[CANCER RESEARCH 59, 4870–4875, October 1, 1999]

Breast Cancer Risk Associated with Genotype Polymorphism of the Estrogen-metabolizing Genes *CYP17*, *CYP1A1*, and *COMT*: A Multigenic Study on Cancer Susceptibility

Chiun-Sheng Huang, Herng-Der Chern, King-Jen Chang, Chun-Wen Cheng, Su-Ming Hsu, and Chen-Yang Shen[1]

Departments of Surgery [C-S. H., K-J. C.] and Pathology [S-M. S.], National Taiwan University Hospital, Taipei, Taiwan; Graduate Institute of Pharmaceutical Sciences, College of Medicine, National Taiwan University, Taipei, Taiwan [H-D. C., C-W. C.]; and Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan [C-W. C., C-Y. S.]

ABSTRACT

Estrogen has been proposed to trigger breast cancer development via an initiating mechanism involving its metabolite, catechol estrogen (CE). To examine this hypothesis, we conducted a multigenic case-control study to determine whether polymorphisms of the genes responsible for CE formation via estrogen biosynthesis (*CYP17*) and hydroxylation (*CYP1A1*) and CE inactivation (*COMT*) are associated with an elevated risk for breast cancer in Taiwanese women, and whether the association between genotype and risk may be modified by estrogen exposure. One hundred and fifty breast cancer patients and 150 healthy controls were recruited. PCR-based RFLP assays were used to determine the genotypes of estrogen-metabolizing genes. The breast cancer risk associated with individual susceptibility genotypes varied among the three genes and was highest for *COMT*, followed by *CYP1A1* and *CYP17*. After simultaneous consideration of all three genes and other well-established risk factors of breast cancer, the *COMT* genotype remained the most significant determinant for breast cancer development and was associated with a 4-fold increase in risk (95% confidence interval, 1.12–19.08). Furthermore, a trend of increasing risk for developing breast cancer was found in women harboring higher numbers of high-risk genotypes ($P = 0.006$), including the high activity *CYP17* (*CYP17* A_2/A_2), high inducibility *CYP1A1* (*CYP1A1 Msp*I *vt/vt*), and low activity *COMT* (*COMT L/L*) genotypes. The association of risk with the number of susceptibility genotypes was stronger in women with prolonged estrogen exposure (indicated by a higher number of estrogen exposure years or a higher number of estrogen exposure years between menarche and first full-term pregnancy), women with higher estrogen levels (implied by early menarche), and women with a higher body mass index (≥22.5). On the basis of comprehensive profiles of estrogen metabolism, this study supports the possibility that breast cancer can be initiated by estrogen exposure.

INTRODUCTION

Both epidemiological and cell biology studies have documented the contribution of estrogen to the development of breast cancer. Well-established risk factors for breast cancer, including age at menarche, age at menopause, parity, and age at FFTP[2] (1–3), are operative via a hormonal mechanism. Hypotheses in which estrogen is involved in tumorigenesis are based on the general concept that cell division plays a crucial role in cancer development and that reproductive factors that increase mitotic activity in the breast epithelium also increase cancer risk (4). On this basis, the role of reproductive hormones during tumorigenesis would be largely related to epigenetic alteration and tumor promotion. However, recent studies have shown that estrogen metabolites can bind to DNA and trigger damage (5–7), suggesting that estrogen might be a complete carcinogen (8) that can directly cause genetic alteration and effect tumor initiation. This possibility is supported by the finding that women with reduced amounts of the enzymes responsible for removing reactive estrogen metabolites are at higher risk of developing breast cancer (9). To comprehensively elucidate the estrogen initiating mechanism of tumorigenesis in breast cancer and to dissect the contribution of individual estrogen-metabolizing genes involved in this mechanism, this molecular epidemiological study sought to determine whether polymorphisms in the genes involved in estrogen biosynthesis (*CYP17*) and hydroxylation (*CYP1A1*) and inactivation of the reactive metabolites (*COMT*) may be associated with an elevated risk of breast cancer, and whether the association between genotypes and risk may be modified by estrogen exposure.

CYP17 encodes the enzyme cytochrome P450c17, which catalyzes steroid 17α-hydroxylase and 17–20 lyase activities at key points in estrogen (estradiol) biosynthesis (10). The 5′-untranslated region of *CYP17* contains a single-base polymorphism (a T-to-C transition) that creates a SP1-type (CCACC box) promoter (11). This change also generates a *Msp*A1 restriction site; therefore, *Msp*A1 digestion identifies two alleles, A_1 (wt) and A_2 (vt). Because the number of 5′ promoter elements correlates with promoter activity, the A_2 allele may increase *CYP17* expression and thus increase estradiol biosynthesis (12). This suggestion is supported by evidence that A_2 is associated with elevated levels of circulating estradiol in young women (13) and is related to a markedly increased risk of advanced breast cancer (12).

Hydroxylation of estrogen to hormonally inactive, water-soluble metabolites, HEs, is an important means of eliminating estrogen (6, 14). Oxidation occurs via two major pathways, one of which involves C-2 (and/or C-4) of estradiol, resulting in the formation of the 2-HE and 4-HE, whereas the other involves C-16, resulting in the formation of 16α-HE. Whereas 2-HE binds much more weakly to the estrogen receptor, resulting in much weaker hormonal potency, 4-HE and 16α-HE retain potent hormonal activity (15–17). Furthermore, 4-HE and 16α-HE are able to bind to DNA, creating adducts and subsequently causing gene mutations (5, 6, 18, 19). Thus, increased formation of 4-HE and 16α-HE has been associated with an elevated risk of breast cancer (6, 14, 20, 21). *CYP1A1* is among the major enzymes participating in estrogen hydroxylation (6, 14, 22) and thus may play an important role in determining the relative distribution of the metabolites. To date, at least four polymorphisms have been described in the human *CYP1A1* gene (23). Two of these, *m1* (a C substituted for T in the 3′-noncoding region, giving rise to a *Msp*I restriction site) and *m2* (a point mutation in codon 462 of exon 7, leading to a substitution of valine for isoleucine), are associated with increased breast cancer risk (22, 24–26). Because the *m2* vt in cancer risk may be unrelated to estrogen metabolism (24, 25), and because our previous study (26) has shown that *m1*, but not *m2*, is related to breast cancer in Taiwan, we focused on the role of the *m1* polymorphism in our analysis. *CYP1A1* activity is more readily inducible in lymphocytes with the *m1* genotype than in wt lymphocytes, resulting in the high inducibility phenotype (27, 28).

