Null Results in Brief

No Association of Polymorphisms in *CYP17*, *CYP19*, and *HSD17-B1* with Plasma Estradiol Concentrations in 1,090 British Women

Ruth C. Travis,[1] Michael Churchman,[2] Sarah A. Edwards,[2] Gillian Smith,[3] Pia K. Verkasalo,[4] C. Roland Wolf,[3] Helga Wolf,[3] and Timothy J. Key[1]

[1]Cancer Research UK Epidemiology Unit and [2]Cancer Research UK Genotyping Facility, Department of Clinical Pharmacology, University of Oxford, Oxford, United Kingdom; [3]Cancer Research UK Molecular Pharmacology Unit, Biomedical Research Centre, Ninewells Hospital and Medical School, Dundee, United Kingdom; and [4]Unit of Environmental Epidemiology, Department of Environmental Health, National Public Health Institute, Kuopio, Finland

Introduction

The production of endogenous estradiol, and thus the risk of breast cancer, may be affected by functional polymorphisms in the genes coding for enzymes in the steroid biosynthesis pathway. Potentially important polymorphisms have been identified in three genes: *CYP17*, which encodes for a P450 enzyme that catalyzes 17-hydroxylase and C_{17-20} lyase activities in the biosynthesis of androgens; *CYP19*, which encodes for aromatase, the enzyme involved in the conversion of androgens to estrogens; and *HSD17-B1*, which encodes for the 17-hydroxysteroid oxidoreductase type 1 enzyme that reduces estrone to estradiol. Several studies have observed an association between risk for breast cancer and these polymorphisms (1). However, other studies have not found such an association, and the biological relationship of these polymorphisms with circulating estrogens has yet to be established. We assessed whether polymorphisms in *CYP17*, *CYP19*, or *HSD17-B1* are associated with circulating levels of estradiol, the most biologically potent estrogen. Specifically, we examined a T to C substitution 34 bp upstream of the translation initiation site in the 5′ untranslated region of *CYP17* among 634 premenopausal and 455 postmenopausal British women. Among the postmenopausal women, we also examined a $[TTTA]_n$ repeat allele beginning at bp 682 (intron 4) of *CYP19*, an associated TCT insertion/deletion polymorphism, a C to T substitution in the untranslated region of exon 10 of *CYP19*, and an A to G substitution in exon 6 of *HSD17-B1* 1,954 bp downstream of the first nucleotide in the translation initiation codon.

Cancer Epidemiol Biomarkers Prev 2004;13(12):2282–4

Received 1/29/04; revised 4/29/04; accepted 5/20/04.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Ruth C. Travis, Cancer Research UK Epidemiology Unit, University of Oxford, Oxford OX2 6HE, United Kingdom. Phone: 44-1865-302211; Fax: 44-1865-310545. E-mail: ruth.travis@cancer.org.uk

Materials and Methods

Study participants included 636 premenopausal and 456 postmenopausal British women recruited to the Oxford arm of the European Prospective Investigation into Cancer and Nutrition and previously studied in a cross-sectional analysis of sex hormones and risk factors for breast cancer (2, 3). Details of the estradiol assays are described elsewhere (2). One postmenopausal and two premenopausal women were excluded from the current study due to insufficient buffy coat being available for molecular analyses.

Genotyping on DNA extracted from buffy coat samples was conducted blind after the hormone assays had been completed. Eight hundred and ninety-eight samples were genotyped at the Cancer Research UK Genotyping Facility in Oxford. Blinded quality control samples (3.4%) were inserted to validate genotype identification procedures: concordance for the blinded samples was 100% for the *CYP17* assay, 93.3% for the *CYP19* intron 4 assay, 100% for the *CYP19* exon 10 assay, and 100% for the *HSD17-B1* assay. One hundred and ninety-eight samples were genotyped for the *CYP17* single nucleotide polymorphism at the Biomedical Research Centre in Dundee as part of a previous unpublished study using similar methods. Genotype frequencies from the two laboratories were statistically similar: *CYP17* TT, TC, and CC genotype frequencies were 40.0%, 45.9%, and 14.1%, respectively, for samples assayed in Oxford and 38.4%, 47.0%, and 14.7%, respectively, for samples assayed in Dundee ($\chi^2 = 0.18$, $P = 0.9$). Seven samples were genotyped for *CYP17* at both laboratories and there was 100% concordance between the two sets of results.

