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+331G/A variant in the progesterone receptor gene, postmenopausal hormone use and risk of breast cancer

Joanne Kotsopoulos[1,*], **Shelley S. Tworoger**[1,2], **Immaculata DeVivo**[1,2], **Susan E. Hankinson**[1,2], **David J. Hunter**[1,2], **Walter C. Willett**[1,2,3], and **Wendy Y. Chen**[1,4]

[1]Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA

[2]Department of Epidemiology, Harvard School of Public Health, Boston, MA

[3]Department of Nutrition, Harvard School of Public Health, Boston, MA

[4]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA

Abstract

A functional promoter polymorphism in the progesterone receptor (PR) gene previously has been associated with an increased risk of postmenopausal breast cancer. Whether the relationship between genetic variation in *PR* and risk of breast cancer is modified by postmenopausal hormone (PMH) use is unknown. Thus, we conducted a case-control study nested within the prospective Nurses' Health Study to evaluate if the risk of breast cancer associated with having the *+331 A* risk allele was modified by PMH use. Genotyping of this SNP was available for 1664 postmenopausal breast cancer cases and 2391 controls. Logistic regression was used to estimate the odds ratios (ORs) and 95% confidence intervals (CIs) for breast cancer. Women who were carriers of one or both variant *A* alleles had a 31% increased risk of developing breast cancer (95%CI 1.04-1.65). PMH use significantly modified the association between the *+331G/A* polymorphism and risk (*P*-interaction <0.05). Among never users of PMH, women who were variant carriers had a significantly increased risk of breast cancer compared to those with the wild-type genotype (OR=2.57; 95%CI 1.64-4.02). The *+331G/A* polymorphism was not associated with breast cancer risk among past (OR=1.23; 95%CI 0.77-1.97) or current (OR=1.14; 95%CI 0.84-1.56) PMH users. The data from this large prospective study provide evidence for a two-fold increased risk of developing postmenopausal breast cancer among never users of PMH with the *+331G/A* SNP. This finding adds to the evidence that the progesterone receptor has an important etiologic role in breast cancer and should be evaluated in future studies.

Keywords

breast cancer; progesterone receptor; postmenopausal hormones

INTRODUCTION

The single-copy human progesterone receptor (hPR) gene is a member of the steroid-receptor superfamily of nuclear receptors 1 that has separate promoters and translational start sites to produce two isoforms, hPR-A and hPR-B 2-4. These isoforms are identical except for an additional 165 amino acids present in the N terminus of hPR-B 5, 6. Although

[*]**ADDRESS CORRESPONDENCE TO:** Joanne Kotsopoulos: Channing Laboratory, 181 Longwood Avenue, Boston, MA 02115; Phone: 617-525-2691; Fax: 617-525-2008; nhjok@channing.harvard.edu

the two isoforms share various structural domains, they are functionally distinct transcription factors 7 that mediate their own response genes and physiological effects with little overlap 8, 9. In the normal developing breast, progesterone is required for ductal branching and alveolar development of the mammary gland and these effects are mediated through binding to the PR 10. Progesterone acting through PR-A and PR-B is also necessary for normal breast development during pregnancy, lactation, and involution 11

A functional polymorphism in the promoter region of the progesterone receptor (PR) gene previously has been described 12. The *+331 G/A* SNP creates a unique transcription start site leading to increased expression of the hPR-B isoform, and has been associated with a two-fold increased risk of endometrial cancer 12, an increased risk of postmenopausal breast cancer 13, and a higher number of failed attempts at *in vitro* fertilization 14. In normal breast tissue, both PR-A and PR-B are present in equimolar concentrations; some hypothesize that a disruption to the ratio of these two isoforms is important in breast cancer etiology because the two isoforms have different responses to their shared ligand, progesterone 15.

Results from both observational studies and randomized controlled trials have consistently reported that postmenopausal hormone (PMH) use increases the risk of breast cancer 16, 17, especially with use of combined estrogen plus progesterone formulations versus use of unopposed estrogens 18, 19. The effect of PMH is stronger with increasing duration of use, with current versus past use, as well as with the development of estrogen receptor (ER) -positive/PR-positive breast tumors, suggesting that PMH may only alter risk in the context of hormone responsive tumors 16, 20, 21.