O-Methylation mediated by *COMT* is an important mechanism for

Received 4/5/99; accepted 8/6/99.

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] To whom requests for reprints should be addressed, at Institute of Biomedical Sciences, Academia Sinica, Taipei, 11529, Taiwan. Fax: 886-2-2782-3047; E-mail: bmcys@ccvax.sinica.edu.tw.

[2] The abbreviations used are: FFTP, first full-term pregnancy; BMI, body mass index; CE, catechol estrogen; CI, confidence interval; HE, hydroxyestrone; HRT, hormone replacement therapy; OR, odds ratio; aOR, adjusted OR; wt, wild-type; vt, variant.

inactivating CE, including 2-HE and 4-HE (6, 14). Reduced COMT activity might increase the risk of breast cancer, secondary to the accumulation of CE, which causes oxidative DNA damage (6, 29, 30). In addition, 2-HE and 4-HE may be oxidized to CE quinones, which react with DNA to form adduct. These adducts, especially CE-3,4-quinone derived from 4-HE, can cause depurination leaving apurine sites (5), which is the major type of genetic damage leading to mutation and genomic deletion during tumorigenesis (31). A G-to-A transition at codon 158 of *COMT*, which leads to a substitution of methionine for valine (32), was recently linked to low COMT activity, designated the *L* (low activity) allele (in contrast to the wt *H* high activity allele). Three epidemiological studies (9, 33, 34) were therefore carried out to examine a possible correlation between low activity genotypes of *COMT* (*L/L* or *L/H*) and breast cancer risk. Two studies (9, 33) found a positive correlation but gave inconsistent results as to whether it was premenopausal or postmenopausal women who had an elevated risk due to possession of the high-risk genotypes.

The dosage of target agents might affect the associations of cancers with polymorphisms of metabolic genes among different study groups. For example, the greatest incremental lung cancer risk (7-fold) for the high-risk *CYP1A1* genotype was seen in light smokers, whereas heavy smokers with this genotype had less than twice the risk of heavy smokers without the genotype (35). Because Asian women have, on average, 20% lower serum estradiol levels than Western women (36), elucidating the ethnicity-specific effects of genetic polymorphisms of estrogen-metabolizing genes on cancer risk in Taiwanese women might yield valuable clues on the association of breast tumorigenesis with estrogen. This study reports such an investigation.

MATERIALS AND METHODS

Study Population. This case-control study is part of an ongoing cooperative study aimed at understanding the causes of breast cancer in Taiwan (26, 37–41), which is characterized by low incidence (37), early tumor onset (39), and novel genomic alterations (38, 39). One hundred and fifty female breast cancer patients and 150 healthy female controls who had given their informed consent were enrolled. All breast cancer patients had pathologically confirmed primary breast carcinoma, and all were diagnosed and treated at the National Taiwan University Hospital between January 1995 and June 1996. This sample of patients constituted about 50% of all women with breast cancer attending our breast cancer clinic during the study period; the remaining patients were excluded because of lack of adequate blood specimens. No significant differences were found in breast cancer risk factors between the included and excluded women. To avoid any differential recall bias of previous disease history, we purposely randomly selected the controls from the health examination clinic of the same hospital during the same study period. Because the examination was not sponsored by the National Insurance Program, the controls might represent a group of women showing more concern about their health (26). These controls constituted about 10% of all women attending the clinic; no significant differences were found in terms of socioeconomic status between those included and those not included. The control subjects received a 1.5-day comprehensive health examination and showed no evidence of breast cancer, any suspicious precancerous lesions of the breast, or other cancers.

Questionnaire. An experienced research nurse was assigned to administer a structured questionnaire to both case and control subjects. The information collected included age at diagnosis, family history of breast cancer (first-degree relatives), history of breast biopsy, history of breast screening, age at menarche and/or menopause, parity, age at FFTP, number of pregnancies, history of breast feeding, use of oral contraceptives, HRT, history of alcohol consumption and cigarette smoking, ethnic background, residence area, family income, and education level. The BMI and menopausal status were also recorded. Women younger than 55 years who had undergone hysterectomy, but not bilateral oophorectomy, were classified as unknown in terms of menopausal status.

Laboratory Analyses. A 10-ml sample of peripheral blood collected in acetate-citrate dextrose was obtained from each breast cancer patient before treatment and from each control subject. The buffy coats of these specimens were prepared immediately and stored at −80°C until extraction of the genomic DNA. Genomic DNA was obtained by conventional phenol/chloroform extraction followed by ethanol precipitation and stored at −20°C until genotype analysis.

PCR-based RFLP assays (9, 12, 26) were used to determine the *CYP17*, *CYP1A1*, and *COMT* genotypes (Fig. 1) of the subjects. To ensure that the observed polymorphisms were specific and were not the results of experimental variation, the results were confirmed by repeating the assay.

Statistical Analysis. Univariate and multivariate analyses were used to determine the risk factors for breast cancer in this series of study subjects, and the ORs and corresponding 95% CIs were estimated (26, 41). In the present study, increased exposure to CE was hypothesized to contribute to elevated breast cancer risk. Therefore, women harboring high-risk alleles, including the *CYP17* A_2 allele, *CYP1A1 Msp*I *vt*, and/or the *COMT* low activity (*L*) allele, were considered to be at higher risk of cancer. The association between susceptibility genotypes and breast cancer risk was evaluated in multivariate logistic regression models with simultaneous consideration of established risk factors for breast cancer or other significant risk factors. Biological plausibility was the most important criterion for inclusion of variables in the model; therefore, we included all established risk factors in the models, regardless of statistical significance: (*a*) age; (*b*) family history of breast cancer; (*c*) age at menarche; and (*d*) age at FFTP (42). HRT history was also included in the model for postmenopausal women because our previous studies (26, 41) demonstrated a significant effect of this factor in determining breast cancer risk in this series of study subjects. A backward elimination procedure (43) was used to select the optimal model, and multivariate-aORs and their 95% CIs were estimated. All *P*s were two-tailed.

