A Polymorphism in CYP17 and Endometrial Cancer Risk

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ABSTRACT

Among women, the A2 allele of CYP17 has been associated with elevated levels of endogenous steroid hormones; however, it does not seem to be a strong independent risk factor for breast cancer. We assessed the association between the A2 allele of CYP17 and endometrial cancer risk in a case-control study nested within the Nurses’ Health Study cohort (cases: n = 184; controls: n = 554). We also evaluated whether endometrial cancer risk associated with CYP17 genotype was modified by established endometrial cancer risk factors. In addition, we further examined the relationship between CYP17 genotype and endogenous plasma steroid hormone levels among postmenopausal controls not using hormone replacement therapy (HRT). Women with the A2 allele of CYP17 were at decreased risk of endometrial cancer (A1/A1 genotype [reference]; A1/A2 genotype: odds ratio, 0.89; 95% confidence interval, 0.62–1.27; A2/A2 genotype: odds ratio, 0.43; 95% confidence interval, 0.23–0.80; P trend, 0.02). We also observed the inverse association between the A2 allele and endometrial cancer risk to be stronger among women with a first-degree family history of endometrial and/or colorectal cancer (P for interaction, 0.05). Among 165 controls, we did not observe women with the A2 allele to have significantly elevated levels of any steroid hormone fraction. When these women were combined and analyzed with those women on whom we had previously examined the relationship between CYP17 genotype and circulating hormone levels (total n = 469), only modest associations were observed for the A2/A2 genotype and steroid hormone fractions estrone (versus A1/A1 genotype: +10.9%; P = 0.05) and estradiol (+8.5%; P = 0.17). These data suggest that the A2 allele of CYP17 decreases endometrial cancer risk, but has only weak effects on endogenous estrogen levels among postmenopausal women.

INTRODUCTION

The endometrium is highly responsive to hormonal stimuli; cyclic production of estrogen and progesterone during the menstrual cycle and declining sex steroid hormone levels after menopause are directly correlated with endometrial proliferation and/or atrophic morphological changes (1, 2). Excess exposure to endogenous and exogenous estrogens unopposed by progesterone increases endometrial cell division and is suggested to be the mechanism through which established risk factors for endometrial cancer influence risk (3). The established risk factors known to modify lifetime exposure to estrogen and progesterone include obesity, age at menarche, age at menopause, parity, and HRT (4).

Polymorphisms in genes involved in steroid hormone biosynthesis (CYP17, CYP19, and 17β-HSD) and hormonal signaling (ER-α and PR) are currently being evaluated as genetic biomarkers for hormone-related diseases (5). One such candidate is a single (T to C) nucleotide change in the 5’ region of CYP17, a gene encoding a cytochrome P450 enzyme, P450c17α, involved in androgen biosynthesis (6). The bp change was thought to create a consensus Sp-1 binding site that may have an effect on the transcriptional regulation of CYP17. The variant allele, designated the A2 allele of CYP17, was found by Carey et al. (6) to be significantly associated with familial polycystic ovarian syndrome, a heterogeneous disorder associated with excess androgen production. On the basis of a potential role in altering steroid hormone metabolism, the A2 allele has been studied extensively among women in relation to breast cancer risk (Ref. 7; reviewed in Ref. 5). Positive associations have been reported but not supported in most studies.

We and others have provided preliminary results to support the hypothesis that this genetic variant may be involved in regulating steroid hormone biosynthesis among women (7, 8). Among healthy pre- and postmenopausal women, the A2/A2 CYP17 genotype has been associated with elevated levels of circulating androgens, estrogens and progesterone. In addition, it has been observed that women with the A2 allele are less likely to be current users of HRT, which suggests that genetic control of lifetime exposure to circulating hormone levels may influence HRT use (9). Experimental evidence, however, does not support the idea that this nucleotide change creates an Sp-1 motif, as Kristensen et al. (10) did not observe Sp-1 binding to this polymorphic site. Whether other tissue-specific regulatory factors differentially bind to this motif remains unknown.

