CYP17 Genotype and Breast Cancer Risk[1]

Ainsley Weston,[2] Ching-fei Pan, Ira J. Bleiweiss,
H. Barbara Ksieski, Noah Roy, Nell Maloney, and
Mary S. Wolff

National Institute for Occupational Safety and Health, Morgantown, West
Virginia 26505 [A. W.], and Mount Sinai Medical Center, New York, New
York 10029 [C-f. P., I. J. B., H. B. K., N. R., N. M., M. S. W.]

Abstract

**The *MspAI* polymorphism in the 5′ untranslated region
of *CYP17* has been evaluated as a breast cancer risk
factor in a hospital-based case-control study in New York
City. The study population consisted of 363 women [123
breast cancer patients and 240 patient controls (123
benign breast disease without atypical hyperplasia, 117
women without breast disease)]. There were 224
Caucasians (76 cases, 148 controls), 55 African-Americans
(20 cases, 35 controls) and 84 Hispanics (27 cases, 57
controls); 142 premenopausal women and 221
postmenopausal women. Consistent with a previous
report (Feigelson *et al.*, Cancer Res., *57:* 1063–1065, 1997)
we found no evidence to implicate the minor variant
(restriction site present allele, designated *A2*) as a breast
cancer risk factor. Furthermore, we sought evidence to
implicate the minor variant of *CYP17* in the development
of more aggressive breast cancers ($n = 38/121$) as had
been reported previously. Although confidence intervals
(CI) overlap, the data presented here do not provide
support for previously reported findings (odds ratio, 0.9;
95% CI, 0.4–2.0; $n = 38$ *versus* odds ratio, 2.5; 95% CI,
1.1–5.2; $n = 40$). Clearly this question needs to be
resolved in a larger study. No evidence was found to
support the contention that inheritance of the minor
variant is a predictor of early age at menarche. Allelic
frequencies between different ethnic groups were not
found to be different with the exception of Hispanic
controls, in which the genotypic distribution was not
consistent with the Hardy-Weinberg equilibrium.**

Introduction

Epidemiological studies have provided a plausible basis for an
association between breast cancer risk and steroid/estrogen or
xenoestrogen exposure (1, 2). A recent report (3) further con-
sidered the possibility that a common polymorphism in *CYP17*,
a 17α-steroid hydroxylase and 17/20-lyase, is a determinant of
human breast cancer risk. The minor variant (*A2*) differs from
the major variant (*A1*) by one nucleotide in the 5′ untranslated
region (T↔C at position 1931, Ref. 4). It was reasoned that the
C-allele may have enhanced transcriptional activity because of
the creation of an Sp-1-type promotor motif. Further, because
CYP17 has a fundamental role in steroid metabolism, it was
further reasoned that this allele may be associated with in-
creased breast cancer risk. In that study, three ethnic groups
were analyzed: African-Americans, Latinos, and Asians. Be-
cause the genotypic distributions were similar in each ethnic
group (~35:50:15), data were combined for analysis (3). It was
reported that the *A2* allele was over-represented in women with
a diagnosis of advanced breast cancer (*A1* homozygotes *versus*
A2 homozygotes and heterozygotes; OR,[3] 2.5; 95% CI, 1.1–
5.9). Furthermore, it was observed that among controls, *A1*
homozygotes were likely to have later age at menarche. In this
study, we report the results of a hospital-based case-control
study of breast cancer in which the *CYP17* genotypes of the
participants have been determined. The current study focuses
on Caucasians, African-Americans, and Latinos recruited in
New York City.

Materials and Methods

Human Samples. The design and recruitment of the breast
cancer case-control study has been described previously (4).
Briefly, women were enrolled at the Mount Sinai Medical
Center (New York) between September 1994 and February
1996. All of the cases were approached before, or within 2
months after, diagnostic biopsy. The participation rate (64.5%)
was comparable in cases and controls and among ethnic groups.
Samples were frequency-matched on age, race, and diagnosis;
and genotypes were determined for 363 women. The case group
was matched to each of two separate control groups (benign
breast disease without atypia and women without breast dis-
ease). Ethnicity was self-described, and the study included 224
Caucasians (76 cases, 148 controls), 55 African-Americans (20
cases, 35 controls), and 84 Hispanics (27 cases, 57 controls).
Menstrual history was determined by questionnaire. For 121
breast cancer cases in which full histopathology was available,
classification of tumors as aggressive or nonaggressive was
possible. Women presenting with stage-3 or -4 disease and who
had undergone chemotherapy without lymph node dissection
were considered to have aggressive tumors. Infiltrating carci-
nomas with lymph node involvement or lymphatic invasion
were also designated as aggressive. Small invasive carcinomas
with favorable histology and ductal carcinoma *in situ* (in which
lymph nodes were not dissected) were regarded as nonaggres-
sive.

