Vitamin D Receptor Polymorphisms and Breast Cancer Risk in a High-Incidence Population: A Pilot Study

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BACKGROUND: Marin County, CA has very high incidence of breast cancer. Traditional risk factors, such as those included in the Gail model, do not effectively stratify breast cancer in this population. This retrospective case-control pilot study evaluates DNA from volunteers from a previous Marin County breast cancer epidemiology study. A polyfactorial risk model (OncoVue; InterGenetics Incorporated) that incorporates 22 polymorphisms in 19 genes and 5 clinical risk factors was used to stratify risk in Marin County women.

STUDY DESIGN: DNA genotyping was performed on 164 Caucasian women diagnosed with primary breast cancer in Marin County from 1997 to 1999 and 174 age- and ethnicity-matched control subjects. Individual lifetime risks were determined using the polyfactorial risk model and genotype frequencies in women at elevated risk were compared with the overall genotypes.

RESULTS: The vitamin D receptor *VDR ApaI A2/A2* (rs7975232) homozygous polymorphism was present in high frequency in elevated-risk women. Sixty-four percent of elevated-risk women had the *VDR Apa1 A2/A2* genotype compared with only 34% in the overall study, a statistically significant 1.9-fold difference ($p = 0.0003$). *VDR Apa1 A2/a1* and *a1/a1* genotypes were also present, but in lower frequencies.

CONCLUSIONS: The high frequency of the *VDR Apa1 A2/A2* homozygous polymorphism in women designated as elevated risk for breast cancer by the polyfactorial risk model might be related to the high incidence rates of breast cancer in Marin County, CA. Vitamin D supplementation could modify risk of breast cancer in this population. (J Am Coll Surg 2012;215:652–657. © 2012 by the American College of Surgeons)

The Marin County, CA population of Caucasian women is known to have a high incidence of breast cancer and traditional epidemiological risk factors in the National Cancer Institute Breast Cancer Risk Assessment Tool or Gail model do not effectively stratify risk in this population.[1-3] A polyfactorial risk model (PFRM) (OncoVue; InterGenetics Incorporated) has been found to stratify risk in the Marin County population.[4] This proprietary model combines 5 traditional risk factors with 22 functional single nucleotide polymorphisms (SNPs) in 19 genes associated with metabolic pathways involved in breast cancer.

In the 1997 to 1999 Marin County, CA risk factor study, the incidence of breast cancer was reported as 199/100,000 women, which is one of the highest rates in the world.[1,2] A subset of women from this study donated their buccal cell DNA for future examination and we have analyzed their DNA using the PFRM and report our findings. We postulated that evaluation of the genotype frequencies of individuals placed at high risk by the

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Abbreviations and Acronyms
BMI = body mass index
PFRM = polyfactorial risk model
SNP = single nucleotide polymorphism
VDR = vitamin D receptor

PFRM would provide insight into the genetics influencing breast cancer development in Marin County, CA.

METHODS

Study design and population

The study population has been described in detail previously.[1,2] Briefly, eligible subjects included any female resident of Marin County, CA diagnosed with primary breast cancer between July 1997 and June 1999, if age younger than 50 years, or between July 1997 and March 1999, if age older than 50 years. Cases were chosen from the Northern California Cancer Center's Surveillance, Epidemiology and End Result database.[2] Eligible control subjects included women without breast cancer selected by random digit dialing. Controls were age-matched to cases by age at diagnosis and ethnicity. All participants were enrolled under informed consent, completed a comprehensive questionnaire on clinical risk information, including personal medical history, family history of cancer, and lifestyle factors. The Investigational Review Board at University of California San Francisco approved the study design and protocol. Interested participants in this study were requested to provide a buccal cell sample collected on cytobrushes for future study.

Our study reports on 164 cases and 174 control subjects in the subset of the Caucasian participants in the Marin County epidemiology risk factor study who donated their buccal cells for future DNA analysis. DNA isolation, genotyping, and PFRM score calculations were conducted with InterGenetics Incorporated blinded to case-control status. Cytobrush samples and personal factor data were anonymously coded to remove case-control status before being provided to InterGenetics for laboratory and risk-stratification analyses.

DNA isolation and genotyping

Genomic DNA was isolated using the Gentra PureGene DNA purification kit (Qiagen). Genotyping assays were performed using microbead-based allele-specific primer extension, followed by analysis on the Luminex 100 (Luminex, Inc.) essentially as described previously.[5,6] As a quality-control measure, internal reproducibility was routinely confirmed by repeating the genotyping on 5% of the specimens. Genotyping assays had reproducibility rates of >99.9%. Buccal cell DNA from a total of 164 cases diagnosed with primary breast cancer and 174 ethnicity- and age-matched controls were genotyped to determine the variation in a total of 22 SNPs that compromise the genetic part of the PFRM.

Risk stratification with the polyfactorial risk model

The PFRM is a single integrated model that has previously been shown to provide substantial improvements in breast cancer risk evaluation for sporadic breast cancer.[4,7] The PFRM uses weighted combinations of 22 SNPs in 19 distinct functional genes in metabolic pathways, including steroid hormone metabolism, DNA repair, and growth control, that influence breast cancer development (Table 1) and includes most of the clinical risk factors in the Gail model (risk of breast cancer based on a woman's age at the time of calculation of risk, age at first live birth, number of first-degree relatives with breast cancer, number of breast biopsies, and outcomes) to estimate breast cancer risk. For each individual analyzed in the study, the percent absolute risk was determined from their current age through age 69 years. The starting age used for cases was their age at the time of diagnosis and for controls their age at enrollment. The PFRM individual risk-estimation scores were determined with InterGenetics Incorporated being blinded to case-control status and were returned to the University of California, San Francisco study investigators for addition of case-control status and analysis. The elevated risk threshold was defined as ≥12% risk that is 1.5 times the Surveillance, Epidemiology and End Results mean breast cancer risk (8%) for the age range of 30 to 69 years.[8]

Statistical analysis

Genotype frequencies for the markers in the PFRM (Table 1) were determined both overall and in risk-stratified groups. We looked at the subtype frequencies of all cases and controls in the study along with the overall population genotype frequencies (Table 2). One genotype that stood out in this analysis was that of the vitamin D receptor (VDR), *VDR Apa1 A2/A2*. To test the hypothesis that the *VDR Apa1 A2/A2* proportion in the designated risk group was statistically different from the overall population proportion, we performed a 1-sample *t*-test of proportions using Proc Freq with binomial option in SAS 9.1 software (SAS Institute).

The 5 clinical risk factors included in the Gail model (ie, risk of breast cancer based on a woman's age at the time of calculation of risk, age at first menses, age at first live birth, number of first-degree relatives with breast cancer, and number of breast biopsies) are listed in Table 3. Table 3 also includes body mass index (BMI) and alcohol consumption history. These factors were shown to be significant in the