On the basis of the observed associations between the A2 allele of CYP17 and endogenous hormone levels, we selected CYP17 as a candidate gene to study in relation to endometrial cancer risk. Independent of a relationship between CYP17 genotype and the pattern of HRT use, we hypothesized that carriers of the A2 allele would be at increased risk of endometrial cancer. In a small preliminary multietnic case-control study (11), however, a significant inverse association was observed between the A2 allele and endometrial cancer risk among women taking estrogen replacement therapy (A1/A1 reference; A1/A2; OR, 0.39; 95% CI, 0.14–1.09; A2/A2; OR, 0 (no A2/A2 cases; P trend, 0.02).

We set out to determine whether CYP17 genotype is associated with risk of endometrial cancer among Caucasian women in a case-control study within the NHS cohort. We investigated potential interactions between endometrial cancer risk factors, CYP17 genotype, and endometrial cancer risk. We also further examined the relationship between CYP17 genotype and endogenous plasma steroid hormone levels among a relatively large sample of postmenopausal women.

MATERIALS AND METHODS

Study Population. The NHS began 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 years, including data on reproductive variables, oral contraceptive and postmenopausal hormone use, cigarette smoking, and, since 1980, dietary intake. Between 1989 and 1990, blood samples were collected from 32,826 women. Approximately 97% of the samples were returned within 26 h of blood draw, immediately centrifuged, aliquoted into plasma, RBCs, and buffy coat fractions, and stored.
in liquid nitrogen freezers. Follow-up has been >90% in all of the subsequent questionnaire cycles for this subcohort.

In this study, we included both incident and prevalent endometrial cancer cases from the blood subcohort of the NHS. Eligible incident cases consisted of women with pathologically confirmed invasive endometrial cancer diagnosed anytime after blood collection up to June 1, 1996, with no previously diagnosed cancer except for nonmelanoma skin cancer. Prevalent cases were defined as having pathologically confirmed invasive endometrial cancer diagnosed between 1976 and the date of blood collection, with no previously diagnosed cancer except nonmelanoma skin cancer. Controls for both incident and prevalent cases were randomly selected participants who gave a blood sample, but had not had a hysterectomy, and were free of diagnosed cancer (except nonmelanoma skin cancer) up to and including the interval in which the case was diagnosed. Controls were matched to cases (3:1 or 2:1) on year of birth, menopausal status at blood draw and diagnosis, and HRT status at blood draw (current versus not current users) as well as time of day, month, and fasting status at blood draw. The case-control study consisted of 68 incident invasive endometrial cancer cases, 116 prevalent endometrial cancer cases, and 554 matched controls. The study samples for the plasma hormone analyses are composed of 165 postmenopausal control women from the NHS nested breast cancer case-control study 1996 follow-up cycle, in addition to the breast cancer controls from the 1989–1994 cycles (for a total of 469 women). None of the women had used HRT within 3 months of blood draw. The protocol was approved by the Committee on Human Subjects, Brigham and Women’s Hospital.

CYP17 Genotyping Assay. CYP17 genotyping analysis was performed by the Taqman Allelic Discrimination method (12) using the ABI 7700 Sequence Detection System (Applied Biosystems, Foster City, CA). This assay measures fluorescent intensity released from allele-specific fluorogenic probes and allows for high-throughput genotyping without post-PCR processing. A 97-bp fragment that included the T to C polymorphism was amplified in a 96-well format using the following primers: 5′-AGGCTTCCCTGTGCTTCTAGA-3′ and 5′-GAGCCACGGCTTCCACAT-3′. The fluorescently labeled allele-specific probes are as follows: FAM 5′-CTTCTACTCCACTGGCTTGGCCTGCTG-3′ TAMARA and VIC 5′-CTTCTACTCCACGGCTTGGCCTGCTG-3′ TAMARA [bp change is highlighted (boldface and italic)]. Primers and probes were designed using Primer Express software (Applied Biosystems). Genomic DNA was used (40 ng per 25 μl of reaction) with 900 nM each primer, 100 nM FAM probe, 200 nM VIC probe, and 1× Taqman Universal PCR Master mix (Applied Biosystems). Amplification conditions were 2 min at 50°C, 10 min at 95°C, and 40 cycles of 15 s at 95°C, and 1 min at 63.5°C. The MspAI restriction enzyme and DNA sequencing were used to identify homozygous (CC and TT) controls required for this genotyping assay. Sequence Detection System software (Applied Biosystems) was used to determine CYP17 genotype. All of the genotyping was performed by laboratory personnel unaware of case-control status, and blinded quality control samples were inserted to validate genotyping identification procedures; concordance for blinded samples was 100%.

