[CANCER RESEARCH 58, 585–587, February 15, 1998]

Advances in Brief

Cytochrome P450c17α Gene (*CYP17*) Polymorphism Is Associated with Serum Estrogen and Progesterone Concentrations[1]

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Abstract

An increased level of serum estrogen is one marker of breast cancer risk. We have recently reported that increased risk of advanced breast cancer is associated with a common allele of the cytochrome P450c17α gene (*CYP17*), designated *A2*. We now show that *CYP17* genotype is associated with serum hormone levels among 83 young, nulliparous women. Serum estradiol (E_2) levels measured around day 11 of the menstrual cycle were 11 and 57% higher ($P = 0.04$), respectively, among women hetero- and homozygous for the *CYP17 A2* allele compared to *A1/A1* women. Similarly, around cycle day 22, E_2 levels were 7 and 28% higher ($P = 0.06$), and progesterone levels were 24 and 30% higher ($P = 0.04$), respectively. These data provide direct evidence of genetic control of serum hormone levels.

Introduction

Most of the risk factors for breast cancer that have been identified manifest their effects by influencing the level of and/or length of exposure to sex-steroid hormones. We have proposed a multigenic model of breast cancer predisposition that includes several genes involved in estrogen biosynthesis based on the assumption that individual variation in the levels of endogenous steroid hormones will result in differences in breast cancer risk (1). We have hypothesized that this individual variation results from genetic variation (*i.e.*, polymorphisms) in crucial genes that control hormone biosynthesis.

One such gene involved in hormone biosynthesis is the *CYP17* gene. It codes for the cytochrome P450C17α enzyme, which mediates both steroid 17α-hydroxlyase and 17,20-lyase activities and functions at key branch points in human steroidogenesis (2). The 5′-UTR of *CYP17* contains a 1-bp polymorphism 34 bp upstream from the initiation of translation and 27 bp downstream from the transcription start site (3). This bp change creates a recognition site for the *Msp*AI restriction enzyme and has been used to designate two alleles, *A1* (the published sequence) and *A2*.

We have recently reported an association between risk of breast cancer and this *CYP17* polymorphism (4). In a case-control study of incident, mainly postmenopausal, breast cancer among Asian, African-American, and Latina women, we found a 2.5-fold increased risk of advanced breast cancer associated with the *CYP17 A2* allele. This suggested that serum hormone levels may differ by *CYP17* genotype.

We report here a study of serum E_2[3] and Prg levels and CYP17 genotype among healthy, premenopausal, nulliparous women.

Materials and Methods

Study Population. This study is part of a larger ongoing study of exercise and ovarian function in premenopausal women (for which L. S. S. is Principal Investigator). Study participants were recruited from advertisements around universities and city colleges in Los Angeles County, CA. African-American, Latina, and white female volunteers residing in Los Angeles County who were nulliparous and between the ages of 18 and 33 were eligible for the study, which was designed to investigate diet and exercise in relation to sex-steroid hormone levels. Women were ineligible for the study if they had taken oral contraceptives or other hormonal medications within the previous 3 months, had ever had a pregnancy that lasted 12 weeks or longer, or weighed 200 pounds or more.

Serum Collection and Hormone Assays. Women were asked to record the 1st day of their menstrual cycle (defined as the first day of menses) and then on cycle days 11 (range, 10–12) and 22 (range, 21–23), fasting morning blood samples were collected. The start of their next menstrual period was also recorded to determine cycle length and to permit adjustment of hormone values by number of days to next menses. Blood components were separated and stored at −40°C within 4 h of collection.

E_2 and Prg levels were measured in serum by our previously validated assays (5–7). E_2 was quantified by RIA following the addition of an internal standard (1000 dpm of [^{3}H]E_2) for monitoring procedural losses to 0.6 ml of serum and extraction with ethyl acetate:hexane (3:2). Interassay coefficients of variation, determined in pooled serum samples, were 13, 11, and 9% for low, medium, and high quality control E_2 pools, respectively. Prg was also measured by RIA after addition of an internal standard (1000 dpm of [^{3}H]Prg) to 0.1 ml of serum and extraction with diethyl ether. The interassay coefficients of variation for Prg were 26, 18, and 10% for low, medium, and high quality control pools, respectively.

