Advances in Brief

A Functional Polymorphism in the Progesterone Receptor Gene Is Associated with an Increase in Breast Cancer Risk[1]

Immaculata De Vivo,[2] Susan E. Hankinson, Graham A. Colditz, and David J. Hunter

Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, and Harvard Medical School [I. D., S. E. H., G. A. C., D. J. H.], and Departments of Epidemiology [I. D., S. E. H., G. A. C., D. J. H.], Nutrition [D. J. H.], and Harvard Center for Cancer Prevention [I. D., D. J. H., G. A. C.], Harvard School of Public Health, Boston, Massachusetts 02115

Abstract

Biological and epidemiological data suggest that progesterone has an important role in mammary tumorigenesis. Because the effects of progesterone require the progesterone receptor (*PGR*), which exists in two isoforms, PR-A and PR-B, we sought to determine whether the functional polymorphism, +331 *G/A,* which causes an increase in the expression of the hPR-B isoform, is related to breast cancer risk. Using a nested case-control study design within the Nurses' Health Study cohort, we genotyped 990 cases and 1,364 controls and observed a statistically significant increased risk of breast cancer among carriers of the +331 *A* allele (odds ratio, 1.33; 95% confidence interval, 1.01–1.74) compared with subjects with the *GG* genotype. We also observed a potential interaction between genotype and body mass index (BMI) among postmenopausal women, with the highest risk (odds ratio, 2.30; 95% confidence interval, 1.02–5.21) among obese women (BMI $\geq$30 kg/m^2) with the *GA* or *AA* genotype compared with lean (BMI <25 kg/m^2) women with the *GG* genotype. Our findings suggest that the increased production of hPR-B by the +331 *G/A* polymorphism may predispose women to breast cancer development through increased hPR-B-dependent stimulation of mammary cell growth.

Introduction

Progesterone and estrogen are the main steroid hormones involved in normal breast development and tumorigenesis (1). Although the proliferative effects of estrogen on mammary gland development and tumorigenesis are well recognized, the contribution of progesterone to these processes is less well understood. Evidence from epidemiological studies has shown that early onset of menarche, late menopause, nulliparity, a late first birth and reproductive states that are affected by the absence or presence of progesterone, increase a woman's risk for breast cancer. More recently, data from epidemiological studies revealed a higher risk of breast cancer in postmenopausal women who used a combination of estrogens and progestins, as compared with those women who used estrogens alone (2, 3).

As demonstrated in PRKO[3] mice, the physiological effects of progesterone are completely dependent on the presence of the human *PGR*, a member of the steroid-receptor superfamily of nuclear receptors (4). The single-copy *PGR* gene, located on chromosome 11q22–23, uses separate promoters and translational start sites to produce two protein isoforms, hPR-A and hPR-B (5–7), that are identical except for an additional 165 amino acids present only in the NH$_2$ terminus of

hPR-B (8, 9). Although hPR-B shares many important structural domains with hPR-A, the two isoforms are functionally distinct transcription factors (10) that mediate their own response genes and physiological effects with little overlap (11, 12). The PRKO mouse, in which the functional activity of both hPR-A and hPR-B were simultaneously ablated, revealed that progesterone is required for the formation of ductal and alveolar structures during pregnancy (4, 13). The PRKO mouse, when used in the context of an established carcinogen-induced mammary tumor model, showed that removal of *PGR* function results in a significant reduction in susceptibility to 7,12-dimethylbenz(*a*)anthracene-induced mammary tumors (13). Studies of transgenic mice that carried either an additional -A or -B form reported that mammary development was abnormal and characterized by excessive lateral ductal branching and inappropriate alveolar growth (14). Considering the epidemiological and biological evidence described above for the role of progesterone in breast cancer causation, we hypothesized that variation in the *PGR* gene may predispose women to breast cancer. Several polymorphisms have been identified in *PGR,* they include S344T, G393G, V660L, H770H, and the *PRO-GINS* allele (15). In this study, we evaluated the promoter polymorphism, +331 *G/A,* for two reasons: its association with endometrial cancer, a hormonally related disease, and because it has established function. We do not plan to evaluate the other *PGR* polymorphisms because they are not known to be functional, and we did not observe an association with endometrial cancer (15).

