Short Communication

Association between *CYP17* Polymorphisms and the Development of Breast Cancer[1]

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Abstract

A nested case-control study was conducted to determine whether a genetic polymorphism in the *CYP17* gene, which encodes for an enzyme that mediates steroid hormone metabolism, was associated with an increased risk of breast cancer. No association was found between the presence of an *A2* allele and the subsequent development of breast cancer [*A1/A2* odds ratio, 0.61 (95% confidence interval, 0.33–1.14); *A2/A2* odds ratio, 0.89 (95% confidence interval, 0.41–1.95)]. No significant association was observed with risk factors presumed to be surrogates for endogenous estrogen exposure, nor was there an association observed with the stage of disease at diagnosis. Genotype frequencies in this Caucasian population were similar to those reported for African-American, Asian, and Latino women. Additional studies of larger size are needed to achieve a consensus regarding the relevance of *CYP17* genotypes to the risk of developing breast cancer.

Introduction

Endogenous levels of androgens and estrogens have been associated with the development of breast cancer, but the results of these studies have not been consistent (1–11). One possible explanation for divergent results across populations may be differences in the prevalence of common inherited variations, or polymorphisms, in genes that code for enzymes involved in steroid hormone production and metabolism. An example of such a gene is the cytochrome P450c17 gene (*CYP17*) whose enzyme product mediates the production of sex steroids from steroid precursors (12). This enzyme catalyzes the rate-limiting step in the androgen biosynthesis pathway in the ovary and the adrenal gland. Androgens produced via this pathway may then be converted to estrone and estradiol. Polymorphic alleles of *CYP17* have been identified (*A1* and *A2*), and the sequence present in the *A2* allele creates a new Sp1 promoter site (CCACC) that is hypothesized to enhance basal transcription of the gene (13–14). An association between the *A2* allele and polycystic ovary syndrome, a condition associated with high androgen levels, anovulatory infertility and hirsutism, has been observed (13) and would be consistent with enhanced transcription of *CYP17*. Higher serum estradiol and progesterone levels have been observed among nulliparous premenopausal women carrying at least one *A2* allele compared with women homozygous for the *A1* allele. However, in that study, androgen levels were not measured, and the sample size was small (15).

It has been hypothesized that polymorphisms in *CYP17* may play a role in the onset of menarche and with the development of breast cancer by increasing androgen and estradiol levels in women with the putative high transcription allele (16). Feigelson *et al.* (16) found that among women of Asian, African-American and Latino descent the presence of an *A2* allele was significantly associated with the presence of advanced stage breast cancer but not with local or *in situ* breast cancer. Among controls, women with an *A2* allele were slightly more likely to experience menarche before 13 years than *A1* homozygotes (49% *versus* 39%; not statistically significant). The protective association between later age at menarche and breast cancer was stronger among women with the *A1/A1* genotype than women with at least one *A2* allele (OR,[3] 0.47 *versus* 0.80; Ref. 16). In the present study we analyzed *CYP17* genotypes in a nested case-control study of women of European-American descent from Washington County, Maryland.

Materials and Methods

Study Population. In 1989, a research specimen bank was established with 32,898 individuals donating a blood sample after signing an informed consent. Of the participants, 25,081 (14,625 women) were residents of Washington County and formed the study cohort. Compared with the Washington County population, participation rates were higher among women and older individuals. Incident breast cancer cases (n = 115) occurring through 1995 were identified by linkage to the Washington County Cancer Registry. The registry identifies cases from discharge records of the Washington County Hospital (Hagerstown, MD), the only hospital in the county, and from death certificates. To estimate the completeness of ascertainment, the number of cancer cases obtained through the Washington County Registry was compared with the number reported to the Maryland Cancer Registry for Washington

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[3] The abbreviations used are: OR, odds ratio; CI, confidence interval; BMI, body mass index.

