行政院國家科學委員會專題研究計畫成果報告

兒茶酚氧位甲基轉移遺傳　多形性為台灣乳癌致癌感受性因子

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INTRODUCTION.

Both epidemiologic 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 first full-term pregnancy (1, 2,3), are operative by way of 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 showed 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 epidemiologic study sought to determine if polymorphisms in the genes involved in estrogen biosynthesis (*CYP17*) and hydroxylation (*CYP1A1*) and inactivation of the reactive metabolites (*COMT*) may be associated with elevated risk of breast cancer, and if the association between genotypes and risk may be modified by estrogen exposure.

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, 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 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.

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 drinking alcohol and smoking cigarettes, ethnic background, residence area, family income and education level. The body mass index (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, prior to treatment, and from each control subject. The buffy coats of these specimens were prepared immediately and stored at -80^{o}C until extraction of the genomic DNA. Genomic DNA was obtained by conventional phenol/chloroform extraction, followed by ethanol precipitation, and was stored at -20^{o}C until genotype analysis.

PCR-based RFLP assays (9,10,12) were used to determine the *CYP17*, *CYP1A1* and *COMT* genotypes of the subjects.

Statistical analysis. Univariate and multi-variate 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 (12,15). 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 Msp1* variant and/or *COMT* low activity (*L*) allele, were considered to be at higher risk of cancer. The association of susceptibility genotypes and breast cancer risk was evaluated with simultaneous consideration of established risk factors for breast cancer or other significant risk factors, in a multivariate logistic regression models. Biologic 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: age, family history of breast cancer, age at menarche and age at FFTP (16). The history of HRT was also included in the model for postmenopausal women, since our previous studies (12,15) demonstrated a significant effect of this factor in determining breast cancer risk in this series of study subjects. A backward elimination procedure (17) was used to select the optimal model, and multivariate-adjusted ORs (aORs) and their 95% CIs were estimated. All p values 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 indexes to estimate the estrogen exposure level: (i) total estrogen exposure years (representing the number of years exposed to menstrual cycles). It was calculated according to the age at menarche and age at interview for premenopausal women, and ages at the time of menarche and menopause for post-menopausal women; (ii) the number of years between menarche and FFTP. A more advanced age at FFTP is generally accepted as a major risk factor for breast 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 (18); this suggests that the period between menarche and the age at FFTP may be also critical; (iii) the age at menarche. This was used because women whose menarche occurred early have higher levels of estrogen during menstrual cycle (19), as well as a longer duration of exposure to estrogen; (iv) the BMI, as 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 indexes.

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 (12,15). The frequency distributions of the genetic polymorphisms of *CYP17*, *CYP1A1,* and *COMT* are shown in Table 1. Association between the various polymorphisms and breast cancer varied in women having heterozygous wild-type genotypes (*CYP17* A_1/A_2, *CYP1A1 Msp1 wt/vt,* and *COMT H/L*). In contrast, the risk of breast cancer was consistently elevated in those harboring homozygous variants of the individual genes (*CYP17* A_2/A_2, *CYP1A1 Msp1 vt/vt* and *COMT L/L*). Because there is very little conclusive evidence in the literature regarding the phenotypic manifestations of heterozygous wild-type susceptibility genes, and because the absence of a gene-dose effect is common in this type of genotype-based study (13), 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 Msp1 vt/vt* and *COMT L/L*) were considered as high-risk genotypes; this basis for definition has been used in previous molecular epidemiologic studies. Overall, an increased risk of breast cancer associated with individual high-risk genotypes was consistently found (Table 1). Individually, breast cancer risk associated with susceptibility genotypes varied for the three genes, being 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 of 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 the risk.