Estradiol values were logarithmically transformed to reduce the positive skewness of the distributions, and geometric mean hormone values and the corresponding 95% confidence intervals are presented. ANOVA was used to evaluate the association between genotypes and estradiol concentrations. Estradiol concentrations in postmenopausal women, classified by their number of putative high-risk alleles, including single nucleotide polymorphisms in *CYP17*, *CYP19* exon 10, and *HSD17-B1*

Table 1. Multivariable-adjusted geometric mean hormone levels by *CYP17*, *CYP19*, and *HSD17-B1* genotypes

Menopausal status		*n* (%)	Estradiol (pmol/L), mean (95% confidence interval)	*P* for heterogeneity/trend
Premenopausal*				
CYP17	TT	204 (38.9)	362 (338-387)	0.6/0.7
	TC	244 (46.6)	346 (326-368)	
	CC	76 (14.5)	359 (321-401)	
Postmenopausal†				
CYP17	TT	174 (42.2)	18.1 (16.8-19.6)	0.7/0.5
	TC	181 (43.9)	18.3 (16.9-19.7)	
	CC	57 (13.8)	19.4 (16.9-22.1)	
CYP19	7/7	73 (21.0)	18.9 (16.9-21.1)	0.9/9
	7/8	38 (10.9)	17.9 (15.3-21.0)	
	7/11	125 (35.9)	17.5 (16.1-19.1)	
	8/11	19 (5.5)	18.8 (15.1-23.5)	
	11/11	53 (15.2)	17.5 (15.3-20.0)	
	Other	40 (11.5)	19.0 (16.3-22.2)	
CYP19 TCT	Ins/Ins	158 (45.4)	18.2 (16.9-19.7)	0.8/1.0
	Ins/Del	154 (44.3)	17.7 (16.4-19.2)	
	Del/Del	36 (10.3)	18.9 (16.0-22.2)	
CYP19 exon 10	CC	76 (19.6)	18.8 (16.8-21.1)	0.7/0.4
	CT	196 (50.5)	18.2 (17.0-19.5)	
	TT	116 (29.9)	17.8 (16.3-19.6)	
HSD17-B1	GG	68 (51.7)	18.3 (16.2-20.7)	0.9/0.8
	AG	193 (18.2)	18.1 (16.9-19.5)	
	AA	112 (30.0)	18.6 (16.9-20.4)	
Postmenopausal†				
No. high-risk alleles‡	0	3 (0.9)	20.6 (11.7-36.2)	1.0/0.6
	1	38 (10.8)	18.3 (15.6-21.4)	
	2	89 (25.3)	17.8 (16.0-19.8)	
	3	106 (30.1)	17.5 (15.9-19.2)	
	4	79 (22.4)	18.8 (16.8-21.0)	
	5	30 (8.5)	18.9 (15.8-22.5)	
	6	7 (2.0)	19.5 (13.5-28.3)	

*Adjusted for age group, body mass index, day of cycle (grouped), hours since last meal, smoking, vigorous exercise, and alcohol consumption.
†Adjusted for age group, body mass index, vigorous exercise, and days blood sample was in the post.
‡No. high-risk alleles from *CYP17* (C allele), *CYP19* exon 10 (T allele), and *HSD17-B1* (A allele).

were also examined. Statistical analyses were repeated with adjustment for potential confounders including age (5-year age groups), body mass index [calculated as weight/height^2 (kg/m^2) and grouped <20, 20<22.5, 22.5<25, 25<27.5, ≥27.5], vigorous exercise (0, 1-2, 3-4, ≥5 hours per week), and number of days the blood sample was in the post (0-1, 2, ≥3 days; among postmenopausal women only). In the analyses of data from premenopausal women, adjustment was also made for stage of menstrual cycle (early follicular, late follicular, mid-cycle, early luteal, and late luteal defined as ≥22, 16-21, 12-15, 3-11, and 0-2 days before next menstrual period), smoking status (never, former, current), alcohol consumption (<0.50, 0.50<8.0, 8.0<16.0, ≥16.0 g/d), and hours since last meal (0-1, 2, 3-4, ≥5 hours).

Results

There was no statistically significant variation in plasma estradiol concentrations by *CYP17* genotype in premenopausal or postmenopausal women, either before or after adjustment for potential confounders. Table 1 shows the results from the multivariable model. We also found no association between *CYP19* or *HSD17-B1* variants and estradiol concentrations among postmenopausal women. When subjects were classified according to the number of putative high-risk alleles in *CYP17*, *CYP19* exon 10, and *HSD17-B1*, we did not observe a significant dose-response relationship between estradiol concentrations and number of high-risk alleles, nor did we observe significant heterogeneity in estradiol concentrations by the number of high-risk alleles either before or after adjustment for potential confounders.

Discussion

The results of this study suggest that there is no association between plasma estradiol concentrations and a polymorphism in *CYP17* in either premenopausal or postmenopausal women, in contrast with the findings of two early studies that found the same polymorphism to be significantly associated with estradiol concentrations (4, 5) but in agreement with several more recent investigations (6-10).

Aromatase, encoded by *CYP19*, catalyzes the last stage in estrogen production; therefore, the effects of variants of this gene are of particular interest in relation to plasma estradiol concentrations. Previous studies have found elevated estradiol concentrations in carriers of the *CYP19* 8-repeat allele (10, 11) and the longer repeat alleles (12 and 13 repeats; ref. 12) compared with noncarriers, and reduced estradiol levels in carriers of the 7-repeat allele

(11) and the 3-bp deletion polymorphism (10), although associations reached statistical significance in only one study (10). Our results, however, suggest no association between these polymorphisms in *CYP19* and plasma estradiol concentrations in postmenopausal women. Our null findings for the *CYP19* exon 10 polymorphism in relation to estradiol concentrations are consistent with results from the only other study of this variant and estradiol (13).

