Research article

CYP17 genetic polymorphism, breast cancer, and breast cancer risk factors

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Abstract

Background: Findings from previous studies regarding the association between the *CYP17* genotype and breast cancer are inconsistent. We investigated the role of the *Msp*AI genetic polymorphism in the 5′ region of *CYP17* on risk of breast cancer and as a modifier of reproductive risk factors.

Methods: Questionnaire and genotyping data were obtained from a population-based, case–control study of premenopausal ($n = 182$) and postmenopausal ($n = 214$) European-American Caucasian women in western New York. Cases and controls were frequency matched by age and by county of residence. Odds ratios and 95% confidence intervals were used to estimate relative risks.

Results: The *CYP17* genotype was not associated with breast cancer risk; however, controls with the A2/A2 genotype (associated with higher estrogens) had earlier menarche and earlier first full-term pregnancy. Premenopausal women with A1/A1 genotypes, but not with A2 alleles, were at significantly decreased risk with late age at menarche (odds ratio = 0.37, 95% confidence interval = 0.14−0.99), and at increased risk with late age at first full-term pregnancy (odds ratio = 4.30, 95% confidence interval = 1.46−12.67) and with use of oral contraceptives (odds ratio = 3.24, 95% confidence interval = 1.08−9.73). Associations were weaker among postmenopausal women.

Conclusion: These results suggest that the effects of factors that may alter breast cancer risk through a hormonal mechanism may be less important among premenopausal women with putative higher lifetime exposures to circulating estrogens related to the *CYP17* A2 allele.

Keywords: breast neoplasms, cytochrome P450c17α, epidemiology, genetic polymorphism

Introduction

A number of studies have evaluated possible associations between a polymorphism in the cytochrome P450c17α (*CYP17*) gene and breast cancer risk [1–16]. Cytochrome P450c17α functions at key branch points in human steroidogenesis, catalyzing the ovarian and adrenal biosynthesis pathways for androstenedione, the immediate precursor of testosterone [17]. Three polymorphisms have been described in this gene: a C→T transition at nucleotide 5471 in intron 6 [18], a G→A transition at

nucleotide 47 in the 5′-untranslated region promoter [19], and a thymidine substitution for cytosine at nucleotide 27 in the 5′-untranslated region promoter that creates a *Msp*AI recognition site [20].

The *Msp*AI polymorphism gives rise to three genotypes (A1/A1, A1/A2, and A2/A2). Although it was hypothesized that the polymorphism (A2 allele) could result in an additional Sp1 binding site with enhanced promoter activity and an increased rate of transcription [1], this was not

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bp = base pairs; PCR = polymerase chain reaction.

found in experimental studies [20,21]. Nonetheless, two studies found that both premenopausal and post-menopausal women with the variant A2 allele had higher levels of circulating estrogens than those with common alleles, indicating that the polymorphism may be in linkage disequilibrium with another functional polymorphism or that it may affect hormone levels through some other mechanism [4,22].

Effects of the variant *CYP17* genotype on risk have been investigated in a number of studies, with some corrobora-tive results and some conflicting results. In a multiethnic cohort, while breast cancer risk was not significantly increased for women with the A2 allele [1], the A2 allele did confer more than a twofold increase in risk among those with advanced disease. Furthermore, late age at menarche was protective only among women who were homozygous for the A1 allele. The findings regarding age at menarche were confirmed in three other studies [2,4,9] and in a recent meta-analysis involving 4227 cases and 4730 controls [16]. These studies did not find that the *CYP17* genotype was associated with risk of breast cancer, even when stratifying by stage of disease [4,9,16].

Feigelson *et al.* [23] recently reported that women with A2 alleles were less likely than those with A1 alleles to use hormone replacement therapy, presumably because of fewer menopausal symptoms due to higher circulating estrogens. It is clear that hormonal milieu differs for pre-menopausal women and postmenopausal women, and there is evidence that risk factors for disease diagnosed among the two groups of women may also differ, particu-larly those related to hormonal factors [24].

The majority of previous studies did not evaluate associa-tions separately by menopausal status, particularly for effect modification by *CYP17* on relationships between breast cancer and other risk factors. In the present study, we evalu-ated, separately for premenopausal and postmenopausal women, whether the *CYP17* polymorphism was indepen-dently related to breast cancer risk or could possibly act through modification of other breast cancer risk factors.

Materials and methods
Population
These research data were collected in an earlier case–control study (1986–1991) of 617 premenopausal and 933 postmenopausal Caucasian women in western New York. The detailed methods have been reported else-where [25–27].