Of particular interest was the relationship between estrogen-metabolizing genes and the risk of breast cancer within categories of risk factors representing different levels of estrogen exposure. We adopted four indices to estimate the estrogen exposure level: (*a*) total years of estrogen exposure (representing the number of years of exposure to menstrual cycles), which was calculated according to the age at menarche and age at interview for premenopausal women and age at the time of menarche and age at menopause for postmenopausal women; (*b*) the number of years between menarche and FFTP. A more advanced age at FFTP is generally accepted as a major risk factor for breast

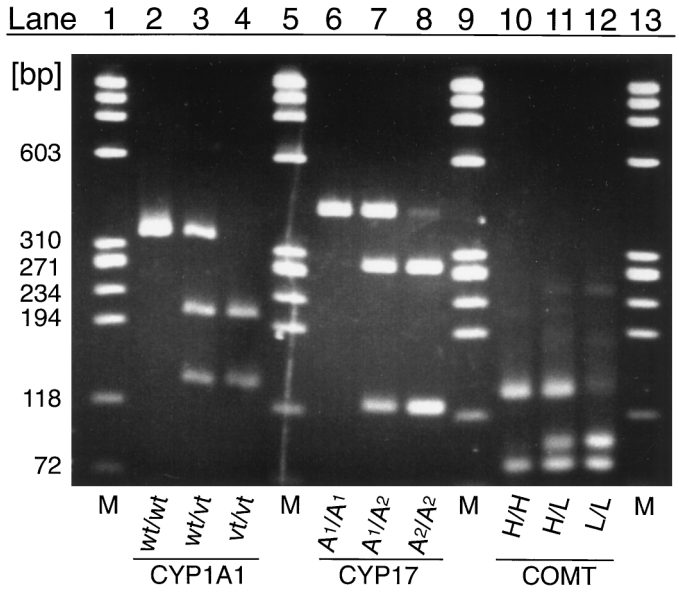

Fig. 1. PCR-based RFLP analysis of genetic polymorphisms of the estrogen-metabolizing genes *CYP17*, *CYP1A1*, and *COMT*. *Lanes 1*, *5*, *9*, and *13*, molecular weight markers (*M*). *Lanes 2–4*, DNA from women homozygous for the *CYP1A1 Msp*I *wt* allele (*Lane 2*), heterozygous for the *vt* (high inducibility) allele (*Lane 3*), and homozygous for the *vt* allele (*Lane 4*). *Lanes 6–8*, *CYP17* polymorphisms in women homozygous for the *wt* allele (*Lane 6*, A_1/A_1), heterozygous for *vt* (high activity) allele (*Lane 7*, A_1/A_2), and homozygous for the *vt* allele (*Lane 8*, A_2/A_2). *Lanes 10–12*, *COMT* polymorphisms in women homozygous for the *wt* (high activity) allele (*Lane 10*, *H/H*), heterozygous for the *vt* (low-activity) allele (*Lane 11*, *H/L*), and homozygous for the *vt* allele (*Lane 12; L/L*).

cancer. Although the mechanism underlying this association has yet to be defined, experimental studies in rats have shown that full-term pregnancy results in permanent differentiation of the vulnerable breast stem cells, altering subsequent susceptibility to hormones (44); this suggests that the period between menarche and the age at FFTP may be also critical; (*c*) age at menarche. This was used because women whose menarche occurred early have higher levels of estrogen during the menstrual cycle (45) as well as a longer duration of exposure to estrogen; and (*d*) BMI, because endogenous estrogen is converted and released from adipose tissue. Subsequently, possible modification of risk by estrogen exposure was evaluated by calculating the risk (OR) of breast cancer in relation to the number of high-risk genotypes within different levels (categories) of estrogen exposure indices.

RESULTS

The risk profiles of this series of study subjects were similar to those in other breast cancer studies, and reproductive risk factors, including early menarche and late FFTP, were significantly associated with increased breast cancer risk (26, 41). The frequency distributions of the genetic polymorphisms of *CYP17*, *CYP1A1*, and *COMT* are shown in Table 1. The association between the various polymorphisms and breast cancer varied in women with heterozygous wt genotypes (*CYP17* A_1/A_2, *CYP1A1 Msp*I *wt/vt*, and *COMT H/L*). In contrast, the risk of breast cancer was consistently elevated in women harboring homozygous variants of the individual genes (*CYP17* A_2/A_2, *CYP1A1 Msp*I *vt/vt*, and *COMT L/L*). Because there is very little conclusive evidence in the literature regarding the phenotypic manifestations of heterozygous wt susceptibility genes, and the absence of a gene-dose effect is common in this type of genotype-based study (33), we defined susceptibility genotypes on the basis of the findings observed in the present study. Thus, in the following analyses, homozygous variants of *CYP17*, *CYP1A1*, and *COMT* (*CYP17* A_2/A_2, *CYP1A1 Msp*I *vt/vt*, and *COMT L/L*) were considered high-risk genotypes; this basis for definition has been used in previous molecular epidemiological studies. Overall, an increased risk of breast cancer associated with individual high-risk genotypes was found consistently (Table 1). Individually, breast cancer risk associated with susceptibility genotypes varied for the three genes and was much higher for *COMT* ($P < 0.05$) than for *CYP17* ($P > 0.05$), with an intermediate value for *CYP1A1* ($P < 0.05$). A more obvious increase in risk associated with high-risk genotypes was found in postmenopausal women, and among premenopausal women, all of the high-risk genotypes were positively but insignificantly associated with risk.