To comprehensively assess the individual contribution of *CYP17, CYP1A1* and *COMT* in the association with breast cancer development, logistic regression analysis considering the effects of individual genes simultaneously was performed (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 epidemiologic 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 proportion of control subjects had a family history of breast cancer (12), 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 only apply to genes with high penetrance, such as *BRCA1* and *BRCA2*. For low-penetrance genes, such as *CYP17, CYP1A1* and *COMT*, the effect might be relatively minor, if any. Furthermore, any such over-representation of genetic predisposition in our control subjects might underestimate the odds ratios, and therefore the odds ratios 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 with women having all three putative low-risk genotypes as the reference groups (Table 3). The reference group represented women least at risk to expose 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 significantly increased as the number of putative high-risk genotypes increased (P based on the Mantel-extension test for a linear trend = 0.006). 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 to 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 that 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 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 who having 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 those with a shorter duration of estrogen exposure, shorter duration from menarche to FFTP, or 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 full understanding of the etiologic 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 showed inconsistent results (e.g. 14,20,21). 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 strength should allow more precise evaluation of the risks associated with individual susceptibility genes and 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*) or estrogen hydroxylation (*CYP1A1 Msp1 vt/vt*), or decreased inactivation of CE, via *O*-methylation (*COMT L/L*). Our epidemiologic 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 estrogen exposure profile (i.e. more years of estrogen exposure or early menarche which implies a higher estrogen level during the menstrual cycle) lends additional support to this hypothesis. These results will shed further light on our understanding of breast tumorigenesis, since, although a link between common carcinogens, including cigarette smoke and environmental polyaromatic hydrocarbons, and breast cancer has been suggested, current views on the agents causing DNA damage 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, both as an initiator (i.e. CE) and a promoter (i.e. estradiol), provides a more direct explanation for breast cancer development.

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 a decrease in anti-carcinogenic 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), *16~-hydroxylase* (involved in 16α-HE formation) or detoxification enzymes in the glutathione *S*-transferase (*GST*) family (6,11). A multigenic study on genetic susceptibility with a larger sample size is the best solution to resolve the current inconsistent study results.

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

Genotype of estrogen-metabolizing gene	No. Cases (%)	No. Control (%)	OR(95%CI)	aOR(95%CI)[a]
Total women				
CYP17				
A_1/A_1	25(20.3)	28(22.2)	1.00(ref)	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]Adjusted odds ratio and 95% confidence intervals were calculated by logistic regression models containing breast cancer risk factors. Risk factors adjusted for were age, family history of breast cancer, age at menarche, age at first full-term pregnancy, and history of hormone replace therapy in the groups of total women and postmenopausal women. For premenopausal women, the history of hormone replace therapy was not included in the model.

Table 2. Unconditional logistic regression analysis of genotypic 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(years)	0.97	0.94-1.00
Family history of breast cancer (Yes vs. No)	1.39	0.34-5.61
Age at menarche(≤13 vs. >13 years)	1.93	1.05-3.58
Age at first full-term pregnancy) (≥30 or nulliparity vs. <30 years)	2.39	1.13-5.24
History of hormone replacement therapy (Yes vs. No)	4.47	1.58-14.76

Table 3. Estimated odds ratio of breast cancer development associated with number of high-risk genotypes of estrogen-metabolizing genes.

No. of high-risk genotypes					
CYP17	*CYP1A1*	*COMT*	Case(%)	Control(%)	OR(95%CI)
No putative high-risk genotype					1.00(ref)
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)	
					P test for trend=0.006

Table 4. Adjusted odds ratio of breast cancer development associated with having additional one high-risk genotype of estrogen-metabolizing genes, stratified by risk factors of estrogen exposure or body mass index (BMI).

Risk factor	Case(%)	Control(%)	aOR[a](95%CI)
Total years of estrogen exposure[b]			
≥30 years	85(61.2)	83(62.4)	1.74(1.05-2.95)
<30 years	54(38.8)	50(37.6)	1.65(0.82-3.51)
Years of estrogen exposure to age at first full-term pregnancy[c]			
≥10 years	95(68.4)	61(45.9)	1.70(1.01-1.03)
<10 years	44(31.6)	72(54.1)	1.63(0.81-3.39)
Age at menarche			
≤13 years	57(41.0)	38(28.6)	1.83(1.00-3.66)
>13 years	82(59.0)	95(71.1)	1.58(0.89-2.87)
Body mass index, 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]Adjusted odds ratio 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 (3 vs. 2 vs. 1 vs. 0), 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 first full-term pregnancy - age at menarche.