The protocol for the present study was reviewed by the Institutional Review Board of the State University of New York at Buffalo and by all of the participating hospitals. Informed consent was received from all participants for the interview and for the medical record review. Women

diagnosed with incident, primary, histologically confirmed breast cancer were identified at all major hospitals in Erie and Niagara counties. They were frequency matched by age and county of residence with controls randomly selected from the New York State Motor Vehicle lists (<65 years) and the Health Care Finance Administration rolls (>65 years). Interview data included medical, repro-ductive, dietary, and lifestyle histories. The majority of women were born in the United States, and all women reported country of birth of their parents to be in Western Europe or, to a lesser degree, in Eastern Europe.

Women were considered postmenopausal if they were younger than age 50 years and had natural menopause, had bilateral oophorectomy or had irradiation to the ovaries. Women aged 50 years and older were consid-ered postmenopausal if they had ceased menstruation. At the end of the interview, women were asked to provide a blood specimen; 45% of premenopausal women and 63% of postmenopausal women agreed. Among pre-menopausal women, there were no significant differences in socioeconomic, hormonal, reproductive, or dietary factors between those who gave blood and those who refused. Among postmenopausal women, controls who gave a blood specimen had a greater mean number of pregnancies (3.5 versus 2.9, $P<0.01$) than those who declined. There were no other significant differences between the groups.

Laboratory analysis
DNA was purified by standard phenol/chloroform extraction followed by ethanol precipitation from frozen blood clots. PCR conditions were based on those of Carey *et al.* [19].

Briefly, genomic DNA (50 ng) was amplified using 50 pmol primers (5′-CAT TCG CAC TCT GGA GTC-3′ and 5′-AGG CTC TTG GGG TAC TTG-3′) in GeneAmp PCR buffer (50 mM KCl, 10 mM Tris–HCl, pH 8.3, 0.001% [w/v] gelatin, 1.5 mM MgCl$_2$; Perkin Elmer, Norwalk, CT, USA), and Amplitaq DNA polymerase (2.5 U; Perkin Elmer) with 2′-deoxynucleoside-3′-triphosphates (1.87 mM; Pharmacia, Piscataway, NJ, USA) in a 50 µl reaction volume. The PCR reaction had an initial melting temperature of 94°C (5 min) followed by 30 cycles of melting (94°C, 1 min), annealing (56°C, 1 min), and extension (72°C, 1 min). An extension period of 7 min at 72°C followed the final cycle. The result-ing product (459 bp) was subjected to *Msp*AI digest (New England Biolabs, Beverly, MA, USA) according to the man-ufacturer's directions. Gel electrophoresis (2.2% agarose; Gibco BRL, Gaithersburg, MD, USA) of the resulting frag-ments revealed either the A1 homozygote (459 bp), the A2 homozygote (335 and 124 bp), or the A1/A2 heterozygote (459, 335, and 124 bp).

Results of genotyping for the study were read from the gel by two independent investigators, and at least 20% of the

samples were repeated for quality control. The assay was validated by confirming polymorphic Mendelian inheritance patterns in seven human family cell lines ($n=134$), each encompassing three generations (data not shown; NIGMS Human Genetic Mutant Cell Repository, Coriell Institute, Camden, NJ, USA).

Statistical analysis
Tests for Hardy–Weinberg equilibrium among controls were conducted using observed genotype frequencies and a χ^2 test with one degree of freedom. To evaluate the role of *CYP17* on breast cancer risk through mediating mechanisms, we first assessed differences in age at menarche, age at first full-term pregnancy, difficulty in becoming pregnant, and use of hormone replacement therapy and oral contraceptives among controls according to the *CYP17* genotype.

Data on hormone replacement use were only available for women who were postmenopausal, and few women beyond menopause had a history of oral contraceptive use, so only premenopausal women were included for those analyses. Odds ratios and 95% confidence intervals were calculated using unconditional logistic regression to evaluate associations between *CYP17* genotypes and breast cancer risk separately for premenopausal and post-menopausal women. The odds ratios were adjusted for age, education, age at menarche, age at first pregnancy, reported family history of breast cancer, body mass index, history of benign breast disease, and age at menopause for postmenopausal women.

Associations between hormonal/reproductive factors and breast cancer were further calculated within genotype strata. We performed tests for interaction by computing the crossproduct of the independent variables (*CYP17* and reproductive factors whose effects on risk varied by genotype) and including it in a regression model with its components entered separately.