To comprehensively assess the individual contribution of *CYP17*, *CYP1A1*, and *COMT* in the association with breast cancer development, we performed logistic regression analysis considering the effects of individual genes simultaneously (Table 2). The high-risk *CYP17* and *CYP1A1* genotypes played a relatively minor role and were not significantly associated with cancer risk. However, consistent with the findings in Table 1, the high-risk *COMT* genotype was strongly associated with breast cancer risk, with an adjusted OR of as high as 4.02. An epidemiological concern of this model is the absence of a significant association between family history and breast cancer. This finding has been confirmed to reflect that a relatively high

Table 1 *Distribution of genotype polymorphisms of estrogen-metabolizing genes, CYP17, CYP1A1, and COMT, and estimated OR and aOR in relation to breast cancer risk*

Genotype of estrogen-metabolizing gene	No. of cases (%)	No. of controls (%)	OR (95% CI)	aOR (95% CI)[a]
Total women				
CYP17				
A_1/A_1	25 (20.3)	28 (22.2)	1.00 (Ref)[b]]	1.00 (Ref)
A_1/A_2	54 (43.9)	63 (50.0)	0.96 (0.48–1.94)	
A_2/A_2	44 (35.8)	35 (27.8)	1.41 (0.66–3.01)	1.28 (0.73–2.27)
CYP1A1 MspI				
wt/wt	48 (35.3)	45 (33.8)	1.00 (Ref)]	1.00 (Ref)
wt/vt	56 (41.2)	71 (53.4)	0.74 (0.42–1.31)	
vt/vt	32 (23.5)	17 (12.8)	1.76 (0.81–3.85)	2.11 (1.08–4.20)
COMT				
H/H	68 (57.6)	66 (52.8)	1.00 (Ref)]	1.00 (Ref)
H/L	37 (31.4)	55 (44.0)	0.65 (0.37–1.16)	
L/L	13 (11.0)	4 (3.2)	3.15 (0.89–12.15)	3.55 (1.15–13.37)
Premenopausal women				
CYP17				
A_1/A_1	10 (19.2)	12 (24.0)	1.00 (Ref)]	1.00 (Ref)
A_1/A_2	25 (48.1)	24 (48.0)	1.25 (0.41–3.87)	
A_2/A_2	17 (32.7)	14 (28.0)	1.46 (0.42–5.06)	1.18 (0.50–2.83)
CYP1A1 MspI				
wt/wt	24 (42.9)	18 (32.7)	1.00 (Ref)]	1.00 (Ref)
wt/vt	21 (37.5)	29 (52.7)	0.54 (0.22–1.35)	
vt/vt	11 (19.6)	8 (12.6)	1.03 (0.30–3.55)	1.79 (0.64–5.24)
COMT				
H/H	29 (60.4)	31 (59.6)	1.00 (Ref)]	1.00 (Ref)
H/L	14 (29.2)	18 (34.6)	0.83 (0.32–2.15)	
L/L	5 (10.4)	3 (5.8)	1.78 (0.33–10.51)	2.00 (0.46–10.36)
Postmenopausal women				
CYP17				
A_1/A_1	14 (20.6)	15 (20.0)	1.00 (Ref)]	1.00 (Ref)
A_1/A_2	27 (39.7)	39 (52.0)	0.74 (0.28–1.95)	
A_2/A_2	27 (39.7)	21 (28.0)	1.38 (0.49–3.85)	1.31 (0.59–2.90)
CYP1A1				
wt/wt	21 (28.0)	27 (35.1)	1.00 (Ref)]	1.00 (Ref)
wt/vt	33 (44.0)	41 (53.2)	1.03 (0.47–2.30)	
vt/vt	21 (28.0)	9 (11.7)	3.00 (1.03–8.89)	2.15 (0.84–5.72)
COMT				
H/H	37 (56.9)	34 (47.2)	1.00 (Ref)]	1.00 (Ref)
H/L	21 (32.3)	37 (51.4)	0.52 (0.24–1.13)	
L/L	7 (10.8)	1 (1.4)	6.43 (0.72–146)	9.34 (1.27–193)

[a] ORs and 95% CIs were calculated by logistic regression models containing breast cancer risk factors. Risk factors were adjusted for age, family history of breast cancer, age at menarche, age at FFTP, and history of HRT in the groups of total women and postmenopausal women. For premenopausal women, the history of HRT was not included in the model.
[b] Ref, reference group.

Table 2 *Unconditional logistic regression analysis of genotype polymorphisms of estrogen-metabolizing genes and multiple risk factors for breast cancer development*

Risk factor	Multivariate-aOR	95% CI
Estrogen-metabolizing gene		
CYP17 (A_2/A_2 *vs.* A_1/A_1, A_1/A_2)	1.23	0.67–2.28
CYP1A1 (vt/vt vs. wt/wt, wt/vt)	1.79	0.86–3.78
COMT (L/L vs. H/H, H/L)	4.02	1.12–19.08
Age (yrs)	0.97	0.94–1.00
Family history of breast cancer (yes *vs.* no)	1.39	0.34–5.61
Age at menarche (≤13 yrs *vs.* >13 yrs)	1.93	1.05–3.58
Age at FFTP (≥30 yrs or nulliparity *vs.* <30 yrs)	2.39	1.13–5.24
History of HRT (yes *vs.* no)	4.47	1.58–14.76

proportion of control subjects had a family history of breast cancer (26), which might be expected because they were selected from a group of women who were probably more concerned about their health (*i.e.*, those attending a self-sponsored health examination clinic). Although this might affect evaluations of genetic factors in breast cancer development, this limitation should apply only to genes with high penetrance, such as *BRCA1* and *BRCA2*. For low penetrance genes, such as *CYP17*, *CYP1A1*, and *COMT*, the effect, if any, might be relatively minor. Furthermore, any such overrepresentation of genetic predisposition in our control subjects might underestimate the ORs, and therefore the ORs contributed by genetic polymorphism determined in our findings would be conservative.

CYP17, *CYP1A1*, and *COMT* are major susceptibility genes sequentially participating in a pathway of estrogen synthesis and inactivation. To determine whether the profiles of these estrogen-metabolizing genes may be associated with breast cancer, we examined the breast cancer risk associated with combinations of these high-risk genotypes using women with all three putative low-risk genotypes as the reference groups (Table 3). The reference group represented women at least risk of exposure to active CE because of lower estrogen synthesis and greater CE inactivation. The presence of at least one putative high-risk genotype was associated with an increased risk of breast cancer. The risk of breast cancer increased significantly as the number of putative high-risk genotypes increased ($P = 0.006$, based on the Mantel extension test for a linear trend). Notably, none of the controls harbored all three high-risk genotypes. Similarly, none of the controls had the high-risk genotypes of both *CYP17* and *CYP1A1*, although these two genes were considered to predispose to a relatively minor risk compared with that for *COMT*.