***CYP17* Genotype.** *CYP17* genotype was determined exactly as
described (3). However, several technical discrepancies should
be noted. The original report describing this polymorphism and

[3] The abbreviations used are: OR, odds ratio; CI, confidence interval; df, de-
gree(s) of freedom.
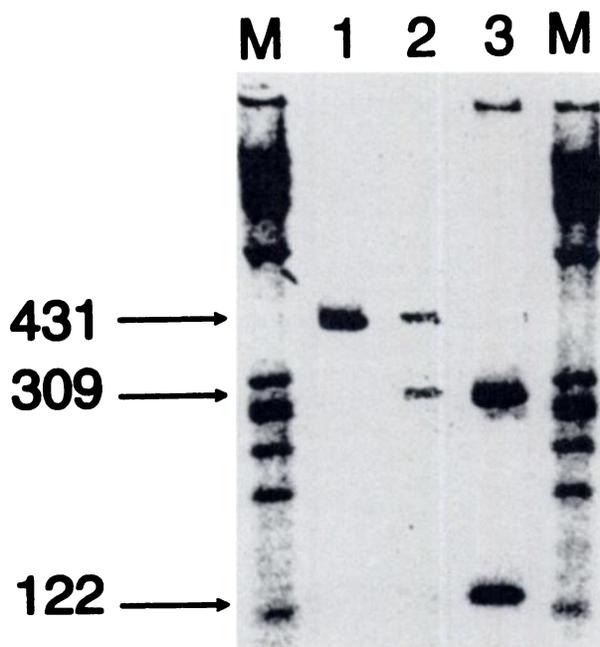
M   1   2   3   M

431 ——→

309 ——→

122 ——→

Fig. 1. Amplification (PCR) and restriction digestion analysis of three constitutive genomic DNA samples derived from peripheral blood donated by participants in a breast cancer case-control study. A molecular weight marker (ϕX174/HaeIII; band sizes 1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118, and 72 bp) is contained in *Lanes* marked *M*. The *A1* homozygote is in *Lane 1* (431-bp band); the *A2* homozygote is in *Lane 3* (122- and 309-bp bands); and the heterozygote is in *Lane 2* (122-, 309-, and 431-bp bands).

Table 1   Age in years at menarche in each study group

Study group	n^a	Mean ± SD	Range
All breast cancer	111	12.6 ± 1.9	8–20
All controls	219	12.6 ± 1.6	9–19
Caucasian women			
Breast cancer	69	12.4 ± 1.4	9–16
Controls	141	12.5 ± 1.5	9–18
African-American women			
Breast cancer	18	12.6 ± 2.0	9–16
Controls	32	12.4 ± 1.8	9–17
Hispanic women			
Breast cancer	24	13.1 ± 2.9	8–20
Controls	46	12.9 ± 1.9	9–19

^{a} *n*, number of women for whom age at menarche was available.

Table 2   *CYP17* genotype and allele frequencies for breast cancer cases and controls

Study group	n^a	A1/A1	A1/A2	A2/A2	F
All breast cancer	123	45 (0.37)	57 (0.46)	21 (0.17)	0.60
All controls	240	92 (0.38)	111 (0.46)	37 (0.15)	0.61
Caucasian women					
Breast cancer	76	29 (0.38)	35 (0.46)	12 (0.16)	0.61
Controls	148	49 (0.33)	74 (0.50)	25 (0.17)	0.58
African-American women					
Breast cancer	20	7 (0.35)	10 (0.50)	3 (0.15)	0.60
Controls	35	15 (0.43)	18 (0.51)	2 (0.06)	0.69
Hispanic women					
Breast cancer	27	9 (0.33)	12 (0.44)	6 (0.22)	0.56
Controls	57	28 (0.49)	19 (0.33)	10 (0.18)	0.66

^{a} *n*, all study subjects; F, frequency of *A1* allele.

associated polycystic ovaries and male-pattern baldness (5) indicated primer sequences exactly consistent with those published by Picado-Leonard and Miller (6), whereas the report of Feigelson *et al.* (3) did not. The amplicon and restriction fragment sizes were not entirely concordant with those reported by Picado-Leonard and Miller (6) but were consistent with Brentano *et al.* (7). Consequently, amplification of constitutive human genomic DNA with primer sequences documented by Feigelson *et al.* (3) gave a product of 431 bp, which was cleaved into fragments of 122 and 309 bp by *MspA1* if the restriction site was present. The molecular weight marker (ϕX174/HaeIII) was obtained from New England Biolabs (Beverly, MA).

