Short Communication

Association of *CYP1B1* Polymorphisms and Breast Cancer Risk[1]

Immaculata De Vivo,[2] Susan E. Hankinson, Lisa Li, Graham A. Colditz, and David J. Hunter

Departments of Epidemiology [I. D., S. E. H., G. A. C., D. J. H.] and Nutrition [D. J. H.], and the Harvard Center for Cancer Prevention [I. D., G. A. C., D. J. H.], Harvard School of Public Health, and the Channing Laboratory [I. D., S. E. H., L. L., G. A. C., D. J. H.], Department of Medicine, Brigham and Women's Hospital, and Harvard Medical School, Boston, Massachusetts 02115

Abstract

Cytochrome P450 1B1 catalyzes the conversion of 17-β-estradiol (E2) to the catechol estrogen metabolites 2-OH-E2 and 4-OH-E2 that have been postulated to be involved in mammary carcinogenesis. We sought to determine whether two common functional polymorphisms in *Cytochrome P450 1B1*, V432L (*m1*), and A453S (*m2*) are related to breast cancer risk. Using a nested case control design within the Nurses' Health Study cohort, we genotyped 453 cases and 456 controls and found no significant association between *m1*[val/leu and leu/leu *versus* val/val, OR = 1 (CI, 0.72–1.45)] or *m2* [asn/ser and ser/ser *versus* asn/asn, OR = 0.8 (CI, 0.62–1.15)] and breast cancer risk. However, we did observe women with the *Val/Val* (*m1*) genotype to have a higher percentage of estrogen receptor-positive tumors ($P = 0.03$). We did not observe any correlation with the *m2* genotypes and estrogen receptor status. The association of the *m1* and *m2* genotypes on plasma hormone levels in postmenopausal control women not using hormone replacement therapy was also evaluated. Carriers of the *m1 leu* and *m2 ser* alleles had modestly higher estradiol levels but similar estrone and estrone sulfate levels. The results presented do not support a strong association between *m1* and *m2* and the risk of breast cancer.

Introduction

E2[3] is thought to have a role in mammary carcinogenesis. E2 is metabolized by either formation of the catechol estrogen derivatives 2-OH-E2 and 4-OH-E2 or by C-16-α hydroxylation. The CYP1B1 enzyme predominately catalyzes the formation of 4-OH-E2 (1–3), the most carcinogenic estrogen in animal models (4). Unlike the 2-OH-E2 derivative, 4-OH-E2 induces uterine adenocarcinoma (5) and can induce DNA single-strand breaks (6). In one study, human breast cancer tissue had a significantly higher ratio of 4-OH-E2/2-OH-E2 compared with adjacent normal tissue (7). In human breast cancer cell lines, the formation of 4-OH-E2 is inducible by dioxin, a common environmental contaminant (8). Although CYP1B1 is expressed in a wide variety of tissues, expression is particularly high in the breast, prostate, and uterus (9, 10), supporting a role for CYP1B1 in hormone-mediated cancer. These findings underscore the importance of the CYP1B1 with regard to metabolism of environmental carcinogens and estrogens and its potential role in the initiation of tumors in estrogen-responsive organs, like the breast.

Two polymorphisms have been examined in relation to breast cancer risk, the *m1* allele (Val to Leu at codon 432) and the *m2* allele (Asn to Ser at codon 453). Recently, biochemical studies determined that the *Val* allele and the *Asn* allele had higher catalytic efficiency for the 4-hydroxylation of estradiol compared with their wild-type counterparts (11, 12). Changes in 4-hydroxylation of 17β-estradiol are of particular interest because of the potential carcinogenicity and estrogenic activity of the 4-OH-E2.

In a case control study, Bailey *et al.* (13), found no association with the *m1* and *m2* alleles and breast cancer risk. They did, however, find an association between the *m1 Val/Val* genotype and Caucasian breast cancer patients who had ER-positive breast cancer ($P = 0.02$); no correlation with the *m2* allele was noted (13). In a second case control study of 186 Asian breast cancer cases and 200 Asian controls, the authors found that women with the *m1 Leu/Leu* genotype had a 2-fold elevated risk of breast cancer compared with women with the *Val/Val* genotype (14). This lack of consistency may be attributable to ethnic differences among studies. These authors were unable to evaluate receptor status.