***CYP17* Assay.** DNA was purified from buffy coats of peripheral blood samples for all cases and controls using a rapid DNA preparation method (8). The *CYP17* assay has been previously described (3). Briefly, a PCR fragment containing the bp change was generated using the following primers: CYP-1, 5-CATTCGCACTCTGGAGTC-3, and CYP-2, 5-AGGCTCTTGGGGTACTTG-3. PCR reactions were carried out in 25-μl aliquots containing about 50 ng of genomic DNA, 50 pmol of each primer, 1× reaction buffer [50 mM KCL, 1.5 mM $MgCl_2$, and 10 mM Tris (pH 9.0 at room temperature)], 100 μM deoxynucleotide triphosphates, and 1 unit of Taq polymerase (Pharmacia). The amplification was for 30 cycles with denaturation at 94°C for 1 min, annealing at 57°C for 1 min, and extension at 72°C for 1 min. An initial denaturation step of 5 min at 94°C and a final extension at 72°C for 5 min were used. The PCR products were digested for 3 h at 37°C using *Msp*AI, separated by agarose gel electrophoresis, and stained with ethidium bromide to identify the bp change.

Data Analysis. Serum concentrations of E_2 and Prg were logarithmically transformed to produce approximately normal distributions. Analysis of covariance was used to test mean differences by genotype while adjusting for ethnicity and other covariates (see text below). All statistical significance levels (*P*s) quoted are two sided.

Received 10/15/97; accepted 1/2/98.

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[1] This work was supported by funds from the California Breast Cancer Research Program of the University of California (Grants 2FB-0212 and 1KB-0060) and by NCI Grant CA14089–21.

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[3] The abbreviations used are: E_2, estradiol; Prg, progesterone; UTR, untranslated region.

Results

The study included 123 women between the ages of 18 and 33 with a day 22 blood sample. Four ethnic groups were represented in the study: African-American, Mexican-American, other Latina [*i.e.*, women who reported a Latina (South and Central America) heritage that was not Mexican], and non-Latina whites (whites). Characteristics of the study population are shown in Table 1.

In a previous study (9), we showed that a serum Prg value within 3–10 days of the next menses can be used to determine, with very high accuracy, whether the particular cycle was or was not ovulatory. We, therefore, restricted attention here to women who had a cycle length of 25–32 days, using the day 22 Prg value to determine ovulatory status. Thirty-three of the women had a cycle length outside the range of 25–32 days, leaving 90 women on whom we could determine whether they had an ovulatory cycle. Seven of the 90 had serum Prg <3.0 ng/ml, indicating an anovulatory cycle, leaving 83 women with an ovulatory cycle. We report here the E_2 values on days 11 and 22 and the Prg values on day 22 for these 83 women. The characteristics of these 83 women were very similar to those of the whole group shown in Table 1.

The geometric mean levels, adjusted for ethnicity, of serum E_2 and Prg levels by genotype for the 83 women with an ovulatory cycle are shown in Table 2. Percentage differences compared to the *A1/A1* genotype are also provided. There is a steady increase in all three serum concentrations depending on the number of *A2* alleles a woman carries, with the *A2/A2* genotype corresponding to the highest concentrations, although the result for day 22 E_2 is not formally statistically significant. Serum E_2 levels measured on day 11 of the menstrual cycle were 11 and 57% higher, respectively, among women hetero- or homozygous for the *CYP17 A2* allele compared to *A1/A1* women. On cycle day 22, E_2 levels were 7 and 28% higher, and Prg levels were 24 and 30% higher. Adjusting the results further for the other characteristics shown in Table 1 and number of days from blood draw to start of next menses, either alone or simultaneously, did not alter these findings.

Discussion

These data provide the first direct evidence of genetic control of serum E_2 and Prg levels in premenopausal, ovulating women. However, replication of these findings in a larger study is warranted, given our small sample size. Our recent study found that the *CYP17 A2* allele was associated with an increased risk of advanced breast cancer (4) in mainly postmenopausal women; the current study suggests that this may be due, at least in part, to elevated serum E_2 and Prg levels in the premenopausal period.

Animal studies have repeatedly demonstrated that estrogens can induce and promote mammary tumors in rodents and that removing the ovaries or administering an antiestrogenic drug has the opposite effect (10). Demonstrating elevated endogenous estrogen levels in premenopausal breast cancer cases has been difficult, probably because of the wide fluctuations in levels during the menstrual cycle, but we have previously found, in a very carefully controlled study, such elevations in two groups of cases (7). Low premenopausal serum E_2 levels have been consistently found in Asian populations at low risk of breast cancer (7, 11, 12). The role of elevated Prg levels in breast cancer etiology is controversial (13), but recent experimental data suggest that progestins are breast mitogens and, as such, are likely to increase breast cancer risk (14).