Results
Data in the present study were available for 182 pre-menopausal and 214 postmenopausal women. The mean age among premenopausal women was 47 years, and the average age of postmenopausal women was 63 years. We evaluated information on the participant's country of origin and their parent's country of origin. In this fairly homogeneous population, all of the women were of European or Eastern European decent. A χ^2 test of observed versus expected genotype frequencies for *CYP17* among premenopausal women suggested a slight deviation from Hardy–Weinberg equilibrium. However, this difference was not statistically significant ($P=0.08$). The genotype frequencies for post-menopausal women followed Hardy–Weinberg equilibrium ($P=0.65$).

Hormonally related factors appeared to vary by *CYP17* genotype (Table 1). Among both premenopausal and postmenopausal women, those with at least one A2 allele were more likely than women with the A1/A1 genotype to experience early menarche, although differences were not statistically significant. There were also slight differences in age at menopause, with a somewhat later average age at menopause for women with either the A1/A2 or A2/A2 genotypes. Interestingly, postmenopausal women with at least one A2 allele were also less likely to experience difficulty in becoming pregnant and, correspondingly, tended to have children at an earlier age. Contrary to findings by Feigelson *et al.* [23], the *CYP17* genotype did not influence the use of hormone replacement therapy or oral contraceptives in the present data.

Neither homozygosity nor heterozygosity for the A2 variant was associated with increased breast cancer risk (Table 2). In fact, there appeared to be inverse associations between the A2 allele and risk, particularly among postmenopausal women, in both unadjusted models and those models adjusted for breast cancer risk factors. Because there were few differences in relationships for those with A1/A2 and A2/A2 genotypes, these categories were collapsed and the associations evaluated between genotype and other breast cancer risk factors.

Associations between breast cancer and hormonal risk factors by *CYP17* genotype are presented in Table 3. For premenopausal women, late age at menarche was significantly protective only among women with A1/A1 alleles (odds ratio = 0.37; 95% confidence interval = 0.14–0.99). This effect was not seen among postmenopausal women. Differences in risk were also noted for other factors. Late age at first full-term pregnancy significantly increased risk among premenopausal women only for those with the A1/A1 genotype. Risk was also elevated among these allele carriers if they reported use of oral contraceptives and difficulty becoming pregnant. For postmenopausal women, it was women with A2 alleles who were most at risk of breast cancer with late age at first full-term pregnancy and with difficulty becoming pregnant. While effects of age at menarche, age at first full-term pregnancy, and oral contraceptive use were only significantly notable among premenopausal women with A1 alleles, interactions were not statistically significant (data not shown).

Discussion
We found in the present study that while the *CYP17* *Msp*AI genetic polymorphism did not increase breast cancer risk, it appears to modify the associations between hormonal and reproductive factors and breast cancer. Interestingly, women with A2 alleles, those associated with higher estrogen levels in both premenopausal and postmenopausal women, were more likely to share char-

Table 1

Distribution of breast cancer risk factors among controls in relation to the *CYP17* genotype: Western New York Breast Cancer Study

CYP17 genotype	Premenopausal (n=86)		Postmenopausal (n = 102)	
	A1/A1	A1/A2 and A2/A2	A1/A1	A1/A2 and A2/A2
Age at menarche				
<13 years	15 (31)	18 (49)	18 (38)	25 (46)
≥13 years	33 (69)	20 (51)	29 (62)	30 (54)
Difficulty getting pregnant				
No	38 (79)	29 (76)	32 (68)*	47 (86)*
Yes	10 (21)	9 (24)	11 (23)	8 (14)
Age at first birth				
<25 years	38 (79)	28 (74)	21 (45)*	40 (73)*
≥25 years	10 (21)	10 (26)	26 (55)	15 (27)
Ever use oral contraceptives				
No	26 (54)	19 (50)		
Yes	22 (46)	19 (50)		
Family history of breast cancer				
No	45 (94)	35 (92)	43 (91)	50 (91)
Yes	3 (6)	3 (8)	4 (9)	5 (9)
Age at menopause				
<48 years			29 (62)	29 (53)
≥48 years			18 (38)	26 (47)
Ever use hormone replacement therapy				
No			32 (68)	39 (71)
Yes			15 (32)	16 (29)

Data presented as *n* (%). * Statistically significant (*P*<0.05).