Hormone Assays. Steroid hormone fractions of estradiol, estrone, estrone sulfate, testosterone, androstenedione, DHEA and DHEAS were measured in four separate batches. Methods for plasma hormone assays and information regarding laboratory precision and reproducibility have been published previously (13, 14). Within-batch laboratory coefficients of variation were ≤13.6%.

Exposure Data. Information regarding endometrial cancer risk factors were obtained from the 1976 baseline questionnaire and were updated with subsequent biennial questionnaires and a questionnaire completed at the time of blood collection. Women were defined as postmenopausal at the time of blood collection if they reported having a bilateral oophorectomy or no menstrual cycle within the last 12 months before blood draw. Menopause status and postmenopausal hormone use, including the dose and duration of current use of conjugated estrogen or estrogen plus progestin, were updated until the date of diagnosis for cases and matched controls based on responses from the biennial questionnaires. First-degree family history of endometrial and colorectal cancer was assessed retrospectively from the 1996 follow-up questionnaire. Histopathological characteristics, such as subtype and differentiation status of epithelial carcinoma, were ascertained from medical records and used in case subgroup analyses when available.

Statistical Analysis. McNemar’s test was used to evaluate differences in endometrial cancer risk factors and carrier status of the A2 allele of CYP17 between cases and controls. ORs and 95% CIs were calculated using conditional and unconditional logistic regression. In addition to the matching variables, we adjusted for endometrial cancer risk factors: BMI (kg/m2); weight gain since age 18 (<5, 5–19.9, or ≥20 kg); age at menarche (<12, 12, 13, or >13 years); parity/age at first birth, (nulliparous; 1–2 children/age at first birth ≥24 years, 1–2/≥24, ≥3/≥24, or ≥3/≥24); duration of postmenopausal hormone use (never, past, or current <5 and ≥5 years); history of oral contraceptive use (ever/never); pack-years of smoking (never smoker, <30, or ≥30 pack-years); first-degree family history of endometrial cancer (yes/no); and first-degree family history of colorectal cancer (yes/no). Indicator variables for all three genotypes were created using the A1/A1 hypothesized low-risk genotype as the reference category in multivariate models. We used the χ2 test to evaluate whether CYP17 genotype was associated with HRT status among controls and, in case-case analyses, to examine associations between CYP17 genotype and the degree of differentiation of endometrial cancer. Interactions between genotype and endometrial cancer risk factors were evaluated by including interaction terms between genotype and risk factor variables in unconditional logistic regression models.

Linear regression was used to evaluate the association between genotype and circulating hormone levels among postmenopausal controls from the nested breast cancer case-control study within the NHS. Analyses were first conducted among the 165 controls matched to cases diagnosed during the 1996 follow-up cycle (1994 to 1996). A second analysis was then performed, which included these women and controls already included in the nested breast cancer case-control study on which we have previously examined the relationship between CYP17 genotype and plasma hormone levels (1989–1994; total n = 469). Differences in hormone levels between genotypes were evaluated with the A1/A1 genotype as the reference category. The natural logarithm of the plasma hormone values was used to reduce the skewness of the regression residuals. Hormone fractions were evaluated in multiple batches. All of the hormone fractions were not assayed in all of the women because of insufficient plasma. Within each batch, hormone values >1 interquartile ranges from the 75th percentile were treated as outliers and were excluded (estrone sulfate, n = 11; estrone, n = 3; estradiol, n = 7; testosterone, n = 2; androstenedione, n = 1; DHEA, n = 3; DHEAS, n = 1). We used the SAS statistical package for all analyses (15).

