in the study and ensure that we contact them.

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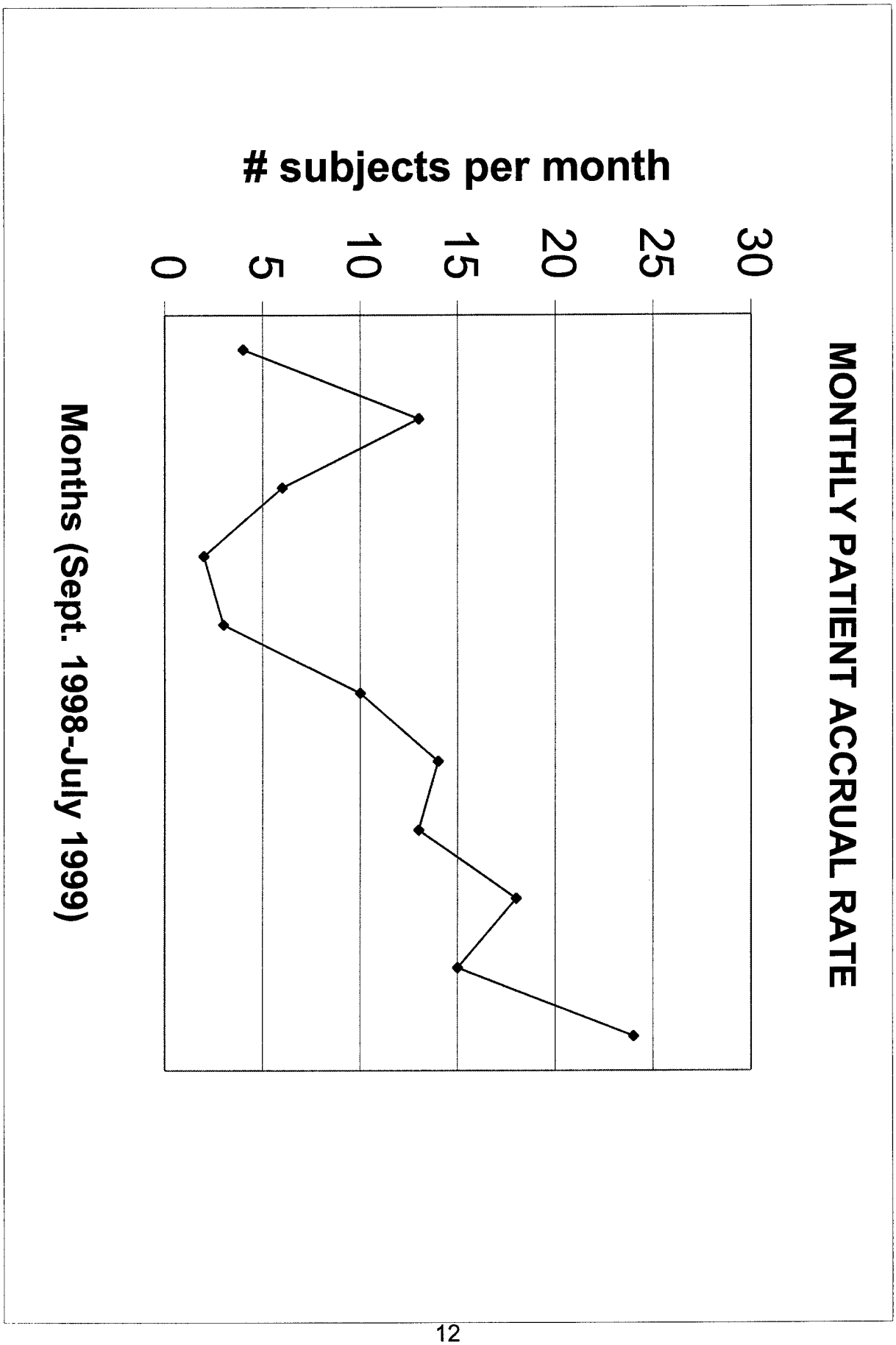
APPENDIX: FIGURE: Monthly recruitment rate from September 1998 through July 1999.


MONTHLY PATIENT ACCRUAL RATE
subjects per month
0
5
10
15
20
25
30
Months (Sept. 1998-July 1999)