Table 2

Associations between *CYP17* genetic polymorphisms and breast cancer by menopausal status: Western New York Breast Cancer Study

	Case (*n* [%])	Control (*n* [%])	Crude odds ratio (95% CI)	Adjusted odds ratio (95% CI)[a]
Premenopausal (*n* = 182)				
A1/A1	58 (61)	48 (56)	1.00	1.00
A1/A2	31 (32)	28 (33)	0.92 (0.48–1.73)	0.85 (0.44–1.63)
A2/A2	7 (7)	10 (11)	0.58 (0.21–1.64)	0.56 (0.20–1.62)
Postmenopausal (*n* = 213)				
A1/A1	51 (46)	47 (46)	1.00	1.00
A1/A2	52 (47)	43 (42)	1.10 (0.63–1.90)	0.97 (0.45–2.20)
A2/A2	8 (7)	12 (12)	0.60 (0.23–1.60)	0.45 (0.13–1.60)

[a] Adjusted for age, education, family history of breast cancer, body mass index, and history of benign breast disease (and age at menopause in postmenopausal women). CI, confidence interval.

Table 3

Relationships between breast cancer and putative risk factors by *CYP17* genotype: Western New York Breast Cancer Study

	All data			A1/A1			A1/A2 and A2/A2		
	Case (n [%])	Control (n [%])	Odds ratio (95% CI)[a]	Case (n [%])	Control (n [%])	Odds ratio (95% CI)[a]	Case (n [%])	Control (n [%])	Odds ratio (95% CI)[a]
Premenopausal									
Age at menarche									
< 13 years	47 (50)	33 (39)	1.00	30 (52)	15 (31)	1.00	17 (46)	18 (49)	1.00
≥ 13 years	48 (51)	52 (61)	0.55 (0.27–1.10)	28 (48)	33 (69)	0.37 (0.14–0.99)	20 (54)	19 (51)	0.84 (0.27–2.61)
Age at first birth									
< 25 years	55 (57)	66 (77)	1.00	34 (59)	38 (79)	1.00	21 (55)	28 (74)	1.00
≥ 25 years	41 (43)	20 (23)	2.69 (1.34–5.39)	24 (41)	10 (21)	4.30 (1.46–12.67)	17 (45)	10 (26)	2.13 (0.76–6.02)
Difficulty getting pregnant									
No	82 (79)	79 (81)	1.00	40 (71)	38 (79)	1.00	33 (89)	29 (76)	1.00
Yes	22 (21)	19 (19)	0.97 (0.44–2.14)	16 (29)	10 (21)	2.23 (0.69–7.24)	4 (11)	9 (24)	0.38 (0.10–1.52)
Ever use oral contraceptives									
No	44 (46)	45 (52)	1.00	24 (41)	26 (54)	1.00	20 (53)	19 (50)	1.00
Yes	52 (54)	41 (48)	1.77 (0.84–3.74)	34 (59)	22 (46)	3.24 (1.08–9.73)	18 (47)	19 (50)	0.67 (0.20–2.26)
Postmenopausal									
Age at menarche									
< 13 years	56 (50)	43 (42)	1.00	25 (48)	18 (38)	1.00	31 (52)	25 (46)	1.00
≥ 13 years	56 (50)	59 (58)	0.78 (0.42–1.48)	27 (52)	29 (62)	0.72 (0.27–1.91)	29 (48)	30 (55)	0.80 (0.33–1.93)
Age at first birth									
< 25 years	60 (54)	61 (60)	1.00	29 (57)	21 (48)	1.00	31 (52)	40 (73)	1.00
≥ 25 years	51 (46)	41 (40)	1.52 (0.83–2.77)	22 (43)	26 (55)	0.68 (0.27–1.68)	29 (48)	15 (27)	2.90 (1.30–6.97)
Difficulty getting pregnant									
No	82 (79)	79 (81)	1.00	42 (86)	32 (74)	1.00	40 (73)	47 (85)	1.00
Yes	22 (21)	19 (19)	1.19 (0.58–2.41)	7 (14)	11 (26)	0.47 (0.14–1.50)	15 (27)	8 (15)	2.59 (0.91–7.38)
Ever use hormone replacement therapy									
No	89 (80)	71 (70)	1.00	40 (77)	32 (68)	1.00	49 (82)	39 (71)	1.00
Yes	23 (20)	31 (30)	0.94 (0.31–2.78)	12 (23)	15 (32)	0.80 (0.14–4.52)	11 (18)	16 (29)	0.98 (0.23–4.26)
Age at menopause									
< 48 years	58 (47)	58 (57)	1.00	24 (47)	29 (62)	1.00	29 (48)	29 (53)	1.00
≥ 48 years	58 (52)	44 (43)	1.60 (0.91–2.84)	27 (53)	18 (38)	1.98 (0.84–4.68)	31 (52)	26 (47)	1.36 (0.61–3.06)

[a] Adjusted for other factors in the table as well as age, education, family history of breast cancer, history of benign breast disease, and body mass index. CI, confidence interval.

acteristics associated with greater fertility. They were more likely to have an earlier age at menarche, less difficulty becoming pregnant, an earlier age at first full-term pregnancy, and later age at menopause.