Materials and Methods

Study Population. The Nurses' Health Study was initiated in 1976, when 121,700 United States registered nurses between the ages of 30 and 55 returned an initial questionnaire reporting medical histories and baseline health-related exposures. Between 1989 and 1990, blood samples were collected from 32,826 women. Incident breast cancers were identified by self-report and confirmed by medical record review. Eligible cases in this study consisted of women diagnosed with pathologically confirmed incident breast cancer after giving a blood specimen up to June 1, 1994. Controls were matched to cases on year of birth, menopausal status, postmenopausal hormone use, and time of day, month, and fasting status at blood draw; menopause was defined as described previously (16). The nested case-control study consists of 990 incident breast cancer cases and 1,364 matched controls. The protocol was approved by the Committee on Human Subjects, Brigham and Women's Hospital. Detailed information on exposure data has been described previously (16, 17).

Laboratory. Genotyping assays were performed by RFLP as described previously (15), and the 5′ nuclease assay (TaqMan) was performed with the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). TaqMan primers, probes, and conditions for genotyping assays are available on request from authors. Genotyping was performed by laboratory personnel blinded to case-control status, and blinded quality control samples were inserted to validate genotyping procedures. Concordance for the blinded samples was 100%.

Statistical Analysis. Student's *t* test and the χ^2 test were used to evaluate differences in breast cancer risk factors between cases and controls. ORs and 95% CIs were calculated by using conditional and unconditional logistic

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Table 1 *Association between PGR polymorphism and breast cancer risk*

PR genotype[a]	Cases, n (%)	Controls, n (%)	OR	OR
GG	864 (87)	1218 (90)	1.0[b]	1.0[c]
AG + AA	126 (13)	139 (10)	1.26 (0.97–1.63)	1.33 (1.01–1.74)
Premenopausal women				
GG	93 (91)	98 (89)	1.0[d]	1.0[e]
AG + AA	9 (9)	12 (11)	0.8 (0.32–2.00)	0.76 (0.28–2.00)
Postmenopausal women				
GG	711 (87)	1047 (90)	1.0[f]	1.0[g]
AG + AA	109 (13)	120 (10)	1.34 (1.02–1.77)	1.41 (1.06–1.87)

[a] Numbers may vary because of missing genotypes.

[b] Conditional logistic regression adjusted for the matching variables: age, menopausal status, postmenopausal hormone use at blood draw, date at blood draw, time at blood draw, and fasting status.

[c] Conditional logistic regression adjusted for matching variables and age at menarche, parity, age at first birth, BMI at age 18, weight gain since age 18, benign breast disease, first-degree family history of breast cancer, and duration of postmenopausal hormone use.

[d] Unconditional logistic regression adjusted for matching variables: age, date at blood draw, time at blood draw, and fasting status.

[e] Unconditional logistic regression adjusted for matching variables in [d] and age at menarche, parity, age at first birth, BMI at age 18, weight gain since age 18, benign breast disease, and first-degree family history of breast cancer.

[f] Unconditional logistic regression adjusted for matching variables in [b].

[g] Unconditional logistic regression adjusted for matching variables in [b] and age at menarche, parity, age at first birth, BMI at age 18, weight gain since age 18, benign breast disease, first-degree family history of breast cancer, and duration of postmenopausal hormone use.

regression. In addition to the matching variables, we adjusted for breast cancer risk factors: BMI (kg/m^2) at age 18 years, weight gain since age 18, age at menarche, parity/age at first birth, duration of postmenopausal hormone use, first-degree family history of breast cancer, and history of benign breast disease. We also adjusted for age at menopause in analyses limited to postmenopausal women. Indicator variables for all genotypes were created by using the wild-type hypothesized low-risk genotype as the reference category in the regression models. Because of the low prevalence of homozygote variants (AA), we combined heterozygotes (AG) and homozygote variants (AA) in the logistic regression analysis. Interactions between genotypes and breast cancer risk factors were evaluated by including appropriate interaction terms in unconditional logistic regression models. The likelihood ratio test was used to assess the statistical significance of these interactions. We used SAS version 8.0 (SAS Institute, Cary, NC) for all analyses. We tested Hardy-Weinberg agreement by using a χ^2 test.