In this study, we evaluated, among primarily Caucasian women, the relationship between the *CYP1B1* alleles and breast cancer risk in a nested case control study within the NHS cohort. Given the role of CYP1B1 in estradiol metabolism, we also evaluated the relationship between the *m1* and *m2* alleles and circulating estrogen levels.

Materials and Methods

Study Population. The NHS was initiated in 1976, when 121,700 United States registered nurses between the ages of 30 and 55 returned an initial questionnaire reporting medical histories and baseline health-related exposures. Between 1989 and 1990, blood samples were collected from 32,826 women. Incident breast cancers are identified by self-report and confirmed by medical record review. Eligible cases in this study consisted of women diagnosed with pathologically confirmed incident breast cancer after giving a blood specimen up to June 1, 1994. Controls were matched to cases on year of birth, menopausal status, and postmenopausal hormone use, as well as time of day, month, and fasting status at blood draw; menopause was defined as described previously (15). The nested case control study consists of 453 incident breast cancer cases and 456 matched controls. The study sample for the plasma hormone

Received 10/12/01; revised 2/15/02; accepted 2/24/02.
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.
[1] Supported by NIH Grants CA65725, CA87969, and CA49449 I.D. is partially supported by a grant from the American Cancer Society (RPG-00-061-01-CCE).
[2] To whom requests for reprints should be addressed, at Channing Laboratory, 181 Longwood Avenue, Boston, MA 02115. E-mail: Devivo@channing.harvard.edu.
[3] The abbreviations used are: E2, 17-β-estradiol; ER, estrogen receptor; CYP1B1, cytochrome P450 1B1; NHS, Nurses' Health Study; OR, odds ratio; CI, confidence interval; BMI, body mass index.

Table 1 Association between *CYP1B1* genotype and breast cancer risk

CYP1B1 genotype	Cases (n = 453)	Controls (n = 453)[a]	ORs (95% CI)	Adjusted OR (95% CI)
m1 (codon 432)	*n* (%)	*n* (%)		
Val/Val	92 (20)	91 (20)	1.00[b]	1.00[c]
Val/Leu	232 (51)	200 (44)	1.19 (0.83–1.69)	1.13 (0.77–1.67)
Leu/Leu	129 (28)	162 (36)	0.80 (0.56–1.15)	0.92 (0.62–1.36)
Val/Leu + Leu/Leu	361 (80)	362 (80)	1.00 (0.72–1.36)	1.00 (0.72–1.45)
m2 (codon 453)				
Asn/Asn	318 (70)	299 (66)	1.00[b]	1.00[c]
Asn/Ser	126 (28)	137 (30)	0.83 (0.62–1.11)	0.88 (0.64–1.20)
Ser/Ser	9 (2)	17 (4)	0.50 (0.21–1.18)	0.57 (0.23–1.42)
Asn/Ser + Ser/Ser	135 (30)	154 (34)	0.80 (0.60–1.06)	0.85 (0.62–1.15)

[a] Three controls were excluded for conditional analysis.
[b] Conditional logistic regression adjusted for the matching variables: age, menopausal status, postmenopausal hormone use, date at blood draw, time at blood draw, and fasting status.
[c] Conditional logistic regression adjusted for matching variables and age at menarche, parity, age at first birth, BMI at age 18, weight gain since age 18, benign breast disease, first-degree family history of breast cancer, and duration of postmenopausal hormone use.

Table 2 Association between *CYP1B1* genotype and breast cancer risk by histological subtype and receptor status