Since the *+331 G/A* polymorphism in PR results in increased production of the PR-B isoform, which is a transcriptional activator 22, it is plausible that the risk of breast cancer from the variant allele may differ by PMH use. We hypothesized that the *+331 G/A* SNP may have a stronger effect among PMH users because the hormone stimulation of PR in these women is likely to be high. Thus, we undertook the current study to evaluate whether PMH use modifies the relationship between genetic variation in *PR* and risk of postmenopausal breast cancer in a case-control study nested within the prospective Nurses' Health Study (NHS).

MATERIALS AND METHODS

Study Cohort

The Nurses' Health Study was initiated in 1976, when 121,700 female registered nurses in 11 US states between the ages of 30 and 55 completed a self-administered, mailed questionnaire, reporting medical histories and baseline health-related exposures 23-25. Every 2 years, information on reproductive variables, medical history, and PMH use was updated through mailed questionnaires. A baseline dietary questionnaire was added in 1980 and subsequent dietary questionnaires were distributed in 1984, 1986 and every four years thereafter. The study was approved by the Institutional Review Board, Brigham and Women's Hospital.

Study Population and Data Collection

Between 1989 and 1990, blood samples were collected from 32,826 women. Details regarding the blood collection methods have been published previously 26. Estradiol and testosterone were measured at Quest Diagnostic's Nichols Institute (San Juan Capistrano, CA) by sensitive and specific radioimmunoassay, after organic hexane-ethyl acetate extraction and Celite column partition chromatography, as described in detail elsewhere 27. Mammographic density has previously been assessed in a subset of the controls in the

current study through the 1998 follow-up cycle. The mammogram collection and quantification has been described in detail 28. In brief, the craniocaudal views of both breasts were digitized at 261 microns/pixel with a Lumysis 85 laser film scanner, which covers a range of 0 to 4.0 optical density. The software for computer-assisted thresholding was developed at the University of Toronto 29 and this measure of mammographic breast density was highly reproducible within this study population 30. We used the average percentage density of both breasts for this analysis.

Incident breast cancers were identified by self-report or death certificate and confirmed by medical record review. The current study was restricted to white women who were postmenopausal at the time of blood collection. Women were considered to be postmenopausal if they reported having a natural menopause (e.g., no menstrual cycles during the previous 12 months) or had a bilateral oophorectomy. Women who had a hysterectomy but had at least one ovary remaining were considered postmenopausal at age 56 (for nonsmokers) or 54 (for smokers) years of age. These were the ages at which natural menopause occurred for 90% of the overall cohort.

Eligible cases in this study consisted of women diagnosed with pathologically confirmed incident breast cancer (invasive and *in situ*) after giving a blood specimen and before June 1, 2006. The nested case-control study consisted of 1,664 incident postmenopausal breast cancer cases and 2,391 postmenopausal controls with genotyping. Controls were matched to cases on year of birth, date of blood draw, time of blood draw, fasting status, and postmenopausal hormone use at blood draw (use within last three months versus non-users/missing) as described previously 31, 32. For each case who reported PMH use within three months prior to blood collection, one control was matched per case; whereas, for each case who had not reported recent PMH use at blood collection, two controls were matched per case. The women were matched on PMH use at blood draw because the assessment of endogenous plasma estrogens was a primary study aim; this matching disallows estimation of relative risks of breast cancer with PMH use but allows valid assessment of effect modification by PMH. The follow-up rate for this sub-cohort of women through 2006 was greater than 96%.

Genotyping

Genotyping assays for the *+331 G/A* polymorphism (rs10895068) were done by the 5′ nuclease assay (TaqMan) and the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). PCR amplification was carried out using forward (CACGAGTTTGATGCCAGAGAAA) and reverse (GCGACGGCAATTTAGTGACA) primers, 200 nmol/L of the FAM-labeled probe (CGGCTCCTTTATC) and 200 nmol/L of the VIC-labeled probe (CGGCTCTTTTATCTC) in a 5 μL reaction; the polymorphic base is underlined. Each reaction was heated to 95°C for 10 minutes, followed by 50 cycles of 92°C for 15 seconds, and 58°C for 1 minute. Blinded quality control samples (10%) were inserted randomly across all the plates to validate genotyping procedures. Concordance for the blinded samples was 100%.