In accordance with previous studies [4,9,12,16], later age at menarche was protective against breast cancer only among women homozygous for the A1 allele, although we noted this association only in premenopausal women. Furthermore, late age at first full-term pregnancy and oral contraceptive use increased risk only among premenopausal women homozygous for A1 alleles. Relationships were not as notable among postmenopausal women, although late age at first birth increased risk only among women with A2 alleles. These apparent discrepancies by menopausal status, although they may be due to chance, could reflect differences in the nature of premenopausal and postmenopausal breast cancer etiology, particularly in relation to steroid hormones.

The initial report of increased risk of breast cancer and modification of other risk factors by variability in *CYP17* was promising for breast cancer research [1]. Steroid hormones clearly play a large role in breast cancer etiology, and identification of risk associated with genetic differences in their biosynthesis and metabolism could greatly elucidate mechanistic pathways and make inroads towards prevention for public health. However, this report was followed by a number of studies that did not corroborate those early results. As more studies have been conducted, relationships between the *CYP17* genotype and breast cancer risk have become clearer, particularly through the repeated findings of modification of risk through other reproductive factors. Because two studies have noted higher estrogen levels among women with A2 alleles [4,23], it is possible that women with these genotypes have more lifetime exposure to circulating steroid hormones that is not abrogated by later age at menarche or by earlier age at first full-term pregnancy. Oral contraceptive use may similarly only impact risk in an environment of lower estrogens.

Experimental studies [21,22] have shown that the *CYP17* polymorphism in the 5′-flanking region of the gene does not influence binding to Sp1, as previously conjectured [1,20]. Nonetheless, studies in both premenopausal women and postmenopausal women have found that those with A2 alleles have higher circulating levels of steroid hormones. Thus, while the mechanism whereby the *CYP17* polymorphism increases serum hormones levels has not been identified, it is probable that there are either effects that have not yet been elucidated or that the site is in linkage disequilibrium with another polymorphism that alters the function of the *CYP17* enzyme.

Results from this study may be impacted by a number of factors, foremost of which may be small sample size.

While we had data on 395 women, stratification by menopausal status and additional stratification by *CYP17* genotype to evaluate differential effects of breast cancer risk factors resulted in small data points in some cells, and therefore results are interpreted cautiously. Nonetheless, results support findings from a number of studies and are consistent with findings from the two largest studies to date: those from the Nurses' Health Study [4] and the study of breast cancer in Finland [9]. In addition, our findings are in accordance with those of a meta-analysis of 15 case–control studies [16].

Furthermore, genotype frequencies among premenopausal women deviated slightly from Hardy–Weinberg equilibrium, although these differences were not statistically significant. Previous studies have shown a range of genotype distributions. In a recent meta-analysis by Ye and Parry [16], the A2/A2 genotype prevalence ranged from 7.7 to 23.4% among Caucasian women in 11 different studies. This variability in estimates could be due, in part, to small sample sizes. In the present study, we found that 11% of premenopausal controls and 12% of postmenopausal controls had the A2/A2 genotype, which falls well within this range. However, the prevalence of heterozygotes was higher in our population than in other studies. It is unlikely that this could be attributed to population stratification, since western New York is a fairly homogeneous area, and women were all of European descent and, for the most part, at least second-generation or third-generation Americans. Another possible explanation for the higher proportion of heterozygotes could be genotyping errors, although two independent investigators read the gels and at least 20% of the samples were repeated for quality control. Furthermore, assays were validated by confirmation of polymorphic Mendelian inheritance patterns in seven human family cell lines ($n = 134$), each encompassing three generations.

In summary, our findings support those of several other studies that variant *CYP17* alleles modify breast cancer risk associated with several hormonal and reproductive factors. These collective findings may point the way toward further investigations into the role of metabolic variability in steroidogenesis and metabolism in relation to breast cancer risk, and target women who may be most at risk for the disease.

Competing interests
None declared.

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