Null Results in Brief

No Association between the Progesterone Receptor Gene *+331G/A* Polymorphism and Breast Cancer

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Introduction

Progesterone is critical to normal breast development, and its activity is mediated by the progesterone receptor (PR) that is part of the steroid-thyroid-retinoic acid receptor superfamily of transcription factors (1). A single copy of the progesterone receptor gene (*PGR*), located on chromosome 11q22-23, uses separate promoters and translation start sites to produce two protein isoforms, PR-A and PR-B, which are functionally distinct (2). Mouse models have demonstrated that PR-mediated signaling pathways are essential for carcinogen-induced mammary gland carcinogenesis and that mammary gland response to progesterone depends on the ratio of PR-A to PR-B (1).

DeVivo et al. (3) resequenced *PGR* and discovered a single nucleotide polymorphism (*+331G/A*) that altered transcriptional activity and favored production of PR-B in an endometrial cancer cell line. Further, they showed that this single nucleotide polymorphism was associated with increased risk of both endometrial cancer and breast cancer (3, 4). Given the strong biological evidence that this single nucleotide polymorphism may alter the PR-A to PR-B ratio, we sought to examine the association between *PGR +331A/G* in a nested case-control study of postmenopausal breast cancer.

Methods

Study Population. Women in this study are participants in the American Cancer Society Cancer Prevention Study II Nutrition Cohort, a prospective study of cancer incidence including ~184,000 U.S. adults. Participants completed a 10-page mailed questionnaire in 1992 that included information on demographics, diet, and other lifestyle factors. The recruitment and characteristics of this cohort are described in detail elsewhere (5). Follow-up questionnaires are sent to surviving cohort participants every 2 years beginning in 1997 to update exposure information and to ascertain occurrence of new cases of cancer. Incident cancers reported on the questionnaires are verified through medical records, linkage with state cancer registries, or death certificates.

From June 1998 to June 2001, blood samples were collected from a subgroup of 39,376 cohort members. After obtaining informed consent, a maximum of 43 mL of nonfasting whole blood was collected from each participant and separated into aliquots of serum, plasma, RBC, and buffy coat. Samples were frozen in liquid nitrogen vapor phase at approximately −130°C for long-term storage.

We selected 507 postmenopausal breast cancer cases diagnosed between 1992 and 2001 and 507 individually matched controls. We selected only cases and controls with no previous history of cancer (other than non-melanoma skin cancer) at diagnosis date. Controls were matched on age (±6 months), race/ethnicity (White, African American, Hispanic, Asian, or other/unknown), and date of blood collection (±6 months). For all cases, exposure information was collected by questionnaire before the cancer diagnosis (i.e., in 1992); however, collection of blood samples occurred after cancer diagnosis or, in rare cases, slightly before cancer diagnosis.

Laboratory. DNA was extracted from buffy coat, and genotyping assays were performed using TaqMan (Applied Biosystems, Foster City, CA) as described previously (4). Genotyping was performed by laboratory personnel blinded to case-control status, and 10% blind duplicates were randomly interspersed with the case-control samples to validate genotyping procedures. Concordance for the quality control samples was 100%. Overall success rate for the genotyping assays was 96%. The genotype distribution was in Hardy-Weinberg equilibrium.

Statistical Analysis. Unconditional logistic regression, Fisher's exact tests, and χ^2 tests were used to examine the association between the *+331A* allele and breast cancer while controlling for matching factors and other possible confounders. Effect modification of the association between breast cancer and *+331* genotype by body mass index (BMI) and hormone replacement therapy (HRT) use was also examined by stratified analysis and logistic regression.

Results and Conclusion

Genotyping data were missing on 28 (5.5%) cases and 13 (2.6%) controls. Cases and controls were largely White

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Table 1. Genotype distribution, ORs, and 95% CIs for the association between *PGR +331* polymorphism and postmenopausal breast cancer

Genotype	Cases (n = 479)	Controls (n = 494)	OR* (CI)	OR† (CI)
G/G	425 (88.7)	445 (90.1)		
G/A	53 (11.1)	48 (9.7)		
A/A	1 (0.2)	1 (0.2)		
G/G	425 (88.7)	445 (90.1)	1.0	1.0
G/A + A/A	54 (11.3)	49 (9.9)	1.19 (0.79-1.79)	1.15 (0.74-1.76)

*OR adjusted for birth date, race/ethnicity, and date of blood draw.
†OR adjusted for birth date, race/ethnicity, date of blood draw, education, personal history of breast cyst, family history of breast cancer, age at menarche, parity, age at first birth, age at menopause, BMI, and type of HRT use.

(99%), with median age of 62 years (range 43 to 75) at enrollment (in 1992). Table 1 shows the genotype frequencies, odds ratios (OR), and 95% confidence intervals (CI) for the association between breast cancer and the *+331A* allele. The *+331* genotype did not differ among cases and controls (Fisher's exact P = 0.76). Because the *A* allele is rare (frequency among controls = 5%), logistic regression models compare the *+331G/G* with the *G/A* and *A/A* genotypes together. Including potential confounders in the logistic models in addition to the matching variables resulted in very little change in the effect estimates or CIs.

Previous data (3, 4) have suggested that the association between both breast and endometrial cancer and the *+331A* allele is stronger among women with higher BMI. We further hypothesized that the association may be stronger among women taking combination estrogen-progestin HRT compared with those not taking combination estrogen-progestin HRT. We did not find evidence of interaction with either BMI (P for interaction = 0.89) or combination estrogen-progestin HRT (P for interaction = 0.86). The ORs for carrying a *+331A* allele (compared with the *+331G/G* genotype) were 1.19 when BMI < 25, 1.32 when BMI = 25 to 30, and 0.98 when BMI > 30. When examined by type of HRT use, the ORs for carrying a *+331A* allele were 1.0 among never users, 0.99 among estrogen-only HRT users, and 1.30 among combination estrogen-progestin HRT users.

We found no association between the *+331G/A* single nucleotide polymorphism and breast cancer. Our results do not support the recent findings of DeVivo et al. who have reported that the *+331A* allele is associated with increased risk of both breast (4) and endometrial (3) cancers.

The primary limitation of this study is limited statistical power to detect a weak association given the low frequency of the risk genotype (10%). When $\alpha = 0.05$, $\beta = 0.20$, and the observed genotype frequency among controls, our study had sufficient power to detect a minimum OR of 1.7. Under the same assumptions, a sample size of 5,000 cases and 5,000 controls would be required to detect an OR of 1.20.

Given the evidence from animal studies that PR is an important regulator of mammary growth and development and further that an imbalance of the ratio between the two PR isoforms may lead to disruption of normal growth and differentiation of mammary cells, further study of the role of PR variation and breast cancer is warranted.

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