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

Immaculata De Vivo, 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, 1.01–4.64) compared with subjects 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

Progestosterone 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 PRKO3 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 NH2 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 PROGRESS 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 χ2 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...
Table 1 Association between PGR polymorphism and breast cancer risk

<table>
<thead>
<tr>
<th>PR genotype</th>
<th>Cases, n (%)</th>
<th>Controls, n (%)</th>
<th>OR</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>864 (87)</td>
<td>1218 (90)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>AG + AA</td>
<td>126 (13)</td>
<td>139 (10)</td>
<td>1.26 (0.97–1.63)</td>
<td>1.33 (1.01–1.74)</td>
</tr>
<tr>
<td>GG</td>
<td>93 (91)</td>
<td>98 (89)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>AG + AA</td>
<td>9 (11)</td>
<td>12 (11)</td>
<td>0.8 (0.32–2.00)</td>
<td>0.76 (0.28–2.00)</td>
</tr>
<tr>
<td>GG</td>
<td>711 (87)</td>
<td>1047 (90)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>AG + AA</td>
<td>109 (13)</td>
<td>120 (10)</td>
<td>1.34 (1.02–1.77)</td>
<td>1.41 (1.06–1.87)</td>
</tr>
</tbody>
</table>

Numbers may vary because of missing genotypes.

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.

Conditional logistic regression adjusted for matching variables: age, menopausal status, 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.

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

Unconditional logistic regression adjusted for matching variables in 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.

Unconditional logistic regression adjusted for matching variables in and at age 18 years, weight gain since age 18 years, BMI at age 18, 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, and duration of postmenopausal hormone use.

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 $\geq 30$ 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 $\geq 30$ 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 $\geq 30$ 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 $\geq 30$ 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-
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/ 

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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 891; 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 O2 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.
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