	Val/Val *(ml)*[a] *n* (%)	Val/ Leu+ Leu/Leu *n* (%)	OR (95% CI)	Adjusted OR (95% CI)	Asn/Asn *(m2)*[a]	Asn/ Ser+ Ser/Ser	OR (95% CI)	Adjusted OR (95% CI)
Controls	91 (20)	365 (80)	1.0[b]	1.0[c]	300 (66)	156 (34)	1.0[b]	1.0[c]
Cases								
Invasive cases only	78 (21)	300 (79)	0.95 (0.68–1.33)	1.0 (0.71–1.45)	266 (70)	112 (30)	0.81 (0.60–1.09)	0.82 (0.60–1.11)
Involved nodes								
≥1	22 (21)	82 (79)	0.90 (0.53–1.54)	0.92 (0.53–1.62)	74 (71)	30 (29)	0.76 (0.47–1.22)	0.76 (0.46–1.25)
≥4	10 (28)	26 (72)	0.69 (0.32–1.51)	0.74 (0.32–1.70)	28 (78)	8 (22)	0.53 (0.23–1.20)	0.57 (0.24–1.33)
Receptor status								
ER+	59 (24)	185 (76)	0.75 (0.51–1.09)	0.76 (0.51–1.12)	169 (69)	75 (31)	0.83 (0.60–1.17)	0.84 (0.59–1.20)
ER−	6 (10)	50 (89)	2.26 (0.93–5.49)	2.35 (0.94–5.89)	41 (73)	15 (27)	0.73 (0.39–1.37)	0.74 (0.38–1.43)
PR+	34 (19)	148 (81)	1.10 (0.69–1.67)	1.04 (0.65–1.65)	124 (68)	58 (32)	0.88 (0.61–1.28)	0.87 (0.59–1.28)
PR−	27 (25)	80 (75)	0.74 (0.45–1.23)	0.81 (0.48–1.37)	78 (73)	29 (27)	0.70 (0.44–1.13)	0.70 (0.42–1.15)

[a] Reference categories.
[b] Unconditional logistic regression adjusted for the matching variables: age, menopausal status, postmenopausal status, postmenopausal hormone use, data at blood draw, time at blood draw, and fasting status.
[c] Unconditional logistic regression adjusted for the matching variables and age at menarche, parity, age at first birth, BMI at age 18, weight gain since age 18, benign breast disease, first-degree family history of breast cancer, and duration of postmenopausal hormone use.
[d] PR, progesterone receptor.

analysis was restricted to postmenopausal controls not using hormone replacement therapy within 3 months of blood draw. The protocol was approved by the Committee on Human Subjects, Brigham and Women's Hospital. Detailed information on exposure data and hormone assays have been described previously (15, 16).

Molecular Analysis. All analyses were conducted with laboratory personnel blinded to case status. DNA was extracted from buffy coat fractions using the Qiagen QIAamp Blood Kit (Qiagen, Inc., Chadsworth CA). Genotyping was performed by automated DNA sequencing on the ABI 377X using BigDye-terminator Cycle Sequencing Ready Reaction Kit (PE Biosystems, Foster City, CA). Because of the close proximity of *m1* to *m2* within 60 bp, we were able to amplify both polymorphisms within one amplicon. PCR amplification of *m1* and *m2* was generated using primers 5′-CCAACACCTCTGTCTTGGGA-3′ and 5′-GCTCATTTGGGTTGGCCCTG-3′. Heterozygotes were called at positions where the secondary peak height was ≥45–50% of the primary peak height in both forward and reverse sequence reads for a subset of the samples. After establishing the criteria for base calling, the forward PCR primer was used for all subsequent sequencing reactions. All of the genotyping was performed by laboratory personnel unaware of case control status. For quality control, a random 10% of the samples were inserted to validate genotyping identification procedures; concordance for blinded samples was 100%.

Statistical Methods. ORs and 95% CIs were calculated using conditional and unconditional logistic regression. In addition to the matching variables, we adjusted for the following breast cancer risk factors: BMI (kg/m^2) at age 18 (continuous), weight gain since age 18 (<5 kg, 5-<20, and ≥20), age of menarche (<12 years, 12, 13, and ≥13), parity/age at first birth (nulliparous, one to two children/age at first birth ≤ 24 years, one to two children/age at first birth ≥ 24, three children/age at first birth ≤ 24 years, and three or more children/age at first birth ≥ 24 years), first-degree family history of breast cancer (yes/no), history of benign breast disease (yes/no), and duration of postmenopausal hormone use (never; past, <5 years and ≥5; and current, <5 years and ≥5). We also adjusted for age at menopause (continuous in years) in analyses limited to postmenopausal women. Indicator variables for CYP1B1 *m1* and *m2* were created using the *Val/Val* and *Asn/Asn* as the reference category in the regression models. These reference categories were used to be consistent with studies published previously

(13, 17). Genotype was also evaluated using dichotomous variables, *Val/Leu + Leu/Leu* combined and *Asn/Ser + Ser/Ser* combined, as a gene dosage effect on breast cancer risk was not apparent. Unconditional multivariate models controlling for the matching factors enabled all controls to be included in analyses, limiting the cases to specified histopathological characteristics. Interactions between genotypes and breast cancer risk factors were evaluated by including interaction terms in multivariate logistic regression models. The likelihood ratio test was used to assess the statistical significance of these interactions.