If these susceptibility genes were associated with breast cancer development via the hypothesized mechanism involving estrogen metabolism, the relationship between cancer risk and susceptibility genotypes would be expected to be more significant in the subset of women with a longer period of estrogen exposure or higher estrogen levels. We therefore investigated the potential importance of estrogen exposure in conjunction with the three susceptibility genotypes. Our suggestion is supported by the findings shown in Table 4, which showed that estrogen might modify the association between the number of high-risk genotypes and elevated cancer risk. A consistently significant association of an increased cancer risk predisposed by high-risk genotypes was seen in women with longer years of total estrogen exposure (≥30 years), greater duration from age at menarche to FFTP (≥10 years), or younger age at menarche (≤13 years; all $P < 0.05$). In contrast, among women with a shorter duration of estrogen exposure, a shorter duration from menarche to FFTP, or an older age at menarche, there was no significant association (all $P > 0.05$). Furthermore, the increased cancer risk conferred by high-risk genotypes was significant in women with a higher BMI (≥22.5), but not in those with a lower BMI (Table 4).

DISCUSSION

A complete understanding of the etiological role of estrogen in breast tumorigenesis will require studies that evaluate both the genes participating in estrogen metabolism and the extent to which estrogen exposure modifies the associations of these genes with breast cancer risk. This understanding is likely to emerge slowly as research is extended from single-gene studies to multigenic or to etiological pathway-wide studies. Thus, the discrepancies regarding the degree and nature of cancer risk related to various genetic polymorphisms among current studies are not surprising. In fact, several previous studies have shown no evidence of a relationship between breast cancer and the high-risk genotypes of *CYP17*, *CYP1A1*, and *COMT* or have shown inconsistent results (*e.g.*, Refs. 34, 46, and 47). To the best of our knowledge, ours is among the first studies to address the issue of estrogen metabolism in relation to breast cancer risk in a multigenic model. This should allow a more precise evaluation of the risks associated with individual susceptibility genes and a more comprehensive insight into tumorigenesis initiated by estrogen exposure.

Exposure of the breast epithelium to CE, which is suggested to trigger DNA damage and genetic mutations directly (5–7), underlies the tumorigenic mechanism evaluated in the present study. In an attempt to address this issue, we defined the role of susceptibility genotypes as contributing to increased formation of CE via increased biosynthesis of estrogen (*CYP17* A_2/A_2), estrogen hydroxylation (*CYP1A1 Msp*I *vt/vt*), or decreased inactivation of CE via *O*-methylation (*COMT L/L*). Our epidemiological observations fit this model remarkably well. The significant association between the number of high-risk genotypes and breast cancer risk supports the hypothesis that breast cancer can be caused by an initiating effect that is due to CE. The modification of this association by the estrogen exposure profile (*i.e.*, more years of estrogen exposure or early menarche, which

Table 3 *Estimated OR of breast cancer development associated with number of high-risk genotypes of estrogen-metabolizing genes*[a]

No. of high-risk genotypes					
CYP17	*CYP1A1*	*COMT*	No. of cases (%)	No. of controls (%)	OR (95% CI)
No putative high-risk genotype					1.00 (Ref)[b]
A_1/A_1, A_1/A_2	*wt/wt, wt/vt*	*H/H, H/L*	47 (44.3)	69 (58.0)	
One putative high-risk genotype					1.47 (0.81–2.66)
A_2/A_2	*wt/wt, wt/vt*	*H/H, H/L*	27 (25.5)	30 (25.2)	
A_1/A_1, A_1/A_2	*vt/vt*	*H/H, H/L*	4 (3.8)	3 (2.5)	
A_1/A_1, A_1/A_2	*wt/wt, wt/vt*	*L/L*	14 (13.2)	12 (10.1)	
Two putative high-risk genotypes					3.52 (1.06–12.4)
A_2/A_2	*vt/vt*	*H/H, H/L*	2 (1.9)	0 (0)	
A_2/A_2	*wt/wt, wt/vt*	*L/L*	7 (6.6)	5 (4.2)	
A_1/A_1 A_1/A_2	*vt/vt*	*L/L*	3 (2.8)	0 (0)	
All three putative high-risk genotypes					
A_2/A_2	*vt/vt*	*L/L*	2 (1.9)	0 (0)	—

[a] P for trend = 0.006.
[b] Ref, reference group.

Table 4 *aOR of breast cancer development associated with having additional one high-risk genotype of estrogen-metabolizing genes, stratified by risk factors of estrogen exposure or BMI*

Risk factor	Case (%)	Control (%)	aOR[a] (95% CI)
Total years of estrogen exposure[b]			
≥30 yrs	85 (61.2)	83 (62.4)	1.74 (1.05–2.95)
<30 yrs	54 (38.8)	50 (37.6)	1.65 (0.82–3.51)
Years of estrogen exposure before FFTP[c]			
≥10 yrs	95 (68.4)	61 (45.9)	1.70 (1.01–1.03)
<10 yrs	44 (31.6)	72 (54.1)	1.63 (0.81–3.39)
Age at menarche			
≤13 yrs	57 (41.0)	38 (28.6)	1.83 (1.00–3.66)
>13 yrs	82 (59.0)	95 (71.1)	1.58 (0.89–2.87)
BMI (kg/m^{-2})			
≥22.5	66 (47.5)	89 (66.9)	1.91 (1.07–3.51)
<22.5	73 (52.5)	44 (33.1)	1.42 (0.78–2.68)

[a] aOR of breast cancer development associated with the number of high-risk genotypes (of *CYP17, CYP1A1, and COMT)* was calculated in a multivariate logistic regression model containing the number of high-risk genotypes (three *versus* two *versus* one *versus* zero), age, and family history of breast cancer.

[b] For premenopausal women, total years of estrogen exposure = age − age at menarche; for postmenopausal women, total years of estrogen exposure = age at menopause − age at menarche.