Serum hormone measurements are subject to variation by many factors, *e.g.*, time of day, age, disease status, and laboratory imprecision. Numerous controls were applied to this study to ensure that the serum hormone levels were as comparable as possible. All samples were collected before 10:00 a.m., and the subjects were asked to fast for at least 8 h before sample collection. All subjects were healthy. Adjustment for age in the multivariate model did not alter our findings. As described above, laboratory precision was carefully monitored using internal standards and pooled quality control samples.

The *Msp*AI polymorphism that defines the *A1* and *A2* alleles at this locus is either in linkage disequilibrium with other, as yet unidentified, genetic variations located near this site or has a direct quantitative effect on *CYP17* gene expression. Using single-strand conformational polymorphism, we have screened all eight exons and several fragments in the promoter region of 28 *A1/A2* heterozygous women and have been unable to identify any polymorphism or mutation that can explain our findings.[4] This indicates that the expression of the *CYP17* gene may be directly affected by this *Msp*AI polymorphism. In the original description of this polymorphism, Carey *et al.* (3) considered the possibility that the 1-bp change T→C that creates an Sp1 type (CCACC) promoter site in allele *A2* may lead to an increased rate of transcription initiation due to Sp1 binding to it. This hypothesis has not been tested formally, and although it remains a possibility, other effects on gene expression might be considered as well. These might be at the level of transcription elongation or translation rate of the corresponding mRNA. Although none of these possibilities have been tested experimentally, precedence exists for effects by 5′-UTR regions on transcription elongation and translation. For example, in the case of the androgen receptor gene, a *cis*-acting suppressor element has been shown to exist in its 5′-UTR that interacts with single-stranded DNA-binding proteins to possibly modulate transcription elongation (15). In the same gene, other areas of the 5′-UTR seem to induce mRNA translation (16). Therefore, it is possible that the 1-bp difference at the *Msp*AI site of the *CYP17* 5′-UTR exerts similar effects and, thus, influences the overall expression.

There are several important consequences of these findings. In the field of cancer prevention, there is likely to be substantial interest in

Table 1 *Characteristics of study population*

Total sample size	123
Ethnic groups *(n)*	
African American	22
Mexican American	32
Other Latina	28
White	41
Age (yr)[a]	22.0 (19–24)
Age at menarche (yr)[a]	12.4 (12–13)
Height (m)[a]	1.62 (1.57–1.65)
Weight (kg)[a]	59.5 (53.1–65.5)
Body mass index (kg/m^2)[a]	22.8 (20.1–24.5)
Ever pregnant (%)	18

[a] Mean and interquartile range.

Table 2 *Geometric mean serum E_2 and Prg concentrations among young nulliparous women*[a]

Variable	*A1/A1* (*n* = 28)	*A1/A2* (*n* = 45)	*A2/A2* (*n* = 10)	*P*[b]
E_2,[c] day 11	148[d] (4.998 ± 0.099)	165 (11%)[e] (5.103 ± 0.078)	233 (57%) (5.452 ± 0.172)	0.04
E_2,[c] day 22	212 (5.355 ± 0.066)	227 (7%) (5.423 ± 0.051)	271 (28%) (5.602 ± 0.106)	0.06
Prg,[f] day 22	13.6 (2.611 ± 0.081)	16.8 (24%) (2.823 ± 0.065)	17.7 (30%) (2.872 ± 0.135)	0.04

[a] Natural log and SE are shown in parentheses below geometric means.
[b] Two-sided test for trend against number of *A2* alleles.
[c] pg/ml.
[d] Adjusted for ethnicity.
[e] Percentage increase compared to *A1/A1* genotype.
[f] ng/ml.

[4] G. A. Coetzee, L. E. Crocitto, and H. S. Feigelson, unpublished data.

the interaction between this *CYP17* polymorphism and exogenous estrogen use, *i.e.*, oral contraceptives and hormone replacement therapy. As a genetic marker of risk, *CYP17* genotype may provide another tool for identifying high-risk groups of women for prevention and treatment protocols. Finally, understanding the genetic control of steroid hormone biosynthesis can provide new insights into reproductive biology and endocrinology.

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