Results

Our study included a total of 990 incident breast cancer cases and 1,364 controls. Eight hundred twenty cases and 1,167 controls were postmenopausal, and 102 cases and 110 controls were premenopausal; menopausal status was uncertain in 68 cases and 87 controls. The mean age of cases at blood draw was 57.2 years; for controls, it was 57.9 years. Cases and controls had similar mean BMI at blood draw (25.4 *versus* 25.5 kg/m^2) and weight gain since age 18 years (11.7 *versus* 11.5 kg). Compared with controls, cases had similar ages at menarche (12.5 *versus* 12.6 years), first birth (23.0 *versus* 22.9 years), and menopause (48.2 *versus* 48.0 years). The proportion of women with a first-degree family history of breast cancer was significantly higher among the cases (21.1% *versus* 14.5%; *P* = 0.001). Cases were also more likely to have a history of benign breast disease (64.8% *versus* 49.3%; *P* < 0.001) and a longer duration of postmenopausal hormone use (30.3% *versus* 22.6% current users for 5 or more years; *P* = < 0.001).

The prevalence of the *AA* carriers was similar to a previous report for Caucasian women (15), 13% for the cases and 10% for the controls. The genotype distribution of the +331 *G/A* polymorphism among the cases and controls was in Hardy-Weinberg equilibrium (*P* = 0.11). We observed a statistically significant increased risk of breast cancer among carriers of the +331 *G/A* polymorphism; compared with the +331 *G/G* wild-type genotype, the adjusted OR for women with +331 *G/A* and +331 *A/A* was 1.33 (95% CI, 1.01–1.74; Table 1). After stratifying by menopausal status, the association was similar among postmenopausal women (adjusted OR, 1.41; 95% CI, 1.06–1.87) but not among premenopausal women (Table 1). Too few homozygote variants were available to analyze the heterozygous and homozygous women separately. The +331 *G/A* polymorphism has been shown to modify the association between BMI and endometrial cancer risk (15), a hormonally related cancer, and obesity is directly related to breast cancer only among postmenopausal women (18). Huang *et al.* observed a modest nonsignificant association between postmenopausal obese women (BMI ≥ 30 kg/m^2) and breast cancer risk. Compared with lean women (<25 kg/m^2), the OR for postmenopausal women with a BMI of 25 to <30 and ≥30 kg/m^2 was 1.06 (95% CI, 0.82–1.37) and 1.24 (95% CI, 0.78–1.95), respectively (18). We sought to determine whether the +331 *G/A* genotype modified the effect of BMI on breast cancer risk among postmenopausal women. We observed a statistically significant association between postmenopausal obese women (BMI ≥30 kg/m^2) who carried at least one *A* allele and breast cancer risk (Table 2). Compared with wild-type (+331 *G/G*) lean women (<25 kg/m^2), the OR for postmenopausal women carriers with a BMI of 25 to <30 kg/m^2 and ≥30 kg/m^2 was 1.98 (95% CI, 1.16–3.39) and 2.30 (95% CI, 1.02–5.21), respectively (Table 2). The test for interaction, however, was not statistically significant (*P* for interaction, likelihood ratio test = 0.10). We observed no significant interactions with first-degree family his-

Table 2 *PR polymorphisms and BMI for postmenopausal women only: frequencies and ORs for breast cancer risk*

Genotype	BMI <25 kg/m^2, n (%)			25 ≤ BMI < 30 kg/m^2, n (%)			BMI ≥30 kg/m^2, n (%)		
	Cases	Controls	OR (95% CI)	Cases	Controls	OR (95% CI)	Cases	Controls	OR (95% CI)
+331 GG[a]	400 (88)	536 (88)	1.0	210 (85)	344 (91)	1.00 (0.76–1.31)	101 (85)	167 (91)	1.19 (0.75–1.90)
+331 AG + AA	55 (12)	72 (11)	1.06 (0.72–1.56)	36 (14)	32 (9)	1.98 (1.16–3.39)	18 (15)	16 (9)	2.30 (1.02–5.21)

Unconditional logistic regression adjusted for matching variables and age at menarche, parity, age at first birth, BMI at age 18, weight gain since age 18, benign breast disease, and first-degree family history of breast cancer. ORs are independent risks for breast cancer following joint variables: +331 *GG* and 25 ≤ BMI ≤ 30, +331 *GG* and BMI >30, +331 *AG + AA* and BMI <25, +331 *AG + AA* and 25 ≤ BMI ≤ 30, +331 *AG + AA* and BMI>30, compared to +331 *GG* and BMI <25 (reference group). Likelihood ratio test for interaction of BMI and +331 is 0.10; Wald test result is 0.11.

[a] Reference category.

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tory of breast cancer, a history of benign breast disease, or hormone replacement therapy use.