Ascertainment of Hormone Use

In 1976, women were asked about current use and duration of PMH use. Beginning in 1978 and on all subsequent biennial questionnaires, women also were asked about the type of PMH they used during the preceding 2 years as well as updated information about current use. We categorized PMH use as never use, past PMH use, current use of estrogen alone, estrogen and progesterone, and other current PMH use. Women were considered current users of therapy if they reported current use of PMH at the beginning of the 2-year follow-up cycle prior to diagnosis. Duration of PMH use was the summation of PMH use across

questionnaire cycles. The initial questionnaire asked participants how long they had used hormones previously. From 1978 on, respondents were asked about the number of months they used hormones since the previous 2-year cycle. We created two categories for duration of PMH use (< 5 and ≥ 5 years) to match cut-points at which associations were observed in our previous studies 20 and to maximize power.

Statistical Analysis

We tested Hardy-Weinberg agreement by using a chi-square (χ^2) test. Conditional logistic regression was used to estimate the multivariable-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) associated with the main effects of the *PR* genotype. Because of the low prevalence of homozygous variants (*AA*), we combined heterozygotes (*GA*) and homozygotes (*AA*) in the analysis. For analyses by ER/PR tumor status, we used unconditional logistic regression adjusting for matching factors to maximize power.

To assess the presence of effect modification of the association between *PR* genotype and the risk of breast cancer by PMH status, stratum-specific ORs for each genotype (*GG* versus *GA* + *AA*) and multivariate interaction terms were estimated across categories of PMH use using unconditional logistic regression. We calculated the *P* - value for interaction using the log-likelihood test comparing models with and without interaction terms between PMH use and genotype.

In addition to the matching variables (e.g., age, date of blood draw), all the models were adjusted for the following *a priori* breast cancer risk factors: age at menarche (continuous), age at menopause (continuous), age at first birth/parity (nulliparous; 1-2 children and age ≤22 years at first birth; 1-2 children and age 23-25 years at first birth; 1-2 children and age ≥26 years at first birth; ≥3 children and age ≤22 years at first birth; ≥3 children and age 23-25 years at first birth; ≥3 children and age ≥26 years at first birth; or data on parity and/or age at first birth unavailable), BMI at age 18 (<21, ≥21-<23, ≥23-<25, >25, missing), weight gain since age 18 (<5, ≥5-<20, ≥20 kg), history of benign breast disease (yes or no), first-degree family history of breast cancer (yes or no), and alcohol consumption (0, >0.1-<5 grams per day, ≥5 grams per day).

In a supplementary analysis, we calculated the mean percent mammographic density stratified by *+331G/A* PR polymorphism and PMH use status using a generalized linear model adjusting for age and BMI. This analysis was conducted in a subset of the controls with mammographic density in the current study identified through 1998. Effect modification by plasma estradiol and testosterone levels was evaluated by using batch-specific medians based on the distribution in the control subjects for PMH current and never users separately. We modeled a two-way interaction between the PR genotype and circulating hormone levels (< median, ≥ median) and compared models with and without the multiplicative interaction terms (degrees of freedom = 1) to assess significance. For effect modification by BMI, we assessed a three-way interaction between PMH use (never, past, current), BMI (< 25, ≥25), and PR polymorphism by including the main effects of each exposure and all two-way and three-way multiplicative interaction terms. The *P* for interaction was based on the likelihood ratio test comparing unconditional logistic regression models with and without interaction terms (degrees of freedom = 5).

All analyses were conducted using SAS version 9.1 (SAS Institute INC., Cary, NC). All *P* values were based on two-sided tests and were considered statistically significant if $P \leq 0.05$.