[c] For postmenopausal nulliparous women, this index = age at menopause − age at menarche; for other women, this index = age at FFTP − age at menarche.

implies a higher estrogen level during the menstrual cycle) lends additional support to this hypothesis. These results will shed additional light on our understanding of breast tumorigenesis, because although a link between common carcinogens, including cigarette smoke and environmental polyaromatic hydrocarbons, and breast cancer has been suggested, current views on the DNA-damaging agents responsible for breast cancer initiation have been largely inconclusive. Our findings certainly do not exclude the well-established mechanism by which estrogen triggers cell proliferation and tumor promotion. Rather, because only cells undergoing cell division have the potential to fix genetic damage and to accumulate the genomic instability essential for driving cancer development, the dual role of estrogen as both an initiator (*i.e.,* CE) and a promoter (*i.e.,* estradiol) provides a more direct explanation for breast cancer development.

The most inconsistent findings regarding the association between breast cancer risk and *COMT* polymorphisms are those on the extent of risk modification by BMI during different menopausal states. Breast cancer risk has been associated with the *COMT* genotype in three particular subsets of women: (*a*) postmenopausal women with the highest BMI; (*b*) postmenopausal women with the lowest BMI; and (*c*) premenopausal women with the highest BMI (9, 33). Various biological effects of CE causing either direct DNA damage, leading to mutation, or, conversely, growth inhibition due to its metabolite, 2-methoxyestradiol (7), under either high or low estrogen environments have been speculated to partially explain these seemingly opposing findings (33, 48). However, these studies considered the effect of *COMT* in isolation, and the failure to consider the effects of other key factors (*e.g.,* the *CYP1A1* and/or *CYP17* genotypes) may have resulted in an imprecise estimation of the tumorigenic effect of CE, which may also explain, in part, the inconsistent findings. Our findings, which are based on a more comprehensive picture of the entire estrogen-metabolizing pathway, confirmed only that beast cancer risk is associated with the low activity *COMT* genotype in women with a high BMI. Aromatase enzyme releases estrogen from adipose tissue into the circulation (48). Therefore, obese women would have higher levels of circulating estrogen, creating an environment in which the genes participating in the CE production and inactivation pathway are most likely to manifest their tumorigenic effects. Due to the relatively small number of women in this study, further stratification of the study subjects based on their BMI and menopausal status would result in an imprecise estimation of risk. Additional, larger, more powerful studies of women with different menopausal and BMI profiles are needed to address this issue.

If the proposed model of estrogen metabolism correctly describes breast cancer pathogenesis, it would be interesting to examine whether it can explain in part the ethnic variation in breast cancer incidence. The breast cancer incidence in Taiwan (20 of 100,000 women/year) is only about 20–25% that of American whites. However, Taiwanese women have several genetic factors, including higher frequencies of the *CYP17* A_2 and *CYP1A1 Msp*I *vt* alleles, that contribute to increased formation of CE, which would seem to increase the risk of tumors and is inconsistent with the low incidence of breast cancer. Our finding that harboring a high-risk *COMT* genotype is a stronger predictor of breast cancer risk than harboring a high-risk *CYP17* or *CYP1A1* genotype is therefore particularly intriguing and may indicate that inactivation is more important than formation of CE in breast cancer development in Taiwanese women. It is biologically plausible that the breast cancer predisposition conferred by high-risk genotypes of *CYP17* or *CYP1A1* in Taiwanese women is minimized because of a relatively low level of estrogen, which could be related to late menarche, early menopause, low fat or cholesterol intake from Eastern diets, or a combination of these factors. On the other hand, the high frequency of high activity alleles/genotypes of *COMT* in our population [*e.g.,* >95% of controls had *H/H* or *H/L* genotypes in this study *versus* 72–87% in previous reports (9, 33, 34)] should indicate a markedly lower exposure of breast epithelium to CE, which might also explain the reduced risk of breast cancer seen in Taiwanese women.

The present study used a case-control design, which, in theory, might be subject to a variety of biases derived mainly from inappropriate selection of the control group or differential recall bias. However, considerable efforts, including the application of a standardized interview to ensure the validity of information collected by questionnaire, were made to avoid such biases. Our finding that the risk profiles defined in this series of patients (26, 37, 41) were similar to those reported in other breast cancer studies and consistent with the current understanding of breast tumorigenesis provides solid justification of the validity of our study results. The major epidemiological consideration, if any, of the present study was the relatively small sample size. However, as compared with other molecular epidemiological studies addressing similar issues, we do not consider our sample size to have been inadequate to assess the associations of interest. In addition, it should be noted that the 150 case subjects included in this study constituted about 8–10% of the total breast cancer patients annually diagnosed in Taiwan. Finally, the proportion of women with the high-risk *CYP1A1* or *CYP17* alleles was higher in our Taiwanese population than those reported in the Western population. Thus, it should have been easier to obtain adequate statistical power to evaluate the contribution of these two genes. In contrast, only 3–4% of Taiwanese women were found to have the high-risk *COMT* genotype, which is in sharp contrast to the >10% rate reported in previous studies conducted in white populations. However, in the present study, *COMT* was found to be the most significant susceptibility gene associated with elevated breast cancer risk in Taiwan. Thus, the problem of sample size should have had only a minor impact on our findings.

In summary, estrogen and other steroid hormones are undoubtedly involved in the pathogenesis and progression of breast cancer. However, the tumorigenic mechanisms underlying their effects are more complex and go beyond the general concept that they stimulate cell proliferation, which in turn leads to neoplasia. In the present study, we demonstrated that breast cancer may be attributable to susceptibility genotypes of estrogen-metabolizing genes, which lead to increased levels of CE. The elevated cancer risk associated with increased

exposure to CE mediated by susceptibility genotypes observed in the present study may reflect not only a higher level of potentially carcinogenic CE but also a decrease in anticarcinogenic 2-methoxyestradiol concentration, which is converted from CE by COMT (7). Other genes certainly participate in this estrogen-metabolizing pathway. Candidate genes are *CYP1B1* (involved in estrogen hydroxylation), *16α-hydroxylase* (involved in 16α-HE formation), or detoxification enzymes in the glutathione *S*-transferase family (6, 14). A multigenic study on genetic susceptibility with a larger sample size is the best solution to resolve the current inconsistent study results.