RESULTS

The distribution of CYP17 genotypes was first compared between incident and prevalent cases to assess whether the A2 allele influences the duration of endometrial cancer survival, because prevalent cases had to be alive in 1989 to donate blood and be included in the study. CYP17 genotype frequencies were similar between incident cases [A1/A1, n = 27 (40%); A1/A2, n = 34 (50%); A2/A2, n = 7 (10%)] and prevalent cases [A1/A1, n = 49 (42%); A1/A2, n = 58 (50%); A2/A2, n = 9 (8%)]. χ2 = 0.38, df = 2, P = 0.83. Because we did not observe evidence of a “survivor bias,” incident and prevalent cases were combined and analyzed together.

There were 136 premenopausal and 564 postmenopausal women with mean ages of 49.5 (SD, 4.0) and 60.1 (SD, 6.7), respectively. Compared with controls, cases had a similar mean age at menarche (12.4 versus 12.5 years) and a greater BMI (28.0 versus 25.7 kg/m2). The proportion of women that ever used oral contraceptives was similar for both groups (36% versus 38%; P = 0.51). Cases were more likely to be nulliparous, 14% versus 9% (P = 0.03); to be current HRT users at diagnosis, 42% versus 30% (P = 0.01); and to have never smoked, 52% versus 42% (P = 0.02). The proportion of women with a first-degree family history of endometrial or colorectal cancer was also greater among the cases, 8.7% versus 3.6% (P = 0.01) and 22.8% versus 16.3% (P = 0.04), respectively. Self-reported major ethnicity/ancestry was similar between cases and controls (cases versus controls: Southern European, 17.9% versus 16.6%; Scandinavian, 9.0% versus 8.5%; and other Caucasian, 69.0% versus 72.1%). Asians, Hispanics, and African Americans comprised less than 1% of cases or controls.
The prevalence of the A2 allele among the controls was 40%, and identical to that observed among Caucasian women in our previous study (7). The distributions of CYP17 genotypes were in accordance with Hardy-Weinberg equilibrium among both cases ($\chi^2 = 2.4$, $df = 1$, $P = 0.12$) and controls ($\chi^2 = 0.34$, $df = 1$, $P = 0.85$). There was a significant difference in genotype frequencies between cases and controls ($\chi^2 = 6.8$, $df = 2$, $P = 0.03$; Table 1). The A2 allele was underrepresented among women with endometrial cancer; 59% of the cases and 64% of controls carried at least one A2 allele ($P = 0.16$). Crude (not shown) and fully-adjusted ORs (Table 1) were similar; therefore, we did not adjust for all matching variables and potential confounding risk factors to improve the precision of the relative risk estimates. Compared with the A1/A1 genotype, the ORs for women with the A1/A2 and A2/A2 genotypes were 0.89 (95% CI, 0.62–1.27) and 0.43 (95% CI, 0.23–0.80), respectively (Table 1). A significant gene-dosage effect was also suggested ($P$ trend, 0.02). The adjusted OR for women carrying at least one A2 allele (A1/A2 and A2/A2 genotypes combined) was 0.78 (95% CI, 0.55–1.11). Associations were similar by menopausal status (Table 1). In case-only analyses, the A2 allele was nonsignificantly underrepresented among cases with poorly or moderately differentiated tumors compared with cases with well-differentiated tumors (38 (51%) of 75 versus 54 (60%) of 90; $P = 0.23$). We did not observe an association between CYP17 genotype and HRT use; noncarriers of the A2 allele were not more likely to be current HRT users [percentage that were current HRT users by genotype and HRT use; noncarriers of the A1/A1 genotype and HRT use (never, past, or current; Table 2)]. We observed inverse associations between CYP17 genotype and endometrial cancer risk among both never and current HRT users. We observed a borderline significant inverse trend for the A2 allele among women who never used HRT ($P$ trend, 0.08; Table 2). Compared with the A1/A1 genotype, the OR for women with the A2/A2 genotype was 0.23 (95% CI, 0.05–1.06); however, there were only two A2/A2 cases in this stratum. There was no significant interaction between HRT status and CYP17 genotype (LRT, interaction $P = 0.49$). We did not observe evidence of effect modification or a statistical interaction between CYP17 genotype and BMI (LRT, interaction $P = 0.28$; Table 2), oral contraceptive use (LRT, interaction $P = 0.70$), age at menarche (LRT, interaction $P = 0.98$), or parity (LRT, interaction $P = 0.35$).