RESULTS

PR genotype data were available for 1,664 incident breast cancer cases and 2,391 matched controls (Table 1). Compared with controls, cases tended to have an earlier age at menarche, later age at first birth, lower mean parity, greater weight gain since age 18, and higher mean daily alcohol consumption. Cases also were more likely than controls to have a personal history of benign breast disease and a family history of breast cancer.

The allele frequency of the variant *A* allele was 6% and 5% for cases and controls, respectively, and is similar to what has previously been reported for white women 33, 34. The *PR* genotype distribution was in Hardy-Weinberg equilibrium for the cases ($P = 0.85$), but not for the controls ($P < 0.001$); however, evaluation of the data showed no genotyping error. Women who were carriers of one or two copies of the variant *A* alleles had a statistically significant increased risk of breast cancer (Table 2). Women with the *GA* or *AA* genotype had a 31% increased risk of developing breast cancer compared to women with the *GG* wild type genotype (95%CI 1.04-1.65). After limiting our study population to ER+ or PR+ tumors only, the magnitude of the associations were similar, although they did not achieve statistical significance (OR = 1.30; 95%CI 0.95-1.78 and OR = 1.30; 95% CI 0.93-1.82, for ER+ and PR+ tumors only, respectively). The risk of breast cancer associated with the *+331 G/A* SNP did not differ by ER/PR tumor status, although the sample sizes were small after stratification (data not shown).

PMH use significantly modified the association between the *+331 G/A* polymorphism in the PR gene and breast cancer risk in this study population (Tables 3 and 4) (P for interaction < 0.05). Among never users of PMH, carriers of one or both variant *A* alleles had a significantly increased risk of breast cancer compared to those with the wild-type genotype (OR = 2.57; 95% CI 1.64-4.02)(Table 3). The *+331 G/A* polymorphism (versus *G/G*) was not significantly associated with breast cancer risk among either past (OR = 1.23; 95% CI 0.77-1.97) or current (OR = 1.14; 95% CI 0.84-1.56) PMH users. The P for interaction was no longer significant up exclusion of never users ($P = 0.66$; data not shown). When the analyses were stratified by type of PMH used, we did not observe any clear associations between the *PR* genotype and risk of breast cancer among women with past or current use of any of the PMH formulations; however, the P for interaction was significant ($P = 0.02$) likely due to the higher OR among never users. In the analyses stratified by duration of PMH use (< 5 and ≥ 5 years), there was again a significant interaction between PMH use and breast cancer risk by PR genotype ($P = 0.04$) with the significant increased risk of breast cancer with the *+331 G/A* polymorphism limited to never PMH users (Table 4).

In a secondary analysis, we evaluated a modifying role of BMI (BMI < 25 versus ≥ 25 kg/m^2) and plasma sex hormone levels on the association between the *+331 G/A* polymorphism and risk by strata of PMH use (data not shown). We did not observe clear evidence for effect modification by BMI (P for interaction = 0.12). However, we found that the risk of breast cancer among women who were carriers of the variant *A* allele was strongest among never PMH users whose circulating estradiol levels were above rather than below the median (OR = 2.58; 95%CI 1.09-6.09 versus OR = 0.71; 95%CI 0.25-2.01 for estradiol < median and ≥ median, respectively)(P for interaction = 0.05). Risk did not vary by circulating estradiol levels among current PMH users (P for interaction = 0.47) nor by testosterone levels among both never and current PMH users (P for interaction = 0.24 and 0.53, respectively). Based on prior evidence that genetic variation in PR may mediate the effect of PMH use on mammographic density and subsequently breast cancer risk 35, we conducted a supplementary cross-sectional analysis to evaluate mean percent mammographic density stratified by the *+331G/A* PR polymorphism and PMH use status (data not shown). We did not detect any statistical difference in mean mammographic density by PR genotype within

each stratum of PMH use (*P* for interaction = 0.18). The mean mammographic density among the controls with the wild type genotype was 21.1 (n=335), 23.2 (n=186) and 27.9 (n=279) for never, past and current PMH users, respectively. The corresponding values for the variant allele carriers were 21.5 (n=28), 21.1 (n=19), and 31.2 (n=35).