Statistical Analysis. Gene frequencies were calculated for breast cancer cases and combined controls in three ethnic groups. The statistical methods used were Student's *t* test, logistic regression, contingency table χ^2 and, for 2-×-2 tables, Fisher's exact test. Calculations were performed using the JMP software (SAS Institute).

Results

The results of PCR amplification and restriction digestion of constitutive genomic DNA for three representative samples are given in Fig. 1. Constitutive genomic DNA samples derived from peripheral blood of 363 women were analyzed by this method (123 breast cancer patient cases, 123 benign-breast-disease patient controls, and 117 women without breast disease; 224 Caucasians, 55 African-Americans, and 84 Hispanics; 142 premenopausal women and 221 postmenopausal women).

There was no difference between cases and controls for age at menarche, when considered as a continuous variable

(mean age in years ± SD) or as a categorical variable (12 years and under *versus* 13 years and over). This was true whether the study subjects were considered altogether (12.6 ± 1.9 for breast cancer patient cases and 12.6 ± 1.6 for patient controls; OR, 1.15; 95% CI, 0.73–1.81; *n* = 330) or separately by race/ethnicity (Table 1). However, mean age at menarche varied by race when all of the Caucasians, all of the African-Americans, and all of the Hispanics were compared, irrespective of diagnosis. Mean age at menarche was significantly higher in Hispanics [13.1 ± 2.9 for patient cases (*n* = 24) and 12.9 ± 1.9 for control subjects (*n* = 46)] than Caucasians [12.4 ± 1.4 for patient cases (*n* = 69) and 12.5 ± 1.5 for control subjects (*n* = 141); *t* test = 2.25; *P* < 0.025; *df*, 278]. Mean age at menarche for African-Americans was similar to Caucasians [12.6 ± 2.0 for patient cases (*n* = 18) and 12.4 ± 1.8 for patient controls (*n* = 32)], but was not significantly different from Hispanics (Student's *t* = 1.23; *P* > 0.22; *df*, 118). No effect was observed when the study group was stratified on menopausal status (data not shown).

The genotypic distributions were found to conform to Hardy-Weinberg equilibrium laws with the exception of the Hispanic control group, in which there was a marginal excess of homozygotes (*P* = 0.0501, *n* = 57; Table 2). The allelic frequency in Caucasians (*A1* = 0.58, patient controls) was not significantly different from those reported by Feigelson *et al.* (3) for Asian controls (0.54), African-American controls (0.63), and Latino controls (0.56; Table 2). Genotypic distributions were also similar (Table 2). Considered separately by race/ethnicity, marginal differences were found between the allelic frequencies. The genotypic distribution in Hispanic controls in

Table 3 χ^2 values and Ps for association of the $A2$ allele with breast cancer diagnosis

Study group	n (cases/controls)	OR	95% CI	χ^2	P
All study subjects	123/240	1.08	0.69–1.69	0.11	0.75
Caucasian women	76/148	0.80	0.45–1.43	0.56	0.45
African-American women	20/35	1.40	0.44–4.38	0.33	0.57
Hispanic women	27/57	1.93	0.75–5.01	1.85	0.17

Table 4   Distribution of CYP17 genotypes in breast cancer cases and aggressive disease

Study group	$A1/A1$	$A1/A2:A2/A2$	OR (±95% CI)
Caucasian			
Aggressive[a]	6	15	1.7 (0.6–5.1)
Nonaggressive	22	32	
African-American			
Aggressive	3	4	0.6 (0.1–4.0)
Nonaggressive	4	9	
Hispanic			
Aggressive	5	5	0.2 (0.0–1.3)
Nonaggressive	3	13	
Combined			
Aggressive	14	24	0.9 (0.4–2.0)
Nonaggressive	29	54	