Mixed regression models were used to evaluate the association between genotype and circulating hormone levels among postmenopausal controls not currently using postmenopausal hormones, controlling for BMI at blood draw and the matching variables. Hormone fractions were measured in three different batches; laboratory batch was treated as a random effect in all hormone analyses. We calculated least square mean plasma steroid hormone levels to evaluate differences in hormone levels between the genotypes. The natural logarithm of the plasma hormone values was used in the analyses to reduce the skewness of the regression residuals. We used the SAS statistical package for all analyses (Ref. 18; SAS Institute, Inc.).

Results

Our study included 453 incident breast cancer cases and 456 cancer controls. A total of 348 cases and 345 controls were postmenopausal, and 64 cases and 69 controls were premenopausal, whereas menopausal status was uncertain for 41 cases and 42 controls. Self-reported major ethnicity/ancestry was similar between cases and controls (cases *versus* controls; Southern European: 18.1 *versus* 16.4%, Scandinavian: 8.5 *versus* 8.5%, and other Caucasian: 62 *versus* 62%). Asians, Hispanics, and African-Americans comprised <1% of cases or controls.

The prevalence of the *m1 Val* allele was similar to previous reports in Caucasian women (45% NHS cases and 42% NHS controls compared with 40%; Ref. 13). The prevalence of the *m2 Ser* allele was also similar to reports published previously in Caucasian women (16% NHS cases and 18% NHS controls compared with 17.4%; Ref. 13). The calculated frequencies for the *m1* alleles (Val and Leu) were 0.46 and 0.54, respectively, in the cases and 0.42 and 0.58 in the controls. For the *m2* alleles (Asn and Ser), the frequencies were 0.84 and 0.16 in the cases and 0.81 and 0.19 in the controls, respectively. Genotype frequencies for the *m1* alleles were in Hardy-Weinberg equilibrium for cases ($\chi^2 = 0.43$, Df = 1, and $P = 0.51$) but slightly out of Hardy-Weinberg equilibrium for the controls ($\chi^2 = 4.1$, Df = 1, and $P = 0.04$). This finding is most likely attributable to chance as no genotyping errors were detected among the blinded quality control samples, and data for the cases were in equilibrium. For the *m2* alleles, the genotype frequencies for the cases ($\chi^2 = 0.58$, Df = 1, and $P = 0.45$) and the controls ($\chi^2 = 0.09$, Df = 1, and $P = 0.76$) were in Hardy-Weinberg equilibrium. The *m1* and *m2* polymorphisms are not in linkage disequilibrium. We found no significant associations between any genotype frequencies and risk of breast cancer (Table 1). The results remained unchanged after stratifying by menopausal status, hormone use, and age at menarche for all genotypes. We did not find any significant associations between any of the genotypes and specific strata of breast cancer defined by receptor status (Table 2). However, we did observe an association with receptor status and genotype. Women who had ER-positive tumors were more likely to have the *Val/Val* genotype ($\chi^2 = 4.87$, $P = 0.03$) compared with women with ER-negative tumors (24 *versus*10%). These findings are consistent with those reported by Bailey *et al.* (13). Additionally consistent with Bailey *et al.* is the lack of an association with ER status for the *m2* genotypes.

Table 3 Least square geometric mean hormone levels[a] among postmenopausal controls by *CYP1B1* genotypes

Hormones	Genotypes	Overall[b] (*n*)
Estrone sulfate (pg/ml)	*ml:* val/val	193.33 (28)
	val/leu + leu/leu	189.97 (119)
		$P = 0.89$
	m2: asn/asn	187.31 (95)
	asn/ser + ser/ser	200.09 (43)
		$P = 0.54$
Estrone (pg/ml)	*ml:* val/val	29.57 (28)
	val/leu + leu/leu	29.47 (119)
		$P = 0.96$
	m2: asn/asn	28.60 (98)
	asn/ser + ser/ser	31.63 (49)
		$P = 0.10$
Estradiol (pg/ml)	*ml:* val/val	6.44 (28)
	val/leu + leu/leu	7.62 (119)
		$P = 0.05$
	m2: asn/asn	7.06 (97)
	asn/ser + ser/ser	8.22 (49)
		$P = 0.03$

[a] Subjects with missing hormone levels removed from analysis; hence, numbers vary slightly by hormone.
[b] Controlling for age, date of blood draw, time of blood draw, fasting status, BMI, and laboratory batch.