Table 1 Selected characteristics for breast cancer cases (n = 115) and matched controls (n = 115), Washington County, Maryland, 1989

Characteristic	Cases (%)	Controls (%)	Matched OR (95% CI)	P
Menopausal status at blood donation[a]				
Premenopausal	28 (24.4%)	26 (22.6%)		0.76
Post-menopausal	87 (75.6%)	89 (77.4%)		
Age in 1989 (mean ± SD)[a]	60.4 ± 11.7	60.2 ± 11.5		0.91
Age at menarche[b]	12.6 ± 1.5	12.8 ± 1.5		0.21
Age at first birth[c]	21.7 ± 3.9	22.5 ± 4.3		0.23
Family history of breast cancer (mother, sister, offspring, or grandmother)				
No	84 (73.0)	100 (87.0)	1.0 (referent)	
Yes	31 (27.0)	15 (13.0)	2.45 (1.22–4.95)	
BMI (kg/m^2)				
≤24.47	47 (40.9)	58 (50.4)	1.0 (referent)	
>24.47	68 (59.1)	57 (49.6)	1.61 (0.89–2.90)	
History of cigarette smoking				
Never	75 (65.2)	72 (62.6)	1.0 (referent)	
Former	27 (23.5)	24 (20.9)	1.07 (0.56–2.06)	
Current	13 (11.3)	19 (16.5)	0.66 (0.30–1.44)	
Number of cigarettes smoked/day[d]				
1–10	17 (14.8)	13 (11.3)	1.02 (0.44–2.37)	
11–20	10 (8.6)	10 (8.6)	1.03 (0.36–2.97)	
20+	4 (3.5)	2 (1.7)	1.51 (0.25–9.27)	
Missing data	9 (7.8)	18 (15.6)		
History of alcohol drinking				
Never	53 (46.1)	60 (52.2)	1.0 (referent)	
Ever	51 (44.3)	41 (35.6)	1.51 (0.81–2.80)	
Missing data	11 (9.6)	14 (12.2)		
Number of alcoholic drinks/wk				
<1	20 (17.4)	13 (11.3)	1.13 (0.42–3.08)	
1–3	16 (13.9)	16 (13.9)	1.18 (0.51–2.73)	
≥4	15 (13.0)	9 (7.8)	1.72 (0.68–4.47)	
Ever-use of hormone replacement therapy				
Never	72 (62.6)	63 (54.8)	1.0 (referent)	
Ever	24 (20.9)	27 (23.5)	0.79 (0.41–1.53)	
Missing data	19 (16.5)	25 (21.7)		
Ever-use of oral contraceptives				
Never	75 (65.2)	74 (64.4)	1.0 (referent)	
Ever	28 (24.4)	26 (22.6)	1.1 (0.5–2.2)	
Missing data	12 (10.4)	15 (13.0)		
Oophorectomy				
No/one ovary removed	89 (77.4)	76 (66.1)	1.0 (referent)	
Two ovaries removed	14 (12.2)	25 (21.7)	0.46 (0.21–0.98)	
Missing data	12 (10.4)	14 (12.2)		

[a] Matching factors.
[b] Data available for 100 cases and 98 controls.
[c] Age at first birth, data available for 84 cases and 86 controls.
[d] Among former and current smokers.

County during 1993. The Maryland Cancer Registry has mandatory reporting of incident breast cancer cases from hospitals, freestanding pathology laboratories, and physicians throughout Maryland (17). In that year, 89 cases of breast cancer were recorded in the Washington County Cancer Registry and 81 cases were reported to the Maryland Cancer Registry.[4]

Each case was matched to one control on age (within 1 year), race (all were white), menopausal status, day of menstrual cycle, and within 2 weeks of blood donation.

At the time of blood donation, participants completed a brief questionnaire that asked for smoking status, height, weight, and medication use in the previous 48 h. As part of a larger study on environmental risk factors and breast cancer, cases and controls were sent a self-administered questionnaire to obtain more detailed information about breast cancer risk factors. Of the 115 cases, 104 (90.4%) returned a completed questionnaire; of the 115 controls, 103 (89.6%) returned the questionnaire. Exposure to factors, such as duration of hormone replacement therapy, among cases and controls was truncated at the date of diagnosis of the subject.

Laboratory Assays. Blood was collected in 20-ml heparinized tubes and centrifuged within 6 h of collection. Plasma, WBCs, and RBCs were separated and stored at −70°C within 24 h of collection. The buffy coat was kept frozen until thawed for DNA extraction for this study.

DNA was extracted from the thawed WBC fraction from each study subject by high-salt fractionation (18) followed by chloroform/isoamyl alcohol extraction (19). Concentration of DNA was adjusted to 100 μg/ml and stored at −70°C until genotype analysis. DNA of sufficient quality to assay the *CYP17* genotype was successfully extracted from 109 of 115

[4] These data were supplied by the Maryland Cancer Registry, the Maryland State Department of Health and Mental Hygiene, Baltimore, Maryland. The Department of Health and Mental Hygiene specifically disclaims responsibility for any analyses, interpretations, or conclusions.

cases and 113 of 115 controls. The *CYP17* genotype was determined using the PCR-RFLP method of Carey *et al.* (13), in which restriction digest by *Msp*AI identifies the presence of the *A2* allele.

Statistical Analysis. The association between *CYP17* alleles and the development of breast cancer was examined using conditional logistic regression to calculate the ORs and 95% CIs. The *CYP17* assay categorizes individuals into three discrete categories: *A1/A1* homozygous, *A1/A2* heterozygous, or *A2/A2* homozygous. ORs and 95% CI were estimated for each of these categories with the *A1/A1* allele designated as the referent category. Stratified analyses according to categories of risk factors were also conducted to assess the presence of confounding or interactions. The variables examined in these stratified analyses were menopausal status of the case at the time of diagnosis, extent of disease at the time of diagnosis (local *versus* regional), age at menarche, history of oophorectomy, smoking history, history of alcohol use, BMI, ever-use of hormone replacement therapy, and family history of breast cancer in mother, sister, offspring, or grandmother. For all of these variables, except menopausal status and stage at diagnosis, stratified analyses required breaking the matching. Consequently, ORs for the stratified analyses were estimated using unconditional logistic regression, adjusting for the matching factors of age and menopausal status at the time of blood donation. None of the factors studied satisfied the criteria of a confounder. Nevertheless, adjustment of the association between genotypes and breast cancer risk was conducted using conditional logistic regression analysis to adjust for smoking, alcohol consumption, BMI, and hormone replacement therapy. The adjusted ORs were similar to the unadjusted risk estimates, thus the simplified model is presented. Genotype frequency differences and Hardy-Weinberg equilibrium analyses were tested using a χ^2 statistic (SigmaStat, Jandel Scientific, San Rafael, CA).

Women with breast cancer were classified as postmenopausal at diagnosis based on their response to the questionnaire items concerning the date of the last menstrual period as well as history of hysterectomy and oophorectomy. Women with missing information regarding the last menstrual period or women with a history of hysterectomy without oophorectomy were considered to be postmenopausal if diagnosed at age 51 or older.

Results

The association between potential risk factors and the development of breast cancer are shown in Table 1. Cases and controls were closely matched on age and menopausal status at the time of blood donation and had similar ages at menarche and at first birth. Twenty-eight cases, 24 of whom were available for *CYP17* assays, were premenopausal at the time of diagnosis. A family history of breast cancer in a grandmother, mother, offspring or sister was associated with a 2-fold increase in breast cancer risk. Ever-use of oral contraceptives was similar for cases and controls as was age at menarche and age at first birth. Fewer cases than controls reported ever using hormone replacement therapy but the difference was not statistically significant. Although alcohol intake and smoking a pack or more of cigarettes a day at sometime were associated with an increased risk of breast cancer, neither association was statistically significant.

The risk of breast cancer associated with the *A1/A2* and *A2/A2* genotypes was examined for the total study population, stratified by menopausal status at diagnosis and by tumor stage at diagnosis (categorized as local *versus* regional; Table 2). The risk of breast cancer was not increased for carriers of the *A2* allele in either the heterozygous or homozygous state, and in general, the associations were in the protective direction. Furthermore, a similar pattern of associations was observed when the data were stratified by menopausal status and stage of disease at diagnosis. The frequency of the *CYP17* genotypes among controls was 33% *A1/A1*, 51% *A1/A2*, and 16% *A2/A2*, showing no departure from Hardy-Weinberg equilibrium and virtually identical to the distribution among a group of Asian, African-American, and Latino women (16).

Table 2 Association between *CYP 17* alleles and the development of breast cancer according to menopausal status at time of diagnosis of the case and stage of disease

	A1/A1	*A1/A2*	*A2/A2*
Total			
Case	41	47	21
Control	37	58	18
OR[a] (95% CI)	1.0	0.61 (0.33–1.14)	0.89 (0.41–1.95)
Menopausal status at diagnosis			
Premenopausal at diagnosis			
Case	11	9	4
Control	8	13	4
OR[a] (95% CI)	1.0	0.38 (0.09–1.63)	0.70 (0.15–3.19)
Postmenopausal at diagnosis			
Case	30	38	17
Control	29	45	14
OR[a] (95% CI)	1.0	0.69 (0.34–1.39)	0.99 (0.39–2.49)
Stage of disease at diagnosis			
Local disease			
Case	31	30	16
Control	26	38	15
OR[a] (95% CI)	1.0	0.53 (0.24–1.16)	0.76 (0.31–1.88)
Regional disease			
Case	9	16	5
Control	10	19	3
OR[a] (95% CI)	1.0	0.78 (0.26–2.40)	1.39 (0.26–7.28)

[a] Matched ORs from conditional logistic regression.

Risk factors such as age at menarche and at first birth, hormone exposure, BMI, oophorectomy, exogenous hormone exposure, and alcohol intake, and potential risk factors such as smoking may mediate breast cancer risk through their influence on endogenous hormone levels. Because of the possible influence of *CYP17* polymorphisms on estradiol biosynthesis, we assessed the association between *CYP17* genotype and hormone-related breast cancer risk factors. As seen in Table 3, there was no evidence of a significant interaction between any risk factors for breast cancer and the presence of one or more *A2* alleles. In particular, later age at menarche was not associated with a reduced risk of breast cancer among women with *A1/A1* genotypes (OR, 1.47; 95% CI, 0.56–3.88) but was associated in the protective direction among women with an *A2* allele. Among controls, women with an *A2* allele were more likely to have had menarche at age 13 or later than women who were *A1* homozygotes (60% *versus* 42%; $P = 0.15$). An increased risk of breast cancer among women who ever drank alcohol or who had a BMI greater than the median of control women was observed among women with at least one *A2* allele, but the associations were not statistically significant. A history of smoking or ever-use of hormone replacement therapy was associated with an increased risk of breast cancer among women homozygous for *A2*, but again the ORs were not statistically significant.

Table 3 Association between *CYP17* genotypes and breast cancer stratified by selected characteristics[a]

	CYP17 genotypes								
	A1/A1			*A1/A2*			*A2/A2*		
	Case *n*	Control *n*	OR (95% CI)	Case *n*	Control *n*	OR (95% CI)	Case *n*	Control *n*	OR (95% CI)
Family history of breast cancer in mother, sister, offspring or grandmother)									
No	32	33	1.0	30	49	1.0	16	16	1.0
Yes	9	4	2.34 (0.65–8.34)	17	9	3.01 (1.18–7.68)	5	2	2.53 (0.42–15.06)
Age at menarche									
<13 yr	18	18	1.0	23	22	1.0	8	4	1.0
≥13 yr	19	13	1.47 (0.56–3.88)	17	28	0.58 (0.25–1.36)	10	11	0.41 (0.09–1.88)
Hormone replacement therapy									
Never	25	19	1.0	30	31	1.0	13	12	1.0
Ever	11	10	0.92 (0.31–2.72)	6	14	0.45 (0.15–1.37)	6	2	4.99 (0.60–41.24)
History of smoking									
Never	31	22	1.0	29	34	1.0	12	14	1.0
Ever	10	15	0.48 (0.18–1.27)	18	24	0.92 (0.41–2.06)	9	4	2.90 (0.67–12.55)
History of alcohol use									
Never	21	17	1.0	20	33	1.0	8	9	1.0
Ever	17	16	0.82 (0.31–2.15)	22	16	2.50 (1.03–6.05)	11	8	1.66 (0.40–6.87)
Body Mass Index									
≤24.47 kg/m^2	23	19	1.0	14	28	1.0	10	11	1.0
>24.47 kg/m^2	18	18	0.87 (0.35–2.17)	33	30	2.26 (0.99–5.16)	11	7	1.92 (0.50–7.38)
Bilateral oophorectomy									
No	33	22	1.0	35	40	1.0	17	13	1.0
Yes	5	10	0.33 (0.10–1.17)	6	10	0.79 (0.25–2.55)	2	4	0.31 (0.04–2.34)

[a] Adjusted for age and menopausal status in 1989.

Discussion

In this population of European-American women from Washington County, Maryland, no association was observed between the presence of genotypes containing the *CYP17 A2* allele and subsequent development of breast cancer. Thus, this study provides no support for the previous observation of an association between the *CYP17 A2* allele and the risk of advanced breast cancer. Contrary to the previous report by Feigelson *et al.* (15), we observed that women with an *A2* allele were somewhat more likely to have a later age at menarche rather than earlier. When the results from the two studies were pooled, women with at least one *A2* allele were only slightly more likely to have a later age at menarche than *A1* homozygotes (53% *versus* 45%). In further stratified analyses, there was no significant association between *CYP17* genotype and stage of cancer at diagnosis or age at menarche. Some interesting patterns of association were observed between the presence of at least one *A2* allele and an increased breast cancer risk for women of higher BMI, history of hormone replacement therapy, and alcohol intake but none of the associations were statistically significant. These associations have not been previously reported and deserve further exploration in larger studies, particularly given that a presumed mechanism of action of these risk factors is through the endogenous hormonal environment.

The number of cases and controls was relatively small in the present study, and it is possible that sampling might affect genotype frequencies, particularly among the control group. However, this does not seem to be the case. We used Hardy-Weinberg equilibrium analysis to test whether genotype frequencies within control or case groups were in the expected balance, and all of the genotype frequencies were in equilibrium. Differences between the study by Feigelson *et al.* (15) and our study also cannot be attributed to allele frequency differences between the sampled populations. The genotype distributions in the present report (controls, *A1/A1* = 33%, *A1/A2* = 51%, and *A2/A2* = 16%, calculated from Table 2) were not significantly different from those reported by Feigelson *et al.* (16) for any of the ethnic groups in that study (Asian, African-American, and Latino) and were virtually identical to the genotype frequencies of the total controls in their study. In addition, genotype frequencies for the Washington County, Maryland controls were similar to a population of European-Americans observed in central North Carolina (*A1/A1* = 38%, *A1/A2* = 50%, and *A2/A2* = 12%.[5]

The study design from Feigelson *et al.* (16) and the present report are similar; community-based nested case-control studies. They differ mainly in size (~200,000 *versus* 32,898), racial composition (Asian, Hispanic and African-American *versus* primarily Americans of European descent), and timing of sample collection (specimens obtained after diagnosis *versus* before the diagnosis of breast cancer). Both studies had a similar proportion of cases diagnosed with advanced stage disease, 28% and 32%, respectively. It is unlikely that with a much larger group of cases, we might have observed a similar risk for the *A2* allele among women with regional/metastatic disease because, in general, the associations observed in our study were in the direction opposite to those observed by Feigelson *et al.* (16). The genotype frequencies did not vary between the studies, and in this sense the studies are complementary. This would suggest that if there are racial/ethnic differences in disease etiology or prognosis, they are not likely to be due to *CYP17*. In the Feigelson *et al.* study, biological samples were collected from incident cancer cases after diagnosis and from a sample of healthy cohort members (controls), whereas in the present study, biological samples were collected in 1989 before the diagnosis of cancer. Although in the Feigelson *et al.* design,

[5] D. Bell, unpublished data.

phenotypic measurements of hormone levels could be influenced by disease state, genotype would not be, and this aspect of the design would not influence the study outcome.

The important role that the CYP17 enzyme has in mediating androgen biosynthesis makes it a compelling candidate for a susceptibility gene for the development of breast cancer. However, the biological plausibility for attaching risk to the *CYP17 A2* allele needs to be explored mechanistically in more detail. Studies demonstrating that the *A2* allele actually influences the rate of *CYP17* transcription and subsequent levels of enzyme would be useful. A recent study by Feigelson *et al.* (15) examined estradiol and progesterone levels but not androgen levels among 83 healthy nulliparous women under the age of 33. Estradiol levels at day 11 and 22 of the menstrual cycle and day 22 progesterone levels were higher among women heterozygous or homozygous for the *A2* allele compared with women homozygous for the *A1* allele (15). These data need to be replicated in a larger study examining an array of hormones. Although no significant association was observed with breast cancer risk nor with age at menarche, some intriguing patterns with other risk factors such as BMI and alcohol intake deserve further exploration. As results emerge from the numerous other studies in progress, a consensus may emerge as to whether inherited variability in steroid biosynthesis is important in breast cancer or other hormone-associated malignancies.

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