We observed independent inverse associations between the A2 allele and endometrial cancer risk among women with either a first-degree family history of endometrial or colorectal cancer (data not shown), although relatively few cases had a first-degree family history of either of these cancers (endometrial, $n = 16$; colorectal, $n = 42$). Our rationale for combining and evaluating women with a first-degree family history of endometrial and/or colorectal cancer was 2-fold. The ORs for CYP17 genotype were similar among women with a positive family history of endometrial or colorectal cancer. In addition, given that endometrial cancer is a component of hereditary nonpolyposis colorectal cancer, a proportion of the familial clustering of these cancers may share an underlying genetic etiology. The interaction between CYP17 genotype and first-degree family history of endometrial and/or colorectal cancer was borderline significant (LRT, interaction $P$, 0.05; Table 2). Among women with this family history, a significant inverse association of CYP17 genotype was observed ($P$ trend, 0.01), whereas no significant association was seen among women without this family history ($P$ trend, 0.34).

In analyses of the relationship of genotype with plasma hormone levels among controls, we calculated least-squared geometric mean plasma steroid hormone levels for each genotype. We first evaluated

<table>
<thead>
<tr>
<th>CYP17 genotype</th>
<th>Cases (%)</th>
<th>Controls (%)</th>
<th>OR (95% CI)(^a)</th>
<th>OR (95% CI)(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All cases + controls(^c)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1/A1</td>
<td>76 (41)</td>
<td>197 (36)</td>
<td>1.00 (reference)</td>
<td>1.00 (reference)</td>
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<tr>
<td>A1/A2</td>
<td>92 (50)</td>
<td>267 (48)</td>
<td>0.89 (0.62–1.27)</td>
<td>0.96 (0.64–1.44)</td>
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<tr>
<td>A2/A2</td>
<td>16 (9)</td>
<td>90 (16)</td>
<td>0.43 (0.23–0.80)</td>
<td>0.44 (0.22–0.87)</td>
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$P$ trend, 0.02

<table>
<thead>
<tr>
<th>OR (95% CI)(^d)</th>
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<table>
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<tr>
<th>Premenopausal</th>
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<tbody>
<tr>
<td>A1/A1</td>
<td>22 (42)</td>
<td>30 (36)</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>A1/A2</td>
<td>25 (48)</td>
<td>40 (48)</td>
<td>0.78 (0.36–1.71)</td>
</tr>
<tr>
<td>A2/A2</td>
<td>5 (10)</td>
<td>14 (17)</td>
<td>0.44 (0.13–1.49)</td>
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$P$ trend, 0.20

<table>
<thead>
<tr>
<th>OR (95% CI)(^d)</th>
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<tr>
<th>Postmenopausal</th>
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<tbody>
<tr>
<td>A1/A1</td>
<td>52 (41)</td>
<td>158 (36)</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>A1/A2</td>
<td>64 (50)</td>
<td>211 (48)</td>
<td>0.95 (0.62–1.47)</td>
</tr>
<tr>
<td>A2/A2</td>
<td>11 (9)</td>
<td>68 (16)</td>
<td>0.50 (0.24–1.03)</td>
</tr>
</tbody>
</table>

$P$ trend, 0.11

\(\text{a ORs and 95% CIs calculated using conditional logistic regression adjusted for the matching variables, age, menopausal status at diagnosis and blood collection, postmenopausal hormone use status at blood draw, time of day of blood draw, date of blood draw, and fasting status at blood draw.}\)

\(\text{b ORs and 95% CIs calculated using conditional logistic regression adjusted for the matching variables and BMI weight gain since age 18, age at menarche, parity, age at first birth, duration of postmenopausal hormone use, history of oral contraceptive use, pack-years of smoking, first-degree family history of endometrial cancer, and first-degree family history of colorectal cancer.}\)