[a] Stage 3 or stage 4 tumors without lymph node dissection and cases of infiltrating carcinoma with lymph node involvement were designated as aggressive. Small invasive carcinomas with favorable histology and ductal carcinoma in situ were regarded as nonaggressive.

our study was different from that reported previously by Fiegelson et al. (Ref. 3; χ^2 = 6.3; P = 0.044; df, 1). However, numbers in the current study were small (n = 57). It should also be noted that the ratio of $A1$ homozygotes:$A2$ homozygotes and heterozygotes combined, for all controls, was also different between the two studies (χ^2 = 5.5, P = 0.019). However, the overall allelic frequencies were not different between the two studies (Table 2; χ^2 = 0.5, P = 0.489).

There was no association of the $A2$ allele with breast cancer in any ethnic/racial group or with the entire study group combined (Table 3). Pre- and postmenopausal status did not affect the results of these analyses (data not shown). Neither was any evidence found to support the previous report that the minor ($A2$) variant was overrepresented in more aggressive breast cancers (Table 4).

There were no significant differences in age at menarche for $A1$ homozygotes and women with an $A2$ allele, this finding was independent of race/ethnicity and case status (Table 5). However, age at menarche tended to be later among African-American and Hispanic women with an $A2$ allele. When categorical data (age, >13 years) were considered for Caucasians and the group as a whole, a nonsignificant protective effect of later age at menarche was seen in $A1$ homozygotes (OR, 0.6; 95% CI, 0.2–1.5 and OR, 0.6; 95% CI, 0.3–1.3, respectively). No such protective effect was seen in the combined $A1/A2$ + $A2/A2$ groups (OR, 1.8; 95% CI, 0.9–3.8 and OR, 1.7; 95% CI, 0.9–3.0; Table 5).

Discussion

In this study, we present genotype data for $CYP17$ obtained from Caucasian, African-American, and Hispanic women in breast cancer cases and controls. This study was prompted by

Table 5   Comparison of age at menarche with genotype for each study group[a]

Study group	$A1/A1^b$ (n)	$A1/A2 + A2/A2^b$ (n)	P
All study subjects			
Cases	12.1 ± 1.5 (42)	12.8 ± 2.1 (69)	0.07
Controls	12.6 ± 1.7 (80)	12.5 ± 1.6 (139)	0.67
Combined	12.5 ± 1.6 (122)	12.6 ± 1.8 (208)	
OR [95% CI][c]	0.6 [0.3–1.3]	1.7 [0.9–3.0]	
Caucasian women			
Cases	12.3 ± 1.3 (27)	12.5 ± 1.6 (42)	0.54
Controls	12.6 ± 1.7 (47)	12.5 ± 1.4 (94)	0.63
OR [95% CI][c]	0.6 [0.2–1.5]	1.8 [0.9–3.8]	
African-American women			
Cases	11.9 ± 2.0 (7)	13.1 ± 1.9 (11)	0.21
Controls	13.1 ± 1.6 (12)	12.1 ± 1.9 (20)	0.12
Hispanic women			
Cases	12.0 ± 1.9 (8)	13.6 ± 3.1 (16)	0.21
Controls	12.5 ± 1.8 (21)	13.3 ± 2.0 (25)	0.19

[a] Calculated for women for whom age at menarche was available.
[b] Mean age in years ±SD.
[c] OR and 95% CI for a protective effect of later-onset menarche from categorical data (age >13 years).

a recent report indicating that the inheritance of the $MspA1$ restriction site-present allele was a risk factor for aggressive forms of breast cancer and a predictor of early onset of menarche (3). Like the earlier report, no evidence was found to implicate the $MspA1$ polymorphism in breast cancer risk, but we did not find an association between the minor $CYP17$ variant ($A2$) and early age at menarche.

The main issue under investigation was the previously reported association of genotype with aggressive forms of breast cancer as defined by histological and invasive criteria (3). Feigelson et al. (3) observed that the $A2$ allele was overrepresented in a small group of racially mixed cases with aggressive breast cancer (OR, 2.5; 95% CI, 1.1–5.2; n = 40). This subgroup represented only 22% of cases in the previous study; therefore, data were pooled between ethnic groups. Our study suffers from the same subgroup deficiency where 30% of breast cancers met the same histological and invasive criteria. Although the total sample numbers are similar and the CIs for the ORs overlap, our data are not suggestive of an association of $A2$ with aggressive disease. Differences between our results and those reported by Feigelson et al. (3) could be due to racial/ethnic factors inasmuch as the current study focuses primarily on Caucasians, whereas that of Feigelson et al. examined African-American subjects, Latino subjects, and Asian subjects. Clearly this question needs to be examined in more detail; others have also indicated a lack of association of $A2$ with disease outcome (8).