DISCUSSION

In this large nested case-control study, we confirmed a significantly elevated risk of postmenopausal breast cancer among women carrying the *+331 G/A* polymorphism in the PR. More importantly, contrary to our hypothesis, we observed that the increase in risk due to the SNP was limited to women who were never users of PMH where a two-fold increase in risk was observed. There were no clear associations between genotype and risk among women who had ever used PMH, irrespective of PMH formulation or duration of use.

The significant increased risk of postmenopausal breast cancer among carriers of the variant *A* allele is consistent with what has been described previously in an earlier analysis of the NHS, although with a smaller sample size (n=1,110 versus 1,664 cases)13. Moreover, in the Huggins et *al.* paper, the increase in risk associated with the *A* allele was only observed among postmenopausal and not among premenopausal women 13. Other population-based studies conducted in the U.S., as well as in Europe and Australia, generally have reported no association between this SNP and breast cancer risk 36-41, although many of these analyses were limited by either low power, inability to stratify by menopausal status, or population stratification (e.g. this SNP is not found in those of African ancestry). Our analysis was limited to postmenopausal white women to minimize confounding due to ethnic variation in the allelic distribution of this gene[1]. In the largest of these studies conducted among 4,647 cases and 4,564 controls in the United Kingdom, Pooley *et al.* used a comprehensive SNP tagging approach to identify SNPs that may be associated with risk 40. The authors did not find any significant association with the *GA* or *AA* genotype and risk nor any significant difference in the genotype distribution between premenopausal and postmenopausal cases. A limitation of this study was that only univariate results were presented. Johnatty *et al.* similarly found no significant association between *+331 G/A* SNP and risk in an Australian analysis of 1,847 cases and 833 controls. Even though 90% of their population was Caucasian, their inability to replicate our findings may be due to the lack of stratification by menopausal status. Furthermore, the women in their study were on average at least 10 years younger than those in our study. This SNP was not evaluated in the genome-wide association study of breast cancer in the NHS 42. Our results suggest that this variant in *PR* may only influence risk in the setting of a low endogenous hormonal milieu.

PR-A and PR-B are functionally distinct with the A isoform acting as a dominant repressor of transcription of B, and B acting as a potent transcriptional activator of its target genes 7. Using a PR-A knock-out mouse model, Mulac-Jericevic *et al.* have demonstrated increased proliferation of the uterine epithelium with treatment of estrogen alone which was further enhanced with the addition of progesterone 22. More importantly, the PR has been shown to exert transcriptional effects irrespective of ligand stimulation 7, 43 and can be activated by cross-talk with other cell-signaling pathways 44. These biological studies provide evidence that PR-B may stimulate mammary cell growth in the absence or presence of ligand activation, thus contributing to cancer development. Expression of PR-A and PR-B are about equal in the normal breast; however, there appears to be dysregulation of this ratio with breast cancer development 15, 45, 46. In addition, Hopp *et al.* reported that patients with a high PR-A to PR-B ratio are more likely to relapse 45. These studies have

[1]http://snp500cancer.nci.nih.gov/snp.cfm?ethnic=true&hdp=true&snp_id=PGR-27

demonstrated that a balance between these two isoforms may be important in breast carcinogenesis.

De Vivo *et al.* have shown that the *+331 G/A* hPR polymorphism is functional and results in a unique transcriptional start site, increased transcriptional activity, and ultimately increased production of the hPR-B over the hPR-A isoform 12. Since this polymorphism results in increased transcription of PR-B, which is required for ductal and alveolar epithelial cell proliferation 47, we initially hypothesized that the increased risk of breast cancer for women carrying the variant allele would be strongest among women who used combination estrogen + progesterone PMH because high levels of the progesterone ligand in these women would stimulate PR-B activity. Despite this, we observed that the effect of the SNP was limited to never PMH users. One other previous study has evaluated a possible association between genetic variation in PR and PMH use. This was a small nested case-control study of 479 postmenopausal breast cancer cases and 494 controls that did not report an association between the *+331 G/A* SNP and breast cancer risk, nor did they find any evidence for an interaction with PMH use (*P* for interaction = 0.86) 36. However, given that these authors did not report the associated confidence intervals, their results are difficult to directly compare to ours. Further given the rarity of the variant *+331 A* allele, a large sample size such as that in the current study may be needed to observe an interaction.