\(\text{c All cases and controls include premenopausal and postmenopausal women and women with dubious menopausal status.}\)

\(\text{d ORs and 95% CIs calculated using unconditional logistic regression adjusted for age and BMI.}\)
the association among women selected as breast cancer controls during the 1996 follow-up cycle. Compared with women with the A1/A1 genotype, women with the A2/A2 genotype did not have significantly elevated levels of any estrogen or androgen hormone fraction [A2/A2 versus A1/A1 genotype: estrone sulfate, −22.6% (P = 0.08); estrone, +3.1% (P = 0.75); estradiol, +1.5% (P = 0.91); testosterone, +5.3% (P = 0.64); androstenedione, +1.0% (P = 0.94); DHEA, +0.1% (P = 0.99); DHEAS, −9.5% (P = 0.55)]. Controls selected for hormone analyses in the 1996 cycle were similar to the controls previously included in our prior analysis (1989–1994) with respect to demographic characteristics (mean age, 61.5 versus 62.1 years; mean BMI, 26.8 versus 26.2 kg/m²; past HRT use: 32 versus 36%). In a combined analysis of all controls with hormone measurements from our previously published (7) nested case-control study of breast cancer (1989–1996, n = 469), women with the A2/A2 genotype had marginally significantly higher levels of estrone (+10.9%, P = 0.05) and estradiol (+8.5%, P = 0.17; Table 3). No positive associations were observed between the A2/A2 genotype and other hormone fractions.

**DISCUSSION**

In this study, women with the A2 allele of *CYP17* were at decreased risk of endometrial cancer. Among postmenopausal women, we did not detect strong associations between *CYP17* genotype and circulating steroid hormone levels. We did observe a significant interaction between *CYP17* genotype and first-degree family history of endometrial and colorectal cancer, but we did not observe the endometrial cancer risk that is associated with *CYP17* genotype to be substantially altered by other established hormone-related endometrial cancer risk factors.

The established risk factors for endometrial cancer are understood to act by altering chronic estrogen exposure unopposed by progesterone (3). Likewise, the hypothesized mechanism through which genetic variation in *CYP17* may influence endometrial cancer risk is by

<table>
<thead>
<tr>
<th>HRT</th>
<th>Never users, n (%)</th>
<th>Past users, n (%)</th>
<th>Current users, n (%)</th>
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<tr>
<td></td>
<td>CYP17 genotype</td>
<td>Cases</td>
<td>Controls</td>
</tr>
<tr>
<td></td>
<td>A1/A1</td>
<td>21 (45)</td>
<td>66 (36)</td>
</tr>
<tr>
<td></td>
<td>A1/A2</td>
<td>24 (51)</td>
<td>92 (50)</td>
</tr>
<tr>
<td></td>
<td>A2/A2</td>
<td>2 (4)</td>
<td>27 (15)</td>
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</table>

**LRT, P interaction, 0.49**

<table>
<thead>
<tr>
<th>BMI (Kg/m²)</th>
<th>BMI &lt; 25, n (%)</th>
<th>25 ≤ BMI ≤ 30, n (%)</th>
<th>BMI &gt; 30, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Controls</td>
<td>OR (95% CI)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>A1/A1</td>
<td>27 (41)</td>
<td>100 (35)</td>
<td>1.00</td>
</tr>
<tr>
<td>A1/A2</td>
<td>31 (47)</td>
<td>143 (50)</td>
<td>0.80 (0.45–1.42)</td>
</tr>
<tr>
<td>A2/A2</td>
<td>8 (12)</td>
<td>43 (15)</td>
<td>0.71 (0.30–1.68)</td>
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**LRT, P interaction, 0.28**

<table>
<thead>
<tr>
<th>First degree family history of cancer</th>
<th>No family history of endometrial or colorectal cancer, n (%)</th>
<th>Family history of endometrial and/or colorectal cancer, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Controls</td>
</tr>
<tr>
<td>A1/A1</td>
<td>50 (38)</td>
<td>163 (36)</td>
</tr>
<tr>
<td>A1/A2</td>
<td>66 (50)</td>
<td>211 (47)</td>
</tr>
<tr>
<td>A2/A2</td>
<td>15 (11)</td>
<td>75 (17)</td>
</tr>
</tbody>
</table>

**Table 2 Associations<sup>a</sup> between CYP17 genotype and endometrial cancer risk stratified by hormone replacement therapy use, BMI, and first-degree family history of cancer**

**Table 3 Least-squared geometric mean steroid hormone levels<sup>a</sup> among postmenopausal controls not using postmenopausal hormones by CYP17 genotype<sup>b</sup>, including 1990–1994 published data and 1996 data**

<table>
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<tbody>
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<td></td>
<td>Estrone Sulfate (pg/ml)</td>
<td>220.6</td>
<td>188.2</td>
<td>195.1</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.01</td>
<td>0.13</td>
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</tr>
<tr>
<td></td>
<td>Estrone (pg/ml)</td>
<td>25.8</td>
<td>25.1</td>
<td>28.6</td>
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<tr>
<td></td>
<td>P</td>
<td>0.46</td>
<td>0.05</td>
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<tr>
<td></td>
<td>Estradiol (pg/ml)</td>
<td>7.1</td>
<td>6.7</td>
<td>7.7</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.26</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Testosterone (ng/dl)</td>
<td>22.1</td>
<td>21.2</td>
<td>23.5</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.47</td>
<td>0.40</td>
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<tr>
<td></td>
<td>Androstenedione (ng/dl)</td>
<td>58.1</td>
<td>51.3</td>
<td>61.2</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.02</td>
<td>0.50</td>
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</tr>
<tr>
<td></td>
<td>DHEA (ng/dl)</td>
<td>206.6</td>
<td>181.1</td>
<td>221.1</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.07</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DHEAS (ug/dl)</td>
<td>78.4</td>
<td>74.7</td>
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<td>P</td>
<td>0.51</td>
<td>0.82</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Controlling for BMI, age, laboratory batch, date of blood draw, time of blood draw, and fasting status.

<sup>b</sup> The A1/A1 genotype is the reference group for all comparisons.

<sup>c</sup> Numbers vary because of missing data and removal of outliers.

Numbers do not add to 184 cases and 554 controls because of missing data.
altering a woman’s lifetime exposure to endogenous steroid hor-
mones. On the basis of the enzymatic steps catalyzed by CYP17, we
would expect carriers of one or two A2 alleles to have elevated levels
of downstream hormones (androgens and estrogens). We and others
have provided preliminary results to support the proposed hypothesis
that this genetic variant may be involved in regulating steroid hor-
mone biosynthesis among women (7, 8). Feigelson et al. (8) initially
reported premenopausal nulliparous women with the A2 allele to have
higher levels of serum estradiol and progesterone measured during
both phases of the menstrual cycle. Similarly, we observed pre-
menopausal women with the A2/A2 genotype to have elevated levels
of circulating estrogens and androgens (versus A1/A1 genotype: es-
trone, +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; DHEAS, +7.2%, P = 0.26; Ref. 7). In the present
study, we reexamined CYP17 genotype as a predictor of circulating
steroid hormone levels among postmenopausal women. Among the
subset of postmenopausal breast cancer controls from the 1996 fol-
low-up cycle, we did not observe women with the A2/A2 genotype to
have significantly elevated levels of any steroid hormone fraction.
In the combined analysis of previously published data and these new
data, previous modest differences in hormone levels between CYP17
alleles were substantially attenuated. The disparity in study results
between this study and our previously published work are most likely
attributable to chance and are not the result of substantial demo-
graphic or biological differences between the two postmenopausal
control samples. Overall, these combined data provide only weak
support for the prior hypothesis that this polymorphism regulates
steroid hormone biosynthesis among postmenopausal women.
Feigelson et al. (8) initially detected premenopausal nulliparous
women with the A2 allele to have significantly elevated levels of
estradiol and progesterone (a precursor hormone to the steps catalyzed
by CYP17). Compared with women with the A1/A1 genotype
(n = 28), women with the A1/A2 (n = 45) and A2/A2 (n = 10)
genotypes had 24 and 30% (P = 0.04) higher progesterone levels,
respectively, measured at day 22 of the menstrual cycle. Although
based on small numbers, these preliminary data suggest that pre-
menopausal women with the A2 allele of CYP17 may have enhanced
production of all steroid hormones because of heightened steroido-
genesis. An upper limit of effective estrogen action on the rate of
endometrial cell division has been suggested among premenopausal
women; it has been argued that endometrial cell division is not
dramatically increased by increases in estradiol concentration beyond
the basal level produced during the follicular phase of the menstrual
cycle (3). Theoretically, premenopausal women with higher circulat-
ing progesterone levels even in the presence of higher estrone levels
may be at lower risk of endometrial cancer, which could potentially
explain the inverse association that we observed between the A2 allele
of CYP17 and endometrial cancer risk. Our CYP17 genotype-steroid
hormone results among postmenopausal women do not provide strong
support for genetic modulation of steroid hormone levels by CYP17
genotype. However, regulation of CYP17 expression via this poly-
morphism may differ between the ovaries and the adrenals, the pre-
dominant sites of steroid hormone production among premenopausal
and postmenopausal women, respectively.
Although we did not observe significant interactions between
CYP17 genotype and established endometrial cancer risk factors
associated with estrogen exposure, we did observe suggestive evidence
that the inverse association between the A2 allele and endometrial
cancer risk was stronger among women who had never used HRT.
These data suggest that the A2 allele of CYP17 may not play a role
among women who are at a significantly increased risk of endometrial
cancer because of long-time HRT use. However, we did not see a
similar modification of risk with BMI; an inverse association with the
A2 allele was actually stronger among women with greater BMI.
Little is known regarding genetic susceptibility to endometrial
cancer. Endometrial cancer is a component of hereditary nonpolyposis
colorectal cancer, which is caused by mutations in genes involved in
DNA mismatch repair (MSH2, MLH1, PMS1, and PMS2; Ref. 16).
Epidemiological studies have shown that women with a first-degree
family history of colorectal and/or endometrial cancer are at signifi-
cantly increased risk of endometrial cancer (17, 18). In our data,
CYP17 genotype did not account for endometrial cancer risk associ-
ated with a first-degree family history of endometrial or colorectal
cancer; the distribution of CYP17 alleles did not differ significantly
among women with or without a family history of cancer overall, or
among the controls. In addition, the effect estimates for family history
and CYP17 genotype remained unchanged when both variables were
included in multivariate models. However, we did observe a signifi-
cant interaction between first-degree family history and CYP17 gen-
type, which appeared to be driven by the low frequency of the A2
allele among cases with a positive family history. A significant
inverse association between the A2 allele and endometrial cancer risk
was limited to women with a family history. Although preliminary,
our results suggest that CYP17 genotype may be involved in modi-
fying family history-associated endometrial cancer risk.
In view of the modest associations that we observed between
CYP17 genotype and hormones levels, and the strong association
observed between CYP17 genotype and endometrial cancer risk, the
A1 or A2 alleles may be serving as markers of a functional variant in
a nearby gene. However, this polymorphism may also be in linkage
disequilibrium with an unidentified functional polymorphism in
CYP17 that influences endometrial cancer risk, but does not produce
detectable differences in circulating hormone levels among postmeno-
pausal women. The relatively large sample size and the acquisition of
risk factor information prospectively are strengths of this study. We
did not observe evidence that endometrial cancer survival depended
on CYP17 genotype, which allowed us to analyze incident and preva-
lent cases together and increase the statistical power of our study.
One possible limitation was that ascertainment of first-degree family
history of cancer was made retrospectively. Prevalent and incident
cases may be more responsive to this question based on their personal
cancer history; however, we would not expect their response to differ
by CYP17 genotype. Larger studies are warranted to confirm these
observations and to further examine interactions between CYP17
alleles and endometrial cancer risk factors. In summary, we provide
evidence that the A2 allele of CYP17 is associated with decreased risk
of endometrial cancer and that the association may be modified by
first-degree family history of endometrial and colorectal cancer.
However, CYP17 genotype has only modest effects, if any, on steroid
hormone metabolism among postmenopausal women.

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