When evaluating the relationship of genotype with plasma hormone levels among controls, we calculated the least squared geometric mean plasma steroid hormone levels for each genotype (Table 3). Compared with women with the *Val/Val* genotype, women with the *Val/Leu* and *Leu/Leu* genotype did not have elevated levels of estrone-sulfate or estrone; however, there was an increased level of estradiol (8.37%, $P = 0.05$). Similarly, compared with women with the *Asn/Asn* genotype, women with the *Asn/Ser* and *Ser/Ser* genotype did not have elevated levels of estrone-sulfate or estrone; however, there was an increased level of estradiol (16.5%, $P = 0.03$). We further evaluated the combination of *m1* and *m2* on breast cancer risk and observed no association.

Discussion

CYP1B1 has an important role in estrogen metabolism. It catalyzes the formation of 4-hydroxyestradiol, a carcinogenic metabolite that retains significant estrogenic activity (2). Several polymorphisms have been identified in *CYP1B1,* two of which have been studied in relation to breast cancer. The Val432Leu (*m1*) and the Asn453Ser (*m2*) polymorphisms both result in an amino acid change in exon 3, which encodes the heme-binding domain, the region critical to the catalytic function of CYP1B1 (13). Recent functional studies show differences between wild-type CYP1B1 and variants in estrogen hydroxylation; specifically, the ratio of 4-OH-E2:2-OH-E2 was higher for both the *Val* allele and the *Asn* allele (11, 12). Higher levels of 4-OH-E2 have been reported in breast cancer tissue (7). When treating MCF-7 breast cancer cells with 4-OH-E2, the rate of cell proliferation and the expression of estrogen-inducible genes were increased, compared with treatment with 2-OH-E2 (19, 20). In our study, the high activity alleles are not associated with increased breast cancer risk, which is consistent

with one other case control study that included 164 Caucasian cases and controls and 59 African-American cases and controls (13). Additionally consistent with the previous study, and with the recent biochemical data suggesting an increase in the enzymatic activity of the *Val* allele, is the association between the *m1 Val* polymorphism and ER-positive status. We found a marginally significant decrease in estradiol levels in women with the *Val* allele. Assays under development for estrogen metabolites, produced by CYP1B1, could be more informative, as these markers may serve as a more direct measure of CYP1B1 enzymatic activity.

Our results suggest that despite a potential association with estradiol levels, neither the V432L nor the A453S polymorphisms in the *CYP1B1* gene alone or in combination are sufficient to substantially influence breast cancer risk in Caucasian women.

Acknowledgments

We thank the participants of the NHS for their continuing exceptional cooperation, Pamela Lescault for technical assistance, and Frank Speizer, principal investigator of the NHS, for his support.

References

1. Spink, D., Eugster, H-P., Lincoln, D., II, Schuetz, E., Johnson, J., Kaminsky, L., and Gierthy, J. 17β-Estradiol hydroxylation catalyzed by human cytochrome P450 1A1: a comparison of the activities induced by 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin in MCF-7 cells with those from heterologous expression of the cDNA. Arch. Biochem. Biophys., *293:* 342–348, 1992.

2. Hayes, C., Spink, D., Spink, B., Cao, J., Walker, N., and Sutter, T. 17 β-Estradiol hydroxylation catalyzed by human cytochrome P450 1B1. Proc. Natl. Acad. Sci. USA, *93:* 9776–9781, 1996.

3. Spink, D., Spink, B., Cao, J., Gierthy, J., Hayes, C., Li, Y., and Sutter, T. Induction of cytochrome P450 1B1 and catechol estrogen metabolism in ACHN human renal adenocarcinoma cells. J. Steroid Biochem. Mol. Biol., *62:* 223–232, 1997.

4. Li, J., and Li, S. Estrogen carcinogenesis in Syrian hamster tissues: role of metabolism. Fed. Proc., *46:* 1858–1863, 1987.