In the current study, the effect of this SNP was substantially attenuated in the setting of pharmacological doses of hormones (OR = 1.23 and OR = 1.14 for *GA/AA* versus *GG* among past and current PMH users, respectively). Among women who use PMH, circulating hormone levels are high and are primarily dictated by this exogenous source of hormones; whereas among non-users, the source of hormones is entirely endogenous and for the most part, synthesized from the peripheral aromatization of androgens to estrogens in adipose tissue 48. While obesity is an important risk factor for postmenopausal breast cancer, we 49 and others 16, 50, have shown that it is more strongly associated with breast cancer risk among women who have never used PMH. Similarly, our results demonstrate that the *+331 G/A* polymorphism may not be important in the setting of very high hormone levels (postmenopausal women using PMH) and illustrate the continued need for future studies to evaluate breast cancer risk factors separately according to exogenous hormone use as a surrogate for overall estrogen exposure. The already high levels of hormones attributed to PMH use likely obscured any effect of the SNP on breast cancer risk. But in the context of endogenous hormone levels, we found that the risk of breast cancer was strongest among never PMH users with the *GA/AA* genotype whose plasma estradiol levels were above the median indicating a more pronounced downstream effect of the SNP among women with elevated hormone levels. In contrast, the SNP does not appear to influence risk among women with low to normal estrogen levels. This further underscores the important role of endogenous estrogen in the etiology of postmenopausal breast cancer among non-users of PMH.

In our prior analysis with fewer cases, we observed that the *+331 G/A* SNP was only associated with breast cancer risk among postmenopausal women or overweight/obese women, consistent with our observation of a stronger effect in women with higher circulating estradiol levels (*P* for interaction = 0.05), but no significant interaction with PMH use 13. With the additional cases, we no longer observed any effect modification by BMI. However, a key difference between these two analyses of the same population is that in the prior analysis, the authors did not restrict the BMI interaction analysis to never PMH users. This sub-analysis is of particular importance since PMH users are often leaner than non-users. Thus, the apparent interaction observed with BMI could have been predominantly driven by PMH use.

Given that PMH may increase the risk of breast cancer by affecting mammographic density, a known risk factor for postmenopausal breast cancer, van Duijnhoven *et al.* evaluated whether the effects of PMH were modified by polymorphisms in the estrogen receptor (*ESR1*) and PR genes 35. They found that a significant increase in mammographic density between PMH users and never users was limited to women with the *+331 GG* wildtype genotype but not those with the *GA* or *AA* genotype. These data suggest that the wildtype genotype influences individual susceptibility to the effects of PMH on mammographic density. We found no evidence for a difference in mammographic density by PR genotype within each stratum of PMH use. Given the different findings, additional studies of this association should be conducted.

There are several strengths and limitations associated with this study. Limitations include the small sample size within individual PMH use stratum for the variant allele, given the low frequency of the variant allele in this population (~ 5%)13. Even though our allele frequencies among controls were not in Hardy-Weinberg equilibrium, the genotyping results using the Taqman assay were unambiguous and thus any deviation is likely attributed to chance. Also, the prevalence of the minor allele and the distribution of the genotypes in our population are similar to what has previously been reported for Caucasians 36, 40, 41. Since we combined the *GA* and *AA* genotypes in this analysis, we also tested for Hardy-Weinberg equilibrium assuming two genotypes: *GG* and *GA+AA* combined. After collapsing these two genotypes, the allele frequencies in the controls no longer deviated from the Hardy-Weinberg principle ($P = 0.50$). Furthermore, with our large sample size, we had increased power to detect small differences.

The current analysis was limited to white women and may not be generalizable to other populations of different ancestries. Also, we were not able to evaluate a combined role of duration and type of PMH used due to small numbers following stratification. Nonetheless, this is the largest study that has evaluated the joint effect of a common exposure, PMH use, and *PR* genotype on breast cancer risk using a study population that included 1,664 cases and 2,391 controls. The prospective nature of the NHS allowed for the detailed collection of updated risk factor information thus controlling for the majority of the known or suspected breast cancer risk factors and decreasing the influence of confounding.

In summary, in this large population-based study we found that the *+331 G/A* SNP in the *PR* gene was associated with more than a two-fold increased risk of developing postmenopausal breast cancer among never users of PMH. This variant was not associated with risk among PMH users, implying that the high levels of circulating hormones due to exogenous sources (i.e. PMH use) may obscure or counteract any detrimental effect the genetic variation may exert on risk. The adverse effect of this SNP implicates PR as a low-penetrance breast cancer susceptibility gene, a finding that warrants further evaluation in a different dataset. In light of the decreasing use of PMH, the effect of this variant in the etiology of breast cancer might be of greater importance. Future studies should continue to consider PMH use as an important effect modifier of breast cancer risk among women with the high-risk *A* allele.

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Table 1

Descriptive characteristics of cases and matched controls from the Nurses' Health Study

Characteristic	Cases (n = 1664)	Controls (n = 2391)
Age at blood draw, y (mean, SD)[1]	57.95 (6.47)	58.37 (6.37)
Age at menarche, y (mean, SD)	12.48 (1.38)	12.58 (1.39)
Age at menopause, y (mean, SD)	50.24 (4.89)	49.86 (4.67)
Natural menopause, n (%)	791 (47%)	1091 (65%)
Age at first birth, y (mean, SD)[2]	26.46 (9.54)	25.85 (7.49)
Parity, n (mean, SD)[2]	3.21 (1.48)	3.34 (1.60)
BMI at 18, kg/m^2 (mean, SD)	21.16 (2.71)	21.34 (2.87)
BMI, kg/m^2 (mean, SD)[3]	26.46 (5.04)	26.29 (4.99)
Weight gain since age 18 , pounds (mean, SD)	14.48 (12.85)	13.46 (12.51)
Alcohol consumption, g/day (mean, SD)	6.27 (10.55)	5.31 (8.94)
First-degree family history of breast cancer, n (%)	353 (21%)	374 (16%)
Personal history of benign breast disease,n (%)	474 (28%)	428 (18%)

[1] Matching factor (1:1 matching for PMH users and 1:2 matching for non-users). PMH users were older than non-users.

[2] Among parous women only (n = 1549 cases and 2241 controls).

[3] BMI at two-year follow-up cycle prior to case diagnosis and comparable cycle for matched controls.

Table 2

Association between the *+331 G/A* PR polymorphism and postmenopausal breast cancer risk, among all cases and limited to ER+ or PR+ tumors only

PR genotype	Cases, n (%)	Controls, n (%)	OR (95%CI)[1]	OR (95%CI)[2]
All cases	1664	2391		
GG	1463 (88%)	2174 (91%)	1.0 (ref)	1.0 (ref)
AG	195 (12%)	202 (8%)	1.44 (1.15-1.79)	1.34 (1.06-1.69)
AA	6 (0.4%)	15 (1%)	0.62 (0.24-1.62)	0.89 (0.32-2.52)
GG	1463 (88%)	2174 (91%)	1.0 (ref)	1.0 (ref)
AG + AA	201 (12%)	217 (9%)	1.38 (1.11-1.71)	1.31 (1.04-1.65)
ER+ tumors only[3]				
GG	941 (89%)	2174 (91%)	1.0 (ref)	1.0 (ref)
AG + AA	116 (11%)	217 (9%)	1.24 (0.97-1.57)	1.30 (0.95-1.78)
PR+ tumors only[4]				
GG	767 (89%)	2174 (91%)	1.0 (ref)	1.0 (ref)
AG + AA	98 (11%)	217 (9%)	1.28 (1.00-1.65)	1.30 (0.93-1.82)

[1] Conditional logistic regression for all cases. Unconditional logistic regression for ER+ and PR+ tumors only adjusted for matching variables: age, date blood draw, time at blood draw, fasting status and PMH use at diagnosis.

[2] Conditional logistic for all cases adjusted for age at menarche, age at menopause, age at first birth/parity, BMI at age 18, weight gain since age 18, history of benign breast disease, first-degree family history of breast cancer, and alcohol consumption. Unconditional logistic regression for ER+ and PR+ tumors only, adjusting for the matching variables and age at menarche, age at menopause, age at first birth/parity, BMI at age 18, weight gain since age 18, history of benign breast disease, first-degree family history of breast cancer, and alcohol consumption.

[3] ER- tumors = 232, ER status unknown = 29.

[4] PR- tumors = 388, PR status unknown = 33.

Table 3

Association between *+331G/A* PR polymorphism and risk of postmenopausal breast cancer by postmenopausal hormone (PMH) status and type of PMH used

PMH use status	GG			*AG+GG*			
	Cases, n (%)	Controls, n (%)	OR (95%CI)[1]	Cases, n (%)	Controls, n (%)	OR (95%CI)	*P* - int[2]
Never	258 (83%)	607 (92%)	1.00 (ref)	53 (17%)	50 (8%)	2.57 (1.64-4.02)	
Past	336 (90%)	574 (91%)	1.00 (ref)	39 (10%)	54 (9%)	1.23 (0.77-1.97)	
Current	804 (89%)	891 (90%)	1.00 (ref)	97 (11%)	97 (10%)	1.14 (0.84-1.56)	
Estrogen alone	373 (89%)	445 (91%)	1.00 (ref)	47 (11%)	45 (9%)	1.49 (0.95-2.36)	
Estrogen + progesterone	355 (90%)	355 (90%)	1.00 (ref)	38 (10%)	41 (10%)	0.78 (0.47-1.27)	
Other	76 (86%)	91 (89%)	1.00 (ref)	12 (14%)	11 (11%)	1.50 (0.48-4.68)	
							0.02

[1] Unconditional logistic regression adjusted for age, date blood draw, age at menarche, age at menopause, age at first birth/parity, BMI at age 18, weight gain since age 18, history of benign breast disease, first-degree family history of breast cancer, and alcohol consumption.

[2] *P* - int = *P* for interaction based on likelihood ratio test comparing unconditional logistic regression models with and without interaction terms between PMH use status (never, past, current) and genotype (*GG*, *AG+GG*)(degrees of freedom = 2).

Table 4

Association between *+331G/A* PR polymorphism and risk of postmenopausal breast cancer by duration of PMH used

PMH use status	GG			*AG+AA*			^2P - int
	Cases, n (%)	Controls, n (%)	OR (95%CI)[1]	Cases, n (%)	Controls, n (%)	OR (95%CI)	
Never	258 (83%)	607 (92%)	1.00 (ref)	53 (17%)	50 (8%)	2.57 (1.64-4.02)	
Past							
< 5 years	178 (87%)	370 (91%)	1.00 (ref)	26 (13%)	37 (9%)	1.62 (0.89-2.94)	
≥ 5 years	158 (92%)	204 (92%)	1.00 (ref)	13 (8%)	17 (8%)	0.93 (0.41-2.11)	
Current							
< 5 years	174 (89%)	209 (92%)	1.00 (ref)	21 (11%)	18 (8%)	1.60 (0.79-3.24)	
≥ 5 years	630 (89%)	682 (90%)	1.00 (ref)	76 (11%)	79 (10%)	1.06 (0.75-1.51)	
							0.04

[1] Unconditional logistic regression adjusted for age, date blood draw, age at menarche, age at menopause, age at first birth/parity, BMI at age 18, weight gain since age 18, history of benign breast disease, first-degree family history of breast cancer, and alcohol consumption.

[2] P - int = P for interaction based on likelihood ratio test comparing unconditional logistic regression models with and without interaction terms between PMH use status (past or current, <5 or ≥5 years) and genotype (*GG*, *AG+GG*)(degrees of freedom = 4).