REFERENCES

1. Hulka, B. S., and Stark, A. T. Breast cancer: cause and prevention. Lancet, *346:* 883–887, 1995.
2. Kelsey, J. L., Gammon, M. D., and John, E. M. Reproductive factors and breast cancer. Epidemiol. Rev., *15:* 36–47, 1993.
3. Trichopoulos, D., MacMahon, B., and Cole, P. The menopause and breast cancer risk. J. Natl. Cancer Inst., *48:* 605–613, 1972.
4. Pike, M. C., Spicer, D. V., Dahmoush, L., and Press, M. F. Estrogens, progestogens, normal breast cell proliferation, and breast cancer risk. Epidemiol. Rev., *15:* 17–35, 1993.
5. Cavalieri, E. L., Stack, D. E., Devanesan, P. D., Todorovic, R., Dwivedy, I., Higginbotham, S., Johansson, S. L., Patil, K. D., Gross, M. L., Gooden, J. K., Ramanathan, R., Cerny, R. L., and Rogan, E. G. Molecular origin of cancer: catechol estrogen-3,4-quinones as endogenous tumor initiators. Proc. Natl. Acad. Sci. USA, *94:* 10937–10942, 1997.
6. Yager, J. D., and Liehr, J. G. Molecular mechanisms of estrogen carcinogenesis. Annu. Rev. Pharmacol. Toxicol., *36:* 203–232, 1996.
7. Zhu, B. T., and Conney, A. H. Is 2-methoxyestradiol an endogenous estrogen metabolite that inhibits mammary carcinogenesis? Cancer Res., *58:* 2269–2277, 1998.
8. Service, R. F. New role for estrogen in cancer? Science (Washington DC), *279:* 1631–1633, 1998.
9. Lavigne, J. A., Helzlsouer, K. J., Huang, H. Y., Strickland, P. T., Bell, D. A., Selmin, O., Watson, M. A., Hoffman, S., Comstock, G. W., and Yager, J. D. An association between the allele coding for a low activity variant of catechol-*O*-methyltransferase and the risk for breast cancer. Cancer Res., *57:* 5493–5497, 1997.
10. Picado-Leonard, J., and Miller, W. L. Cloning and sequence of the human gene for P450c17 (steroid 17α- hydroxylase/17,20 lyase): similarity with the gene for P450c21. DNA (N. Y.), *6:* 439–448, 1987.
11. 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.
12. 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.
13. Feigelson, H. S., Shames, L. S., Pike, M. C., Coetzee, G. A., Stanczyk, F. Z., and Henderson, B. E. Cytochrome P450c17α gene (*CYP17*) polymorphism is associated with serum estrogen and progesterone concentrations. Cancer Res., *58:* 585–587, 1998.
14. Zhu, B. T., and Conney, A. H. Functional role of estrogen metabolism in target cells: review and perspectives. Carcinogenesis (Lond.), *19:* 1–27, 1998.
15. Fishman, J., Osborne, M. P., and Telang, N. T. The role of estrogen in mammary carcinogenesis. Ann. N. Y. Acad. Sci., *768:* 91–100, 1995.
16. van Aswegen, C. H., Purdy, R. H., and Wittliff, J. L. Binding of 2-hydroxyestradiol and 4-hydroxyestradiol to estrogen receptor human breast cancers. J. Steroid Biochem., *32:* 485–492, 1989.
17. MacLusky, N. J., Barnea, E. R., Clark, C. R., and Naftolin, F. Catechol estrogens and estrogen receptors. *In:* G. R. Merriam and M. B. Lipsett (eds.), Catechol Estrogens, pp. 151–165. New York: Raven Press, 1983.
18. Kabat, G. C., Chang, C. J., Sparano, J. A., Sepkovie, D. W., Hu, X. P., Khalil, A., Rosenblatt, R., and Bradlow, H. L. Urinary estrogen metabolites and breast cancer: a case-control study. Cancer Epidemiol. Biomark. Prev., *6:* 505–509, 1997.
19. Bradlow, L., Telang, N. T., and Osborn, M. P. Estrogen metabolites as bioreactive modulators of tumor initiators and promoters. Adv. Exp. Med. Biol., *387:* 285–296, 1996.
20. Liehr, J. G., and Ricci, M. J. 4-Hydroxylation of estrogens as marker of human mammary tumors. Proc. Natl. Acad. Sci. USA, *93:* 3294–3296, 1996.
21. Fishman, J., and Martucci, C. Biological properties of 16α-hydroxyestrone: implications in estrogen physiology and pathophysiology. J. Clin. Endocrinol. Metab., *51:* 611–615, 1980.
22. Taioli, E., Trachman, J., Chen, X., Toniolo, P., and Garte, S. J. A *CYP1A1* restriction fragment length polymorphism is associated with breast cancer in African-American women. Cancer Res., *55:* 3757–3758, 1995.
23. Garte, S., Zocchetti, C., and Taioli, E. Gene-environment interactions in the application of biomarkers of cancer susceptibility in epidemiology. *In:* P. Toniolo, P. Boffetta, D. E. G. Shuker, N. Rothman, B. Hulka, and N. Pearce (eds.), Application of Biomarkers in Cancer Epidemiology, pp. 251–264. Lyon, France: IARC, 1997.
24. Ambrosone, C. B., Freudenheim, J. L., Graham, S., Marshall, J. R., Vena, J. E., Brasure, J. R., Laughlin, R., Nemoto, T., Michalek, A. M., Harrington, A., Ford, T. D., and Shields, P. G. Cytochrome P4501A1 and glutathione *S*-transferase (M1) genetic polymorphisms and postmenopausal breast cancer risk. Cancer Res., *55:* 3483–3485, 1995.
25. Ishibe, N., Hankinson, S. E., Colditz, G. A., Spiegelman, D., Willett, W. C., Speizer, F. E., Kelsey, K. T., and Hunter, D. J. Cigarette smoking, cytochrome *P4501A1* polymorphisms, and breast cancer risk in the Nurses' Health Study. Cancer Res., *58:* 667–671, 1998.