Polymorphisms in the gene *HSD17-B1* may also be important given the role of 17-hydroxysteroid oxidoreductase type 1 in the reduction of estrone to the more biological potent estradiol, yet to the authors' knowledge, no previously published studies have investigated polymorphisms in this gene in relation to estradiol concentrations. We found no association between a polymorphism in this gene and estradiol concentrations in postmenopausal women. We also found no evidence that the effects of individual polymorphisms in genes involved in steroid biosynthesis were cumulative as has been suggested previously (14).

This study had >80% power at a 0.05 significance level to detect differences of 25% in plasma concentrations between women with different genotypes. The results suggest that there is no large effect of the polymorphisms studied in *CYP17*, *CYP19*, and *HSD17-B1* on endogenous estradiol concentrations; however, more data would be needed to establish whether or not there is a small effect.

References

1. Dunning AM, Healey CS, Pharoah PD, Teare MD, Ponder BA, Easton DF. A systematic review of genetic polymorphisms and breast cancer risk. Cancer Epidemiol Biomarkers Prev 1999;8:843–54.
2. Thomas HV, Davey GK, Key TJ. Oestradiol and sex hormone-binding globulin in premenopausal and post-menopausal meat-eaters, vegetarians and vegans. Br J Cancer 1999;80:1470–5.
3. Verkasalo PK, Thomas HV, Appleby PN, Davey GK, Key TJ. Circulating levels of sex hormones and their relation to risk factors for breast cancer: a cross-sectional study in 1092 pre- and postmenopausal women (United Kingdom). Cancer Causes Control 2001;12:47–59.
4. Feigelson HS, Shames LS, Pike MC, Coetzee GA, Stanczyk FZ, Henderson BE. Cytochrome *P*450c17 gene (CYP17) polymorphism is associated with serum estrogen and progesterone concentrations. Cancer Res 1998;58:585–7.
5. Haiman CA, Hankinson SE, Spiegelman D, et al. The relationship between a polymorphism in CYP17 with plasma hormone levels and breast cancer. Cancer Res 1999;59:1015–20.
6. Haiman CA, Hankinson SE, Colditz GA, Hunter DJ, De Vivo I. A polymorphism in CYP17 and endometrial cancer risk. Cancer Res 2001;61:3955–60.
7. Marszalek B, Lacinski M, Babych N, et al. Investigations on the genetic polymorphism in the region of CYP17 gene encoding 5′-UTR in patients with polycystic ovarian syndrome. Gynecol Endocrinol 2001;15:123–8.
8. Garcia-Closas M, Herbstman J, Schiffman M, Glass A, Dorgan JF. Relationship between serum hormone concentrations, reproductive history, alcohol consumption and genetic polymorphisms in premenopausal women. Int J Cancer 2002;102:172–8.
9. Somner J, McLellan S, Cheung J, et al. Polymorphisms in the *P*450 c17 (17-hydroxylase/17,20-lyase) and *P*450 c19 (aromatase) genes: association with serum sex steroid concentrations and bone mineral density in postmenopausal women. J Clin Endocrinol Metab 2004;89:344–51.
10. Tworoger SS, Chubak J, Aiello EJ, et al. Association of CYP17, CYP19, CYP1B1, and COMT polymorphisms with serum and urinary sex hormone concentrations in postmenopausal women. Cancer Epidemiol Biomarkers Prev 2004;13:94–101.
11. Haiman CA, Hankinson SE, Spiegelman D, et al. A tetranucleotide repeat polymorphism in CYP19 and breast cancer risk. Int J Cancer 2000;87:204–10.
12. Berstein LM, Imyanitov EN, Suspitsin EN, et al. CYP19 gene polymorphism in endometrial cancer patients. J Cancer Res Clin Oncol 2001;127:135–8.
13. Haiman CA, Hankinson SE, Spiegelman D, Brown M, Hunter DJ. No association between a single nucleotide polymorphism in CYP19 and breast cancer risk. Cancer Epidemiol Biomarkers Prev 2002;11:215–6.
14. Feigelson HS, McKean-Cowdin R, Coetzee GA, Stram DO, Kolonel LN, Henderson BE. Building a multigenic model of breast cancer susceptibility: CYP17 and HSD17B1 are two important candidates. Cancer Res 2001;61:785–9.

No Association of Polymorphisms in *CYP17*, *CYP19*, and *HSD17-B1* with Plasma Estradiol Concentrations in 1,090 British Women

Ruth C. Travis, Michael Churchman, Sarah A. Edwards, et al.

Cancer Epidemiol Biomarkers Prev 2004;13:2282-2284.