5. Newbold, R., and Liehr, J. Induction of uterine adenocarcinoma in CD-1 mice by catechol estrogens. Cancer Res, *60:* 235–237, 2000.

6. Han, X., and Liehr, J. DNA single-strand breaks in kidneys of Syrian hamsters treated with steroidal estrogens: hormone-induced free radical damage preceding renal malignancy. Carcinogenesis (Lond.), *15:* 997–1000, 1994.

7. Liehr, J., and Ricci, M. 4-Hydroxylation of estrogens as marker of human mammary tumors. Proc. Natl. Acad. Sci. USA, *93:* 3294–3296, 1996.

8. Spink, D., Hayes, C., Young, N., Christou, M., Sutter, T., Jefcoate, C., and Gierthy, J. The effects of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin on estrogen metabolism in MCF-7 breast cancer cells: evidence for induction of a novel 17 β-estradiol 4-hydroxylase. J. Steroid Biochem. Mol. Biol., *51:* 251–258, 1994.

9. McKay, J., Melvin, W., Ah-See, A., Ewen, S., Greenlee, W., Marcus, C., Burke, M., and Murray, G. Expression of cytochrome P450 CYP1B1 in breast cancer. FEBS Lett., *374:* 270–272, 1995.

10. Murray, G., Taylor, M., McFadyen, M., McKay, J., Greenlee, W., Burke, M., and Melvin, W. Tumor-specific expression of cytochrome P450 CYP1B1. Cancer Res, *57:* 3026–3031, 1997.

11. Shimada, T., Watanabe, J., Kawajiri, K., Sutter, T., Guengerich, F., Gillam, E., and Inoue, K. Catalytic properties of human polymorphic cytochrome P450 1B1 variants. Carcinogenesis (Lond.), *20:* 1607–1613, 1999.

12. Hanna, I., Dawling, S., Roodi, N., Guengerich, F., and Parl, F. Cytochrome P450 1B1 (CYP1B1) pharmacogenetics: association of polymorphisms with functional differences in estrogen hydroxylation activity. Cancer Res., *60:* 3440–3444, 2000.

13. Bailey, L., Roodi, N., Dupont, W., and Parl, F. Association of cytochrome P450 1B1 (CYP1B1) polymorphism with steroid receptor status in breast cancer. Cancer Res., *58:* 5038–5041, 1998.

14. Zheng, W., Xie, D-W., Jin, F., Cheng, J., Dai, Q., Wen, W-Q., Shu, X-O., and Gao, Y-T. Genetic polymorphism of P450 1B1 and risk of breast cancer. Cancer Epidemiol. Biomarkers Prev., *9:* 147–150, 2000.

15. Haiman, C., Hankinson, S., Spiegelman, D., Colditz, G., Willett, W., Speizer, F., Kelsey, K., and Hunter, D. The relationship between a polymorphism in CYP17 with plasma hormone levels and breast cancer. Cancer Res., *59:* 1015–1020, 1999.

16. Hankinson, S., Willett, W., Manson, J., Colditz, G., Hunter, D., Spiegelman, D., Barbieri, R., and Speizer, F. Plasma sex steroid hormone levels and risk of breast cancer in postmenopausal women. J. Natl. Cancer Inst. (Bethesda), *90:* 1292–1299, 1998.

17. Goodman, M., McDuffie, K., Kolonel, L., Terada, K., Donlon, T., Wilkens, L., Guo, C., and Le Marchand, L. Case-control study of ovarian cancer and polymorphisms in genes involved in catecholestrogen formation and metabolism. Cancer Epidemiol. Biomarkers Prev., *10:* 209–216, 2001.

18. SAS Institute, I. Changes and Enhancements. SAS Technical Report, Release 6.07, 1992.

19. Schultze, N., Vollmer, G., Tiemann, I., Geiger, M., and Knuppen, R. Catecholestrogens are MCF-7 cell estrogen receptor agonists. J. Steroid Biochem. Mol. Biol., *46:* 781–789, 1993.

20. Schultze, N., Vollmer, G., and Knuppen, R. Catecholestrogens are agonists of estrogen receptor dependent gene expression in MCF-7 cells. J. Steroid Biochem. Mol. Biol., *48:* 453–461, 1994.