Despite the fact that in the current study, it was necessary to pool data across ethnic backgrounds to readdress the question of an association between $CYP17$ and aggressive breast cancer and although the frequency of this polymorphism has not thus far been found to vary with race, other ethnic factors (genetic and environmental) are dissimilar. In our sample, there was a significant difference in age at menarche between Caucasians and Hispanics for example. In light of this and known gene-gene interactions (9) and gene-environment interactions (10), data from different ethnic groups should clearly be considered separately.

References

1. Wolff, M. S., and Weston, A. Breast cancer risk and environmental exposures. Environ. Health Perspect., *105* (Supp. 4): 891–896, 1997.

2. Wolff, M. S., Toniolo, P., Lee, E. W., and Rivera, M. Blood levels of organochlorine residues and risk of breast cancer. J. Natl. Cancer Inst., *85:* 648–652, 1993.

3. Feigelson, H. S., Coetzee, G. A., Kolonel, L. N., Ross, R. K., and Henderson, B. E. A polymorphism in the *CYP17* gene increases the risk of breast cancer. Cancer Res., *57:* 1063–1065, 1997.

4. Weston, A., Pan, C-f., Ksieski, H. B., Wallenstein, S., Berkowitz, G. S., Tartter, P. I., Bleiweiss, I. J., Brower, S. T., Senie, R. T., and Wolff, M. S. *p53* haplotype determination in breast cancer. Cancer Epidemiol. Biomark. Prev., *6:* 105–112, 1997.

5. Carey, A. H., Waterworth, D., Patel, K., White, D., Little, J., Novelli, P., Franks, S. And Williamson, R. Polycystic ovaries and premature male pattern baldness are associated with one allele of the steroid metabolism gene *CYP17.* Hum. Mol. Genet., *3:* 1873–1876, 1994.

6. Picado-Leonard, J., and Miller, W. L. Cloning and sequencing of the human gene for P450c17 (steroid 17 α-hydroxylase/17,20 lyase): similarity with the gene for P450c21. DNA, *6:* 439–448, 1987.

7. Brentano, S. T., Picado-Leonard, J., Mellon, S. H., Moore, C. C. D., and Miller, W. L. Tissue-specific cyclic adenosine 3′,5′-monophosphate-induced and phorbol ester-repressed transcription from the human *P450c17* promotor in mouse cells. Mol. Endocrinol., *4:* 1972–1979, 1990.

8. Dunning, A. M., Healey, C. S., Pharoah, P. D. P., Foster, N. A., Lipscombe, J. M., Redman, K. L., Easton, D. F., Day, N. E., and Ponder, B. A. J. No association between a polymorphism in the steroid metabolism gene *CYP17* and risk of breast cancer. Br. J. Cancer, *77:* 2045–2047, 1998.

9. Phelan, C. M., Rebbeck, T. R., Weber, B. L., Devilee, P., Ruttledge, M. H., Lynch, H. T., Lenoir, G. M., Stratton, M. R., Easton, D. F., Ponder, B. A. J., Cannon-Albright, L., Larsson, C., Goldgar, D. E., and Narod, S. A. Ovarian cancer risk in *BRCA-1* carriers is modified by the *HRAS-1* variable number of tandem repeat (VNTR) locus. Nat. Gen., *12:* 309–311, 1996.

10. Kawajiri, K., Nakachi, K., Imai, K., Watanabi, J., and Hayashi, S. Germ line polymorphisms of *p53* and *CYP1A1* genes involved in human lung cancer. Carcinogenesis (Lond.), *14:* 1085–1089, 1993.

Cancer Epidemiology, Biomarkers & Prevention

AACR American Association for Cancer Research

CYP17 genotype and breast cancer risk.

A Weston, C F Pan, I J Bleiweiss, et al.

Updated version	Access the most recent version of this article at: http://cebp.aacrjournals.org/content/7/10/941

E-mail alerts	Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions	To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions	To request permission to re-use all or part of this article, use this link http://cebp.aacrjournals.org/content/7/10/941. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.