Discussion

PGR polymorphisms, most notably the *PROGINS* allele, have been studied in relation to breast and ovarian cancer (19–21). Wang-Gohrke *et al.* (20) found an inverse association between breast cancer risk in women younger than 50 years and carriers of the *PROGINS* allele. In contrast, Spurdle *et al.* (21) found no association between Val660Leu, a polymorphism that is in complete linkage disequilibrium with *PROGINS*, and breast cancer risk in women of comparable age. To our knowledge, this is the first population-based study to examine the functional *PGR* polymorphism, +331 *G/A,* in relation to breast cancer risk. We observed a modestly significant increase in breast cancer risk among women with *AG* or *AA* genotypes (adjusted OR, 1.33; 95% CI, 1.01–1.74) compared with women with *GG* genotypes, and the effect was similar in postmenopausal women (adjusted OR, 1.41; 95% CI, 1.06–1.87). We have shown previously that a consequence of this polymorphism is an increase in the expression of the hPR-B isoform (15). Biological studies have convincingly shown that the two *PGR* isoforms, hPR-A and hPR-B, are functionally distinct (10, 11, 22, 23). In contrast to hPR-A, hPR-B is a potent transcriptional activator, and hPR-B alone can promote cell growth by interacting with the estrogen receptor to stimulate the Src/p21ras/extracellular signal-regulated kinase pathway (24). Moreover, recent microarray analysis confirmed that each *PGR* isoform, expressed in a breast cancer cell line, has a unique set of target genes, with little overlap (12). Genes selectively up-regulated by hPR-B that predispose to mammary cell survival and proliferation include *IAP homologue C* and *cyclin D3*, as well as antiapoptotic protein BcL-XL (12). Taken together, these data suggest that increased production of hPR-B by the +331 *G/A* polymorphism may predispose to cancer development through increased hPR-B-dependent stimulation of mammary cell growth.

Acknowledgments

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Announcements

MEETING OF THE RADIATION RESEARCH SOCIETY

The annual meeting of the Radiation Research Society will be held at the State University of Iowa, Iowa City, on June 22–24, 1953. The Society will be the guest of the University, and all meetings will be held on the campus. The program will consist of: (1) Two symposia, one on "The Effects of Radiation on Aqueous Solutions," which includes the following speakers: E. S. G. Barron, Edwin J. Hart, Warren Garrison, J. L. Magee, and A. O. Allen. The second is "Physical Measurements for Radiobiology" and companion talks by Ugo Fano, Burton J. Moyer, G. Failla, L. D. Marinelli, and Payne S. Harris. (2) On Monday night, June 22, a lecture by Dr. L. W. Alvarez on meson physics has been tentatively scheduled. On Tuesday night, June 23, Dr. L. H. Gray of the Hammersmith Hospital, London, will speak on a topic to be announced. Dr. Gray's lecture is sponsored by the Iowa Branch of the American Cancer Society. Those desiring to report original research in radiation effects, or interested in attending or desiring additional information, please contact the Secretary of the Society, Dr. A. Edelmann, Biology Department, Brookhaven National Laboratory, Upton, L.I., New York.

ERRATUM

The following correction should be made in the article by Beck and Valentine, "The Aerobic Carbohydrate Metabolism of Leukocytes in Health and Leukemia. I. Glycolysis and Respiration," November, 1952, page 821; substitute for the last paragraph:

The data in Table 3 permit several interesting calculations. If one compares the amount of glucose actually disappearing with the sum of the amount equivalent to lactic acid produced plus that equivalent to O_2 consumption, it is seen that the amount of glucose "cleavage products" *exceeds* the amount of glucose utilized by 12 per cent in N and 27 per cent in CML and *is exceeded* by the glucose utilized by 16 per cent in CLL. If the assumption is made that, *in this respect*, the myeloid and lymphoid cells of leukemia are similar to those of normal blood, it may be that the computed normal figure represents a summation of the myeloid (M) and lymphoid (L) cells that make up the normal leukocyte population. Thus, if $M = +0.27$ and $L = -0.16$ and the normal differential is 65 per cent M and 35 per cent L, then

$$0.65 \, (+0.27) + 0.35 \, (-0.16) = +0.12 \ ,$$

a figure identical to the observed $+0.12$ for normal leukocytes.

AACR American Association for Cancer Research

A Functional Polymorphism in the Progesterone Receptor Gene Is Associated with an Increase in Breast Cancer Risk

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