26. Huang, C. S., Shen, C. Y., Chang, K. J., Hsu, S. M., and Chern, H. D. Cytochrome P4501A1 polymorphism as a susceptibility factor for breast cancer in postmenopausal Chinese women in Taiwan. Br. J. Cancer, *80:* 1838–1843, 1999.
27. Petersen, D. D., McKinney, C. E., Ikeya, K., Smith, H. H., Bale, A. E., McBride, O. W., and Nebert, D. W. Human *CYP1A1* gene: cosegregation of the enzyme inducibility phenotype and an RFLP. Am. J. Hum. Genet., *48:* 720–725, 1991.
28. Landi, M. T., Bertazzi, P. A., Shields, P. G., Clark, G., Lucier, G. W., Garte, S. J., Cosma, G., and Caporaso, N. E. Association between *CYP1A1* genotype, mRNA expression and enzymatic activity in humans. Pharmacogenetics, *4:* 242–246, 1994.
29. Malins, D. C., Holmes, E. H., Polissar, N. L., and Gunselman, S. J. The etiology of breast cancer: characteristic alterations in hydroxyl radical-induced DNA base lesions during oncogenesis with potential for evaluating incidence risk. Cancer (Phila.), *71:* 3036–3043, 1993.
30. Mallins, D. C., Polissar, N. L., and Gunselman, S. J. Progression of human breast cancers to the metastatic state is linked to hydroxyl radical-induced DNA damage. Proc. Natl. Acad. Sci. USA, *93:* 2557–2563, 1996.
31. Friedberg, E. C., Walker, G. C., and Siede, W. DNA Repair and Mutagenesis, pp. 1–58. Washington DC: ASM Press, 1995.
32. Lachman, H. M., Papolos, D. F., Saito, T., Yu, Y. M., Szumlanski, C. L., and Weinshilboum, R. M. Human catechol-*O*-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. Pharmacogenetics, *6:* 243–250, 1996.
33. Thompson, P. A., Shields, P. G., Freudenheim, J. L., Stone, A., Vena, J. E., Marshall, J. R., Graham, S., Laughlin, R., Nemoto, T., Kadlubar, F. F., and Ambrosone, C. B. Genetic polymorphisms in catechol-*O*-methyltransferase, menopausal status and breast cancer risk. Cancer Res., *58:* 2107–2110, 1998.
34. Millikan, R. C., Pittman, G. S., Tse, C. K. J., Duell, E., Newman, B., Savitz, D., Moorman, P. G., Boissy, R. J., and Bell, D. A. Catechol-*O*-methyltransferase and breast cancer risk. Carcinogenesis (Lond.), *19:* 1943–1947, 1998.
35. Nakachi, K., Imai, K., Hayashi, S., and Kawajiri, K. Polymorphism of the CYP1A1 and glutathione *S*-transferase genes associated with susceptibility to lung cancer in relation to cigarette dose in a Japanese population. Cancer Res., *53:* 2994–2999, 1993.
36. Bernstein, L., Yuan, J. M., Ross, R. K., Pike, M. C., Hanisch, R., Lobo, R., Stanczyk, F., Gao, Y. T., and Henderson, B. E. Serum hormone levels in pre-menopausal Chinese women in Shanghai and white women in Los Angeles: results from two breast cancer case-control studies. Cancer Causes Control, *1:* 51–58, 1990.
37. Yang, P. S., Yang, T. L., Liu, C. L., Wu, C. W., and Shen, C. Y. A case-control study of breast cancer in Taiwan. Br. J. Cancer, *75:* 752–756, 1997.
38. Lou, M. A., Tseng, S. L., Chang, S. F., Yue, C. T., Chang, B. L., Chou, C. H., Yang, S. L., The, B. H., Wu, C. W., and Shen, C. Y. Novel patterns of p53 abnormality in breast cancer from Taiwan: experience from a low incidence area. Br. J. Cancer, *75:* 746–751, 1997.
39. Lo, Y. L., Yu, J. C., Huang, C. S., Tseng, S. L., Chang, T. M., Chang, K. J., Wu, C. W., and Shen, C. Y. Allelic loss of the *BRCA1* and *BRCA2* genes and other regions on 17q and 13q in breast cancer among women from Taiwan (area of low incidence but early onset). Int. J. Cancer, *79:* 580–587, 1998.
40. Tseng, S. L., Yu, J. C., Yue, C. T., Chang, S. F., Chang, T. M., Wu, C. W., and Shen, C. Y. Allelic loss at *BRCA1*, *BRCA2* in relation to *TP53* abnormality in breast cancer. Genes Chromosomes Cancer, *20:* 377–382, 1997.
41. Huang, C. S., Chern, H. D., Shen, C. Y., Hsu, S. M., and Chang, K. J. Association between *N*-acetyl-transferase 2 (*NAT2*) genetic polymorphism and development of breast cancer in postmenopausal Chinese women in Taiwan, an area of great increase in breast cancer incidence. Int. J. Cancer, *82:* 175–179, 1999.
42. Kelsey, J. L. Breast cancer epidemiology: summary and future directions. Epidemiol. Rev., *15:* 36–47, 1993.
43. Kleinbaum, D. G., Kupper, L. L., and Morgenstern, H. Epidemiologic Research. New York: Van Nostrand Reinhold, 1982.
44. Russo, J., Tay, L. K., and Russo, I. H. Differentiation of the mammary gland and susceptibility to carcinogenesis. Breast Cancer Res. Treat., *2:* 5–73, 1982.
45. MacMahon, B., Trichopoulos, D., Brown, J., Andersen, A. P., Cole, P., deWaard, F., Kauraniemi, T., Polychronopoulou, A., Ravnihar, B., Stormby, N., and Westlund, K. Age at menarche, urine estrogens and breast cancer risk. Int. J. Cancer, *30:* 427–431, 1982.
46. 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.
47. Bailey, L. R., Roodi, N., Verrier, C. S., Yee, C. J., Dupont, W. D., and Parl, F. F. Breast cancer and *CYP1A1*, *GSTM1*, and *GSTT1* polymorphisms: evidence of a lack of association in Caucasians and African Americans. Cancer Res., *58:* 65–70, 1998.
48. Bernstein, L., and Ross, R. K. Endogenous hormones and breast cancer risk. Epidemiol. Rev., *15:* 48–65, 1993.