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CYP17 (T-34C) and *CYP19* (Trp39Arg) Polymorphisms and their Cooperative Effects on Breast Cancer Susceptibility

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Abstract. *Background: Breast cancer is the commonest cancer among women in industrialized countries. Most sporadic breast carcinomas are likely to be caused by low-penetrance genes. Genes encoding enzymes involved in estrogen and carcinogen metabolism are among these low-penetrance genes. In this study, for the first time the T/C (A1/A2) polymorphism at the 5' untranslated region (UTR) of CYP17 and the Arg/Trp (T/C) polymorphism at codon 39 of CYP19 among genes regulating endogenous estrogen levels was studied. Patients and Methods: Fifty-five female breast cancer patients and ninety-one controls took part in the study. DNA was extracted from paraffin-embedded tissues for the patients and from blood cells for the controls. The distribution of genotypes was determined using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) techniques. Results: The frequency of the TC genotype of CYP19 was significantly higher in the patient group ($p<0.001$, κ^2: 12.31, OR: 7.30, 95% CI: 2.29-25.64). CYP17 frequencies were similar to those in Caucasian populations. In combined analysis, when the high risk alleles were evaluated together, the results reached significance ($p=0.006$, $\kappa^2=7.01$, OR: 2.53, %95 CI: 1.26-5.07) for the A2 allele of CYP17 and the C allele of CYP19, being more frequent in the patient group compared to the control. The risk possesed by the TC varient of CYP19 was reduced when evaluated with A1, the protective allele of CYP17 ($p=0.082$). The cumulative protective effects of both A1 allele and the TT genotype were ascertained to occur significantly less frequently in the patient group ($p=0.001$, κ^2: 10.53, OR: 8.47, %95 CI: 1.9-37.04). Conclusion: The results were consistent with the individual studies of CYP17 and CYP19 in the literature, however, in combined analysis of the alleles of the two genes, the frequency of high risk alleles was higher and the frequencies of low risk alleles were lower in the patient group. The CYP17 A1 + CYP19 TT haplotype may be protective for breast cancer.*

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Breast cancer is the most abundant cancer among women in industrialized countries (1). Only 10% of the cases are heritable and caused by high-penetrance cancer susceptibility genes, such as BRCA1 (breast cancer 1) and BRCA2 (breast cancer 2). However, most sporadic breast carcinomas that have relatively late age onset, are likely to be caused by low-penetrance genes acting together with endogenous/lifestyle risk factors. The genes encoding enzymes involved in estrogen and carcinogen metabolism are among these low-penetrance genes (2). Most cases are due to increased or prolonged exposure to estrogen, so polymorphisms of the genes which regulate endogenous estrogen levels are candidates as breast cancer risk factors. In this study, polymorphisms of *CYP19* and *CYP17* among the genes regulating endogenous estrogen levels, were investigated.

CYP17 encodes cytochrome P450-17 (CYP17), which catalyzes the 17α-hydroxylation of pregnenolone and progesterone as well as conversion of C21 steroids to C17. *CYP19* encodes aromatase, a key cytochrome P450 enzyme that converts C19 adrenal androgens to C18 estrogens in the ovarian tissues of premenopausal women and in the adipose tissue of postmenopausal women (3). Polymorphic variation in these genes may effect estrogen synthesis by causing slight changes in aromatase and CYP17 activities (4). Such changes may account for differences in pre- and postmenopausal estrogen levels, which can effect an individual's susceptibility to cancer.

In this study, a mutation causing a T/C nucleotide substitution at position 34 in the 5' untranslated region (UTR) promoter of *CYP17* was studied. These T and C alleles are also named A1 and A2 alleles respectively, hence the mutation creates a recognition site for *Msp*AI restriction

enzyme. It was hypothesized that T/C substitution would result in an additional binding site for *Sp1* promoter causing an increased rate of transcription of enzyme in the A2A2 genotype due to enhanced promoter activity (5). Fiegelson *et al*. and Haiman *et al*. have also found that both premenopausal and postmenopausal women with the A2 allele of *CYP17* have higher levels of estrogens (6-7). The A2 allele has been found to be significantly associated with advanced breast cancer (5), male breast cancer (8), and both postmenopausal (9) and early-onset breast cancer (10).

The other polmorphism we studied is a novel mutation first identified by Miyoshi *et al*. in the *CYP19* gene (11). The T/C polymorphism causes a Trp/Arg amino acid substitution at codon 39 and in their study, Miyoshi *et al*. found a significant association with breast cancer risk among Japanese women (11). Hirose *et al*. have shown that homozygous and heterozygous carriers of the variant C allele have significantly increased risk of breast cancer among premenopausal Japanese women with a late age at first full-term pregnancy or a high body mass index (12).

In all these studies, the *CYP19* T/C and *CYP17* A1/A2 polymorphisms have been considered separately. Here we hypothesize that functionally relevant polymorphisms in such genes would exhibit small, but additive, effects on individual susceptibility to breast cancer. Moreover specific combinations could result in a high-risk profile by influencing lifetime levels of estrogen and also influence breast cancer risk. A case-control study was therefore conducted to analyze the association of *CYP19* T/C and *CYP17* A1/A2 polymorphisms together as breast cancer risk factors. It was also the first study of these polymorphisms among Turkish women.

Materials and Methods

Patient selection. A total of 146 unrelated individuals were included in this study: 55 breast cancer patients, (median age: 56.12±12.35, range 31-79 years) and 91 controls, (median age: 37.77±12.19, range 20-91 years). The patients were selected from the Department of Pathology, Istanbul University Cerrahpasa Medicine School, Istanbul from 2002-2007. Among the patients, 47 had invasive ductal, 3 had invasive lobular, 3 had invasive ducto-lobular carcinomas, one had muscinous breast carcinoma and one had medullar breast carcinoma. There was no statistical relation between the pathological features and the polymorphisms (data not shown). The control group was selected from healthy female blood donors.

DNA extraction. The patients' DNA was extracted from paraffin-embedded tissue with the use of a method taