[CANCER RESEARCH 59, 1015–1020, March 1, 1999]

The Relationship between a Polymorphism in *CYP17* with Plasma Hormone Levels and Breast Cancer[1]

Christopher A. Haiman,[2] Susan E. Hankinson, Donna Spiegelman, Graham A. Colditz, Walter C. Willett, Frank E. Speizer, Karl T. Kelsey, and David J. Hunter

Departments of Epidemiology [C. A. H., S. E. H., D. S., G. A. C., W. C. W., D. J. H.], Nutrition [W. C. W.], Environmental Health [F. E. S., K. T. K.], Cancer Cell Biology [K. T. K.], and Biostatistics [D. S.], and Harvard Center for Cancer Prevention [K. T. K., D. J. H.], Harvard School of Public Health, and Channing Laboratory, Department of Medicine, Brigham and Women's Hospital [S. E. H., G. A. C., W. C. W., F. E. S., K. T. K., D. J. H.], Harvard Medical School, Boston, Massachusetts 02115

ABSTRACT

The *A2* allele of *CYP17* has been associated with polycystic ovarian syndrome, elevated levels of certain steroid hormones in premenopausal women, and increased breast cancer risk. We prospectively assessed the association between the *A2* allele of *CYP17* and breast cancer risk in a case-control study nested within the Nurses' Health Study cohort. We also evaluated associations between this *CYP17* genotype and plasma steroid hormone levels among postmenopausal controls not using hormone replacement to assess the biological significance of this genetic variant. Women with the *A2* allele were not at an increased risk of incident breast cancer [OR (odds ratio), 0.85; 95% CI (confidence interval), 0.65–1.12] or advanced breast cancer (OR, 0.84; 95% CI, 0.54–1.32). We did observe evidence that the inverse association of late age at menarche with breast cancer may be modified by the *CYP17 A2* allele. The protective effect of later age at menarche was only observed among women without the *A2* allele (*A1/A1* genotype: for age at menarche ≥13 *versus* <13; OR, 0.57; 95% CI, 0.36–0.90; *A1/A2* and *A2/A2* genotypes: OR, 1.05; 95% CI, 0.76–1.45; *P* for interaction = 0.07). Among controls, we found women with the *A2/A2* genotype to have elevated levels of estrone (+14.3%, *P* = 0.01), estradiol (+13.8%, *P* = 0.08), testosterone (+8.6%, *P* = 0.34), androstenedione (+17.1%, *P* = 0.06), dehydroepiandrosterone (+14.4%, *P* = 0.02), and dehydroepiandrosterone sulfate (+7.2%, *P* = 0.26) compared with women with the *A1/A1* genotype. These data suggest that the *A2* allele of *CYP17* modifies endogenous hormone levels, but is not a strong independent risk factor for breast cancer.

INTRODUCTION

Endogenous steroid hormones are important in the development and progression of breast cancer (1–3). Steroid hormones exert growth-promoting effects and induce breast cell proliferation by binding to intracellular receptors and regulating gene transcription (4, 5). Several breast cancer risk factors are thought to act by influencing lifetime exposure to steroid hormones. The rate of increase in breast cancer incidence declines after menopause, probably due to lower circulating estrogen and progesterone levels (6). Age at menarche, age at menopause, postmenopausal obesity, and postmenopausal hormone use are well established breast cancer risk factors that influence the dose and duration of estrogen and progesterone exposure (7). Obesity increases breast cancer risk among postmenopausal women (8), probably due to the greater peripheral conversion in adipose tissue of precursor androgen steroid hormones such as DHEA,[3] DHEAS, and androstenedione to estrogens, estradiol, and estrone (9, 10).

Tissue-specific expression and altered activity of steroid hormone receptors and steroidogenic enzymes are presently being studied to clarify their role in hormone-related cancers (11, 12). Functional polymorphisms in genes encoding steroid metabolizing enzymes may contribute to this understanding by serving as surrogate markers for altered long-term hormone exposure and, thus, as biomarkers of individual breast cancer susceptibility. Although these polymorphisms may pose small relative risks, those with a high prevalence in the population could result in a substantial attributable risk. One such polymorphism in *CYP17*, a gene encoding the steroidogenic enzyme P450c17α (13), has recently been associated with breast cancer risk (14).

The cytochrome P450 enzyme, P450c17α, has both 17α-hydroxylase and 17,20-lyase activities and catalyzes two distinct steps in steroid hormone production (15). In steroidogenesis, cholesterol is first converted to pregnenolone by an enzyme encoded by *CYP11a*, and then to progesterone by 3β-hydroxysteroid dehydrogenase. 17α-Hydroxylase converts pregnenolone and progesterone to 17-hydroxypregnenolone and 17-hydroxyprogesterone, respectively. In humans, these intermediates are then converted to DHEA and, to a lesser extent, androstenedione by the 17,20-lyase activity. In women, P450c17α is primarily expressed in ovarian theca cells and the adrenal cortex. Rare mutations in the coding region of *CYP17* have been found in patients with 17α-hydroxylase/17,20-lyase deficiency, resulting in various clinical profiles such as congenital adrenal hyperplasia, abnormal sexual development, and irregular menstruation (16).

Feigelson *et al.* (14) reported data on the association between the *A2* allele of *CYP17* and breast cancer in a nested case-control study (n = 174 cases) among a cohort of Asian, African American, and Latino women. These authors observed that women with the *A2* allele were at a nonsignificantly increased risk of breast cancer (OR, 1.32; 95% CI, 0.87–2.00). Among cases with regional or metastatic breast cancer (n = 40), a significant positive association was observed (OR, 2.52; 95% CI, 1.07–5.94). Women with the *A2* allele also had significantly earlier age of menarche, and the inverse association of a late age of menarche with breast cancer risk was not observed among women who were homozygous or heterozygous for the *A2* allele. Subsequent studies do not support the association between the *A2* allele and risk of breast cancer or advanced breast cancer (17–19); however, in one of these studies the inverse association with late age of menarche was limited to women without the *A2* allele (17).

We evaluated the association between the variant *CYP17* allele and breast cancer in a nested case-control study within the Nurses' Health Study, along with the hypothesis that the *A2* allele could influence age at onset of menarche. The relationship of the *A2* allele with plasma steroid hormone levels was also examined among postmenopausal women without breast cancer to assess the biological function of the polymorphism.

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. Updated information has been obtained by questionnaire every 2

Received 10/5/98; accepted 1/4/99.

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[1] Supported by NIH Grants CA40356, CA49449, EH00002, and CA65725. D. J. H. is partially supported by an ACS Faculty Research Award (FRA-455).

[2] To whom requests for reprints should be addressed, at Channing Laboratory, 181 Longwood Avenue, Boston, MA 02115.

[3] The abbreviations used are: DHEA, dehydroepiandrosterone; DHEAS, DHEA sulfate; *CYP17*, *cytochrome p450c17α* gene; BMI, body mass index; OR, odds ratio; CI, confidence interval; LRT, likelihood ratio test.

years, including data on reproductive variables, oral contraceptive and postmenopausal hormone use, cigarette smoking and, since 1980, dietary intake. Incident breast cancers are identified by self-report and confirmed by medical record review. Between 1989 and 1990, blood samples were collected from 32,826 women. Approximately 97% of the blood samples were returned within 26 h of blood draw, immediately centrifuged, aliquoted into plasma, RBC, and buffy coat fractions, and stored in liquid nitrogen freezers. The follow-up has been greater than 98% for this subcohort.

Eligible cases in this study consisted of women with pathologically confirmed incident breast cancer from the subcohort who gave a blood specimen. Cases with a diagnosis anytime after blood collection up to June 1, 1994, with no previously diagnosed cancer except for nonmelanoma skin cancer, were included. Controls were randomly selected participants who gave a blood sample and were free of diagnosed cancer (except for nonmelanoma skin cancer) up to and including the interval in which the case was diagnosed. Controls were matched to cases on year of birth, menopausal status, postmenopausal hormone use, as well as time of day, month, and fasting status at blood draw. Women were defined as postmenopausal at the time of a bilateral oophorectomy or after having no menstrual cycle within the last 12 months before blood draw. Women who had had a hysterectomy with one or both ovaries left intact were classified as premenopausal until the age at which 10% of the cohort had undergone natural menopause (46 years for smokers and 48 years for nonsmokers) and as postmenopausal at the age at which 90% of the cohort had undergone natural menopause (54 for smokers and 56 for nonsmokers); in the intervening years, these women were classified as being of uncertain menopausal status. For postmenopausal cases not using postmenopausal hormones within 3 months before blood draw, we matched a second control to increase our statistical power in plasma steroid hormone analyses. The nested case-control study consists of 464 incident breast cancer cases and 619 matched controls. The study sample for the plasma hormone analysis is composed of 297 postmenopausal control women not using hormone replacement therapy within 3 months of blood draw. The protocol was approved by the Committee on Human Subjects, Brigham and Womens' Hospital.

***CYP17* Assay.** DNA was extracted from buffy coat fractions using the Qiagen QIAamp Blood Kit (Qiagen, Inc., Chatsworth, CA). The *Msp*AI (New England Biolabs, Inc., Beverly, MA) restriction enzyme was used for detection of the *CYP17* RFLP. *CYP17* genotyping analysis was performed as follows: PCR amplification of the polymorphic fragment was generated using primers 5′-CATTCGCACCTCTGGAGTC-3′ and 5′-GCTCTTGGGGTACTTG-3′. Genomic DNA (45 ng) was used per 50 μl of reaction with 50 mM KCl, 20 mM Tris-HCl (pH 8.9), 0.1% BSA, 0.2 mM dNTPs, 100 ng of each primer, and 1.25 units of AmpliTaq DNA polymerase (Perkin-Elmer Corp., Foster City, CA). Amplification conditions were 5 min of initial denaturation at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 57°C, and 1 min at 72°C, followed by a final extension at 72°C for 5 min. Amplified products were incubated overnight with *Msp*AI restriction enzyme, and digested fragments were visualized on a 2.5% agarose gel with ethidium bromide staining. Genotyping was performed by laboratory personnel unaware of case-control status, and blinded quality control samples were inserted to validate genotype identification procedures.

Hormone Assays. Steroid hormone fractions of estradiol, estrone, estrone sulfate, testosterone, androstenedione, DHEA, and DHEAS were assayed in up to three separate batches. Estrone sulfate from batches 1 and 2 were assayed in the laboratory of Dr. C. Longcope (University of Massachusetts Medical Center, Worcester, MA). All other analyses were performed by Nichols Institute (San Juan Capistrano, CA). Methods for plasma hormone assays and information regarding laboratory precision and reproducibility have been previously published (20, 21). Within-batch laboratory coefficients of variation were $\leq$13.6%.

Exposure Data. Information regarding breast cancer risk factors were obtained from the 1976 baseline questionnaire, subsequent biennial questionnaires, and a questionnaire completed at the time of blood sampling. Menopausal status and use of postmenopausal hormones was assessed at blood draw and updated until date of diagnosis for cases and matched controls. Histopathological characteristics such as stage and estrogen and progesterone receptor status were ascertained from medical records and used in case subgroup analyses when available.

Statistical Analysis. ORs and 95% CIs were calculated using conditional and unconditional logistic regression. In addition to the matching variables, we adjusted for the breast cancer risk factors: BMI (kg/m^2) at age 18 (continuous), weight gain since age 18 ($<$5 kg, 5–19.9, $\geq$20), age of menarche ($<$12 years, 12, 13, and $>$13), parity/age at first birth (nulliparous, 1–2 children/age at first birth $\leq$24 years, 1–2 children/age at first birth $>$24, 3+ children/age at first birth $\leq$24, and 3+ children/age at first birth $>$24), first-degree family history of breast cancer (yes/no), history of benign breast disease (yes/no), and duration of postmenopausal hormone use (never; past, $<$5 years and $\geq$5; current, $<$5 years and $\geq$5). We also adjusted for age at menopause (continuous in years) in analyses limited to postmenopausal women. Indicator variables for all three genotypes were created using the *A1/A1* hypothesized low-risk genotype as the reference category in the multivariate models. Genotype was also evaluated using a dichotomous variable with *A1/A2* and *A2/A2* subjects combined because a gene dosage effect on breast cancer risk was not apparent, and because a prior study (14) observed an increase in risk of advanced breast cancer using this categorization of genotype. One case with missing parity and her matched control have been removed from all analyses, and nine women missing age at menarche have been removed from analyses examining the interaction between genotype and age at menarche on breast cancer risk. Unconditional multivariate models controlling for the matching factors enabled all controls to be included in case-subgroup analyses when limiting the cases to specified histopathological characteristics. Interactions between genotype and breast cancer risk factors were evaluated by including interaction terms between genotype and risk factor variables in unconditional multivariate logistic regression models. The LRT was used to assess the statistical significance of these interactions.

To examine if age at menarche is influenced by genotype, the Wilcoxon rank sum test was used to compare the median ages of menarche between genotypes separately for cases and controls.

Mixed regression models were used to evaluate the association between genotype and circulating hormone levels among controls, controlling for BMI at blood draw and the matching variables (22). Differences in hormone levels between the genotypes were evaluated with the *A1/A1* group as the reference category. The natural logarithm of the plasma hormone values were used in the analyses to reduce the skewness of the regression residuals. The robust variance was used for all *P*s based on Wald statistics, to increase the validity of inferences even if regression residuals are nonnormal or heteroscedastic (23). Hormone fractions were measured in two to three different batches; laboratory batch was treated as a random variable in all hormone analyses except for DHEAS among never users of hormone replacement, where a batch effect was not observed. To interpret hormone levels as percentage differences relative to the reference genotype, 1.0 was subtracted from the appropriate exponentiated linear combination of coefficients from the mixed regression models. Subjects with hormone levels below detectable limits ($n = 6$) were assigned the lowest detectable value (estrone, $n = 3$; testosterone, $n = 1$; DHEAS, $n = 2$). Within each batch, hormone values $>$3 interquartile ranges were treated as outliers and excluded (estrone sulfate, $n = 5$; estrone, $n = 2$; estradiol, $n = 3$; testosterone, $n = 2$; DHEA, $n = 1$; and DHEAS, $n = 1$). All hormones fractions were not assayed for all women due to insufficient plasma (estrone sulfate, $n = 18$; estrone, $n = 2$; estradiol, $n = 2$; testosterone, $n = 8$; androstenedione, $n = 11$; DHEA, $n = 30$; DHEAS, $n = 8$). We used the SAS statistical package for all analyses (24).

RESULTS

The mean age of the women was 58.3 (SD, 7.1), ranging from 43–69. There were 188 premenopausal and 804 postmenopausal women with mean ages of 48.1 (SD, 2.8) and 61.4 (SD, 5.0), respectively, at blood draw. Compared with controls, cases had the same mean age of menarche (12.5 *versus* 12.6 years) and the same age at first birth (25.2 *versus* 25.0 years). BMI was similar for both groups (25.5 *versus* 25.5 kg/m^2). Among parous women, controls were more likely to have had more than two full-term pregnancies compared with cases (68.7% *versus* 62.0%; $P = 0.03$). The proportion of women with a family history of breast cancer and a history of benign breast disease were significantly higher among the cases, 17.9% *versus* 13.1% ($P = 0.03$) and 62.0% *versus* 41.9% ($P = 0.001$), respectively. The prevalence of the *A2* allele among the controls was 40.0%, similar to

Table 1 *Associations between* CYP17 *genotype and breast cancer risk (Nurses' Health Study, 1989–94).*

	CYP17 genotype			
	A1/A1 n (%)	*A1/A2 n* (%)	*A2/A2 n* (%)	*A1/A2 + A2/A2 n* (%)
Controls	217 (35)	307 (50)	94 (15)	401 (65)
Cases	178 (38)	212 (46)	73 (16)	285 (62)
OR[a]	1.0	0.84 (0.65–1.11)	0.97 (0.67–1.41)	0.87 (0.68–1.13)
OR[b]	1.0	0.84 (0.63–1.12)	0.91 (0.61–1.34)	0.85 (0.65–1.12)

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

that observed in the previous study composed of Asians, African Americans, and Latinos (40.9%; Ref. 14), and the allele frequencies were in Hardy-Weinberg equilibrium ($\chi^2 = 0.703$, $df = 2$, $P = 0.70$).

Women with the *A2* allele were not at an increased risk of breast cancer; the controls (64.9%) and cases (61.6%) carried at least one *A2* allele ($P = 0.26$). Compared with the *A1/A1* genotype, the adjusted ORs for the *A1/A2* and *A2/A2* genotypes were 0.84 (95% CI, 0.63–1.12) and 0.91 (95% CI, 0.61–1.34), respectively (Table 1). Comparing *A2/A2* homozygotes with all other women, the OR was 1.00 (95% CI, 0.70–1.44). Because a gene dosage effect was not apparent, and for comparison with the data of Feigelson *et al.* (14), women with at least one *A2* allele were analyzed together to increase the power to detect significant main effect associations and gene-breast cancer risk factor interactions. The multivariate adjusted OR for women with at least one *A2* allele (genotypes *A1/A2* and *A2/A2*) was 0.85 (95% CI, 0.65–1.12). Results were similar when cases were restricted to those with invasive disease ($n = 387$; OR, 0.90; 95% CI, 0.68–1.19; Table 2). With all controls included in adjusted unconditional logistic regression models controlling for the matching factors, no association was observed among estrogen receptor-positive cases (OR, 0.91; 95% CI, 0.66–1.24) or progesterone receptor-positive cases (OR, 0.92; 95% CI, 0.66–1.30). The adjusted ORs for women who had ever and never used oral contraceptives were 0.89 (95% CI, 0.60–1.32) and 0.88 (95% CI, 0.61–1.28), respectively. Among the 23.1% of the cases ($n = 107$) with advanced breast cancer, defined as having one or more involved nodes at diagnosis, compared with all controls, the adjusted OR was 0.84 (95% CI, 0.54–1.32). When limiting the cases to those with four or more involved nodes or metastasis ($n = 36$), the adjusted OR showed a similar inverse association (OR, 0.77; 95% CI, 0.37–1.61).

Among premenopausal women, the OR for women with at least one *A2* allele was 0.77 (95% CI, 0.33–1.82), and among postmenopausal women, the OR was 0.92 (95% CI, 0.68–1.23; Table 3). We also stratified postmenopausal women by hormone use status. The OR for the *A2* allele among postmenopausal women who had never used hormones was 1.06 (95% CI, 0.60–1.85; Table 4). A similar result was observed among past users (OR, 0.88; 95% CI, 0.46–1.68). Among present users, risk varied slightly according to duration of use (<5 years: OR, 1.21; 95% CI, 0.51–2.85; and ≥ 5 years: OR, 0.75; 95% CI, 0.42–1.33); however, the interaction between *CYP17* genotype and postmenopausal hormone use was highly nonsignificant (LRT, $P = 0.74$).

Because the data of Feigelson *et al.* (14) suggest that breast cancer risk associated with age at menarche may be modified by *CYP17* allele status, we examined potential interactions between genotype and established breast cancer risk factors. The interaction between age at menarche and genotype approached statistical significance (LRT, interaction $P = 0.07$; Table 5). Among women with the *A1/A1* genotype, a significant inverse association of later age of menarche (≥13) was observed (OR, 0.57; 95% CI, 0.36–0.90), whereas no association was seen among women with the *A2* allele (OR, 1.05; 95% CI, 0.76–1.45). There was no significant evidence of an interaction of *CYP17* genotype with parity (one to two children *versus* 3+; LRT, interaction $P = 0.42$). Among parous women, a significant interaction was observed between age at first birth and the *A2* polymorphism (LRT, interaction $P = 0.03$), however, effect estimates were unstable and sensitive to the cut points used. A significant inverse association for age at first birth ≤24 compared with age at first birth >24 was limited to women without the *A2* allele (OR, 0.44; 95% CI, 0.27–0.73). Among *A2* carriers, no association was observed (OR, 0.94; 95% CI, 0.67–1.34). However, when a cutpoint of ≤22 was used to define early age at first birth, significant associations were not observed among women with or without the *A2* allele (*A1/A1*: OR, 0.83; 95% CI, 0.45–1.51; *A1/A2* or *A2/A2*: OR, 1.29; 95% CI, 0.83–1.93; LRT, interaction $P = 0.16$).

In analyses limited to postmenopausal women, the OR for women with a BMI at blood draw of >24 among carriers of the *A2* allele was 1.16 (95% CI, 0.80–1.69). A BMI >24 was associated with a non-

Table 2 *Associations between the* A2 *allele and breast cancer risk by histological subtype and receptor status*

	CYP17 genotype			
	A1/A1 n (%)	*A1/A2 + A2/A2 n* (%)	OR[a]	Adjusted OR[b]
Controls	217 (35)	401 (65)	1.0	1.0
Cases				
Invasive	145 (37)	242 (63)	0.90 (0.69–1.18)	0.90 (0.68–1.19)
Involved nodes				
≥1	40 (37)	67 (63)	0.91 (0.59–1.41)	0.84 (0.54–1.32)
≥4	14 (39)	22 (61)	0.80 (0.39–1.62)	0.77 (0.37–1.61)
Receptor status[c]				
ER+[d]	98 (37)	166 (63)	0.91 (0.67–1.23)	0.91 (0.66–1.24)
ER−	27 (42)	37 (58)	0.76 (0.44–1.29)	0.74 (0.43–1.29)
PR+	75 (37)	128 (63)	0.91 (0.65–1.27)	0.92 (0.66–1.30)
PR−	45 (39)	68 (61)	0.84 (0.55–1.28)	0.79 (0.51–1.23)

[a] Unconditional logistic regression adjusted for matching variables: age, menopausal status, postmenopausal hormone use, date of blood draw, time of blood draw, and fasting status.
[b] 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, first-degree family history of breast cancer, and duration of postmenopausal hormone use.
[c] Numbers do not add to total due to missing data on receptor status.
[d] ER, estrogen receptor; PR, progesterone receptor.

Table 3 *ORs and 95% CI for breast cancer risk by* CYP17 *genotype and menopausal status*

	CYP17 genotype			
	A1/A1	*A1/A2 + A2/A2*	OR	Adjusted OR
Premenopausal				
Controls	19	51	1.0[a]	1.0[b]
Cases	27	37	0.51[a] (0.24–1.06)	0.77[b] (0.33–1.82)
Postmenopausal				
Controls	180	325	1.0[c]	1.0[d]
Cases	132	225	0.94[c] (0.70–1.25)	0.92[d] (0.68–1.23)

[a] Unconditional logistic regression adjusted for matching variables: age, date of blood draw, time of blood draw, and fasting status.
[b] 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.
[c] Unconditional logistic regression adjusted for matching factors: age, postmenopausal hormone use, date of blood draw, time of blood draw, and fasting status.
[d] 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, age at menopause, benign breast disease, first-degree family history of breast cancer, and duration of postmenopausal hormone use.

Table 4 *ORs and 95% CI for breast cancer risk and* CYP17 *genotype stratified by postmenopausal hormone use*[a]

	Cases	Controls	OR[b]	Adjusted OR[c]
Never users (n = 285)				
CYP17 genotype				
A1/A1	30	69	1.0	1.0
A1/A2 + A2/A2	60	126	1.04 (0.61–1.78)	1.06 (0.60–1.85)
Past users (n = 199)				
CYP17 genotype				
A1/A1	32	47	1.0	1.0
A1/A2 + A2/A2	46	74	0.89 (0.49–1.62)	0.88 (0.46–1.68)
Present users <5 yrs (n = 132)				
CYP17 genotype				
A1/A1	24	23	1.0	1.0
A1/A2 + A2/A2	46	39	1.10 (0.52–2.32)	1.21 (0.51–2.85)
Present users ≥5 yrs (n = 239)				
CYP17 genotype				
A1/A1	45	39	1.0	1.0
A1/A2 + A2/A2	71	84	0.75 (0.43–1.28)	0.75 (0.42–1.33)
LRT,[d] P = 0.74				

[a] Seven subjects with missing hormone status removed from analysis.
[b] Unconditional logistic regression adjusted for matching variables: age, date of blood draw, time of blood draw, and fasting status.
[c] 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, age at menopause, benign breast disease, and first-degree family history of breast cancer.
[d] LRT is for the interaction between genotype and hormone use.

significantly decreased risk of breast cancer (OR, 0.66; 95% CI, 0.39–1.13) among women without the *A2* allele. This interaction also was not significant (LRT, interaction P = 0.09). Among postmenopausal women who never used hormones, we stratified by weight gain since age 18. A nonsignificant elevation in breast cancer risk was observed among women who had gained ≥20 kg and had the *A2* allele (OR, 1.74; 95% CI, 0.43–6.98). Among never users who had gained <20 kg, *A2* carriers were not at elevated risk (OR, 0.95; 95% CI, 0.47–1.90). The interaction between weight gain and *CYP17* genotype was also not significant (LRT, P = 0.42).

Among the controls, the median age of menarche did not differ significantly by genotype (*A1/A1*: 13 years; *A1/A2 + A2/A2*: 12 years; P = 0.97). The median age of menarche was the same among cases with and without the *A2* allele (*A1/A1*: 12 years; *A1/A2 + A2/A2*: 12 years; P = 0.17).

In analyses of the relationship of genotype with hormone levels among controls, we calculated geometric mean plasma steroid hormone levels for each genotype (Table 6). Compared with women with the *A1/A1* genotype, women with the *A2/A2* genotype had elevated levels of estrone (+14.3%, P = 0.01), estradiol (+13.8%, P = 0.08), testosterone (+8.6%, P = 0.34), androstenedione (+17.1%, P = 0.06), DHEA (+14.4%, P = 0.02), and DHEAS (+7.2%, P = 0.26). In analyses limited to never users of postmenopausal hormones (n = 189), the absolute differences were greater. Women with the *A2/A2* genotype had higher levels of estrone sulfate (+6.2%, P = 0.64), estrone (+21.0%, P = 0.04), estradiol (+14.8%, P = 0.23), testosterone (+13.5%, P = 0.54), androstenedione (+20.5%, P = 0.39), DHEA (+30.7%, P = 0.03), and DHEAS (18.6%, P = 0.23).

DISCUSSION

In this study, women with the *A2* allele of *CYP17* were not at an increased risk of breast cancer. The 95% CI (0.65–1.12) excludes the positive association (OR, 1.32) reported in a previous study examining this polymorphism and risk of breast cancer (14). We did not observe the *A2* allele to be positively associated with advanced breast cancer or with breast cancer grouped by estrogen and progesterone receptor status. Among postmenopausal women with no previous use of hormone replacement therapy, where the influence of modest changes in endogenous hormones may be more easily detected, there was also no increased risk of breast cancer associated with the *A2* allele. However, levels of certain steroid hormones were significantly elevated among postmenopausal women homozygous for the *A2* allele.

The *A2* allele of *CYP17* was not a biomarker for an earlier age of menarche. However, our findings are compatible with the potential modification of breast cancer risk due to late age of menarche by genotype observed in two previous studies (14, 17), with the protective effect of late onset of menarche limited to women with the *A1/A1* genotype. The modest inverse association of age at first birth within

Table 5 *ORs and 95% CI for breast cancer risk and age at menarche stratified by* CYP17 *genotype*

	Cases	Controls	OR[a]	Adjusted OR[b]
Menarche[c] (*A1/A1* Genotype)				
<13 yrs	99	104	1.0	1.0
≥13 yrs	79	112	0.71 (0.47–1.07)	0.57 (0.36–0.90)
Menarche[c] (*A1/A2 + A2/A2* genotype)				
<13 yrs	146	202	1.0	1.0
≥13 yrs	135	195	0.99 (0.72–1.35)	1.05 (0.76–1.45)
LRT,[d] P = 0.07				

[a] Unconditional logistic regression adjusted for matching variables: age, menopausal status, postmenopausal hormone use, date of blood draw, time of blood draw, and fasting status.
[b] Unconditional logistic regression adjusted for matching variables, and 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.
[c] Nine subjects with missing age at menarche removed from analysis.
[d] LRT is for the interaction between genotype and age at menarche.

Table 6 *Geometric mean hormone levels*[a] *among postmenopausal controls not using postmenopausal hormones by* CYP17 *genotype*[b]

	Controls (n)[c]		
Hormone	*A1/A1* (n = 104)	*A1/A2* (n = 146)	*A2/A2* (n = 47)
Estrone sulfate (pg/ml)	169.4 (96)	148.5 (136)[d]	164.5 (42)
Estrone (pg/ml)	32.1 (102)	31.2 (145)	36.7 (46)[d]
Estradiol (pg/ml)	8.0 (101)	7.6 (145)	9.1 (46)[e]
Testosterone (ng/dl)	22.2 (100)	21.6 (143)	24.1 (44)
Androstenedione (ng/dl)	63.0 (100)	59.9 (142)	73.8 (44)[e]
DHEA (ng/dl)	227.4 (89)	211.3 (132)	260.1 (45)[f]
DHEAS (μg/dl)	67.0 (99)	70.8 (143)	71.8 (46)

[a] Controlling for BMI, age, date of blood draw, time of blood draw, fasting status, and laboratory batch.
[b] The *A1/A1* genotype is the reference group for all comparisons.
[c] Number varies due to missing data and removal of outliers.
[d] $P \leq 0.01$.
[e] $P \leq 0.1$.
[f] $P \leq 0.05$.

strata of genotype was in the same direction as age at menarche, with benefit observed for women with earlier births who do not carry the *A2* allele; however, this apparent interaction was sensitive to the age at first birth cutpoint selected. These observations are compatible with the hypothesis that the protection against breast cancer by late menarche is reduced among women with the *A2* allele due to elevated baseline levels of circulating steroid hormones.

Enzymatic dysfunction of P450c17α has been evaluated as a cause of excess androgen production involved in the heterogeneous disorder polycystic ovarian syndrome (25, 26). Carey *et al.* (27) identified a common single bp change creating a potential Sp1-type promoter site in the 5′-transcribed, but untranslated, region of *CYP17* . This variant, designated the *A2* allele of *CYP17*, was found to be significantly associated with familial polycystic ovarian syndrome and male pattern baldness among members of the families studied. Sp-1 promoter sites are believed to enhance transcription of genes regulated by the transcriptional control element, Sp1 (28). As a result, this polymorphism may cause elevated expression of P450c17α and, consequently, an increase in P450c17α enzymatic activity, resulting in greater conversion of C-21 steroids into androgens. However, to our knowledge there are no *in vitro* studies correlating this specific polymorphism with Sp1 binding or increased transcription of *CYP17* in humans.

Four other published studies have examined the association between the *A2* allele of *CYP17* and breast cancer risk (14, 17–19). Among 174 cases and 285 controls within a cohort study, Feigelson *et al.* (14) observed a nonsignificant positive association between the *A2* allele and breast cancer risk (OR, 1.32; 95% CI, 0.87–2.00). This association became statistically significant among 40 cases with advanced breast cancer (OR, 2.52; 95% CI, 1.07–5.94). These authors also observed women with the *A1/A1* genotype to have a marginally significant later mean age of menarche (13.4 *versus* 13.0, $P = 0.05$). Our data are consistent with three other studies observing no evidence of these relationships. In the largest case-control study to date, ($n = 835$ cases; $n = 24$ with stage III/IV at diagnosis; $n = 591$ controls) conducted in East Anglia, England, Dunning *et al.* (18) found no evidence of an association between the *A2* allele and breast cancer risk (OR, 1.10; 95% CI, 0.89–1.37) or advanced breast cancer (OR, 0.88; 95% CI, 0.38–2.01). Cases and controls in the large English case-control study were primarily Caucasian with all enrolled cases diagnosed under the age of 55, whereas the mean age of the cases in the multiethnic study by Feigelson *et al.* (14) was 63. Among 123 cases (62% Caucasian, 16% African American, and 22% Hispanic) and 240 controls, Weston *et al.* (17) observed no association between the *A2* allele and breast cancer risk (OR, 1.08; 95% CI 0.69–1.69) or advanced breast cancer (OR, 0.90; 95% CI 0.40–2.00). Likewise, Helzlsouer *et al.* (19) did not observe an association between the *A2* allele and breast cancer or aggressive disease in a smaller nested case-control study ($n = 109$ cases; $n = 113$ controls).

In the study by Feigelson *et al.* (14), the ORs for age at menarche ($\geq$13) was 0.47 (95% CI, 0.22–0.98) among women with the *A1/A1* genotype and 0.80 (95% CI, 0.51–1.27) for *A2* allele carriers. A similar observation was observed in the study by Weston *et al.* (17). Unlike the results of Dunning *et al.* (18) and Helzlsouer *et al.* (19), our data support these findings, with a significant protection from later age at menarche limited to women without the *A2* allele. These observations suggest that associations between age at menarche and breast cancer could depend on *CYP17* genotype. However, given the absence of an overall association, more data are clearly needed to assess this possibility.

This *CYP17* polymorphism has been suggested as a potential modifier of circulating hormone levels among premenopausal women (29). Feigelson *et al.* (29) observed premenopausal nulliparous women with the *A2* allele to have higher levels of serum estradiol and progesterone measured during both phases of the menstrual cycle. We examined the relationship between allele status and steroid hormones levels among postmenopausal women. Among controls, we found women with the *A2/A2* genotype, but not the *A1/A2* genotype, had significantly elevated levels of estrone and DHEA, and modest, nonsignificant elevations in estradiol, testosterone, androstenedione, and DHEAS. Our results provide support for the previous observation that this polymorphism is involved in regulating steroid hormone metabolism. However, these differences in steroid hormone levels were modest and would be expected to have only weak effects on elevating breast cancer risk. Although it is possible that a weak effect does exist, and the available studies are insufficiently large to detect the association, the upper bound of the CIs in the present study (0.65–1.12) and the largest case-control study (0.89–1.37; Ref. 18) exclude all but weak associations.

The higher levels of certain hormones we observed support the hypothesis that the *A2* allele of *CYP17* has increased functional activity. Up-regulation of *CYP17* may have a stronger influence on serum levels of hormones more proximal to the enzymatic steps catalyzed by this enzyme (DHEA, androstenedione). Effects would be expected to be weaker for steroid hormones further removed from 17α-hydroxylase/17,20-lyase activity (*e.g.*, estradiol). In our study, elevated levels were limited to carriers of two *A2* alleles; increased levels were not observed for women with the *A1/A2* genotype, suggesting that the effect of the *A2* allele is not codominant.

Ethnic differences could explain some of the discrepancy between study results as although the prevalence of the *A2* allele is similar in all of the available studies (14, 17–19), the *A2* allele may be linked to other more functionally relevant polymorphisms in non-white populations. In the study of Weston *et al.* (17), nonsignificant elevations in breast cancer risk were observed among African Americans (OR, 1.40; 95% CI, 0.44–4.38) and Hispanics (OR, 1.93; 95% CI, 0.75–5.01), however, among controls in the latter group, genotype distributions were not in Hardy-Weinberg equilibrium. Similar to the other three null studies (17–19), our study was comprised primarily of white women (~95%), whereas the initial study was composed of various non-white ethnic groups (Asian, African American, and Latino; Ref. (14).

The relatively large sample size, prospective design, and blood sample collection before case diagnosis are among the strengths of this study. Larger studies will be needed to confirm potential gene-risk factor interactions. Although we observed evidence of a relationship between the A2 allele of *CYP17* and circulating hormone levels, we observed no association with risk of either early or late stage breast cancer.

ACKNOWLEDGMENTS

We thank Kathryn Starzyk, Jeanne Sparrow, Leiming Li, and Lisa Li for technical assistance. We are also indebted to the participants in the Nurses' Health Study for dedication and commitment.

REFERENCES

1. Bernstein, L., and Ross, R. K. Endogenous hormones and breast cancer. Epidemiol. Rev., *15:* 48–65, 1993.
2. Preston-Martin, S., Pike, M. C., Ross, R. K., Jones, P. A., and Henderson, B. E. Increased cell division as a cause of human cancer. Cancer Res., *50:* 7415–7421, 1990.
3. Colditz, G. A. Relationship between estrogen levels, use of hormone replacement therapy, and breast cancer. J. Natl. Cancer Inst., *90:* 814–823, 1998.
4. Tsai, M. J., and O'Malley, B. W. Molecular mechanisms of action of steroid/thyroid receptor superfamily. Annu. Rev. Biochem., *63:* 451–486, 1994.
5. Beato, M. Gene regulation by steroid hormones. Cell, *56:* 335–344, 1989.
6. Key, T. J. A., and Pike, M. C. The role of oestrogens and progestogens in the epidemiology and prevention of breast cancer. Eur. J. Cancer Clin. Oncol., *24:* 29–43, 1988.

7. Kelsey, J. L. Breast cancer epidemiology: summary and future directions. Epidemiol. Rev., *15:* 256–263, 1993.
8. Hsieh, C. C., Trichopoulos, D., Katsouyanni, K., and Yuasa, S. Age at menarche, age at menopause, height and obesity as risk factors for breast cancer: associations and interactions in an international case-control study. Int. J. Cancer, *46:* 796–800, 1990.
9. Hankinson, S. E., Willett, W. C., Manson, J. E., Hunter, D. J., Colditz, G. A., Stampfer, M. J., Longcope, C., and Speizer, F. E. Alcohol, height and adiposity in relation to estrogen and prolactin levels in postmenopausal women. J. Natl. Cancer Inst., *87:* 1297–1302, 1995.
10. Perel, E., and Killinger, D. W. The interconversion and aromatization of androgens by human adipose tissue. J. Steroid Biochem., *10:* 623–627, 1979.
11. Sourdaine, P., Mullen, P., White, R., Telford, J., Parker, M. G., and Miller, M. R. Aromatase activity and *CYP19* gene expression in breast cancers. J. Steroid Biochem. Mol. Biol., *59:* 191–198, 1996.
12. Khan, S. A., Rodgers, M. A., Khurana, K. K., Meguid, M. M., and Numann, P. J. Estrogen receptor expression in benign breast epithelium and breast cancer risk. J. Natl. Cancer Inst., *90:* 37–42, 1998.
13. Picado-Leonard, J., and Miller, W. L. Cloning and sequence of the human gene for P450c17 (steroid 17α-hydroxylase/17,20-lyase): similarity with the gene for P450c21. DNA, *6:* 439–448, 1987.
14. Feigelson, H. S., Coetzee, G. A., Kolonel, L. N., Ross, R. K., and Henderson, B. E. A polymorphism in the *CYP17* gene increases the risk of breast cancer. Cancer Res., *57:* 1063–1065, 1997.
15. Zuber, M. X., Simpson, E. R., and Waterman, M. R. Expression of bovine 17α-hydroxylase cytochrome P450 cDNA in nonsteroidogenic (Cos 1) cells. Science (Washington DC), *234:* 1258–1261, 1986.
16. Yanase, T. 17α-Hydroxylase/17,20-lyase defects. J. Steroid Biochem. Mol. Biol., *53:* 153–157, 1995.
17. Weston, A., Pan, C., Bleiweiss, I. J., Ksieski, H. B., Roy, N., Maloney, N., and Wolff, M. S. *CYP17* genotype and breast cancer risk. Cancer Epidemiol. Biomark. Prev., *7:* 941–944, 1998.
18. Dunning, A. M., Healey, C. S., Pharoah, P. D. P., Foster N. A., Lipscombe, J. M., Redman, K. L., Easton, D. F., Day, N. E., and Ponder, B. A. J. No association between a polymorphism in the steroid metabolism gene *CYP17* and risk of breast cancer. Br. J. Cancer, *77:* 2045–2047, 1998.
19. Helzlsouer, K. J., Huang, H., Strickland, P. T., Hoffman, S., Alberg, A. J., Comstock, G. W., and Bell, D. A. Association between *CYP17* polymorphisms and development of breast cancer. Cancer Epidemiol. Biomark. Prev., *7:* 945–949, 1998.
20. Hankinson, S. E., Manson, J. E., Spiegelson, D., Willett, W. C., Longcope, C., and Speizer, F. E. Reproducibility of plasma hormone levels in postmenopausal women over a 2–3-year period. Cancer Epidemiol. Biomark. Prev., *4:* 649–654, 1995.
21. Hankinson, S. E., Willett, W. C., Manson, J. E., Colditz, G. A., Hunter, D. J., Spiegelman, D., Barbieri, R. L., and Speizer, F. E. Plasma sex steroid hormone levels and risk of breast cancer in postmenopausal women. J. Natl. Cancer Inst., *90:* 1292–1299, 1998.
22. SAS Institute, Inc. SAS technical report p-229 SAS/STAT software. Changes and enhancements, release 6.07. Cary, NC: SAS Institute, Inc., 1992.
23. White, H. A heteroskedasticity-consistent covariance matrix estimator and a direct test for heteroskedasticity. Econometrica, *48:* 817–838, 1980.
24. SAS Institute, Inc. SAS/STAT user's guide, version 6., Ed. 4. Cary, NC: SAS Institute, Inc., 1989.
25. Franks, S., White, D., Gilling-Smith, C., Carey, A., Waterworth, D., and Williamson, R. Hypersecretion of androgens by polycystic ovaries: the role of genetic factors in the regulation of cytochrome P450c17α. Baillieres Clin. Endocrinol. Metab., *10:* 193–203, 1996.
26. Rosenfield, R. L., Barnes, R. B., Cara, J. F., and Lucky, A. W. Dysregulation of cytochrome P450c17α as the cause of polycystic ovarian syndrome. Fertil. Steril., *53:* 785–791, 1990.
27. Carey, A. H., Waterworth, D., Patel, K., White, D., Little, J., Novelli, P., Franks, S., and Williamson, R. Polycystic ovaries and premature male pattern baldness are associated with one allele of the steroid metabolism gene CYP17. Hum. Mol. Genet., *10:* 1873–1876, 1994.
28. Kadonaga, J. T., Jones, K. A., and Tjian, R. Promoter-specific activation of RNA polymerase II transcription by Sp1. Trends Biochem. Sci., *11:* 20–23, 1986.
29. Feigelson, H. S., Shames, L. S., Pike, M. C., Coetzee, G. A., Stanczyk, F., Z., and Henderson, B. E. Cytochrome P450c17α gene (*CYP17*) polymorphism is associated with serum estrogen and progesterone concentrations. Cancer Res., *58:* 585–587, 1998.

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Cancer Res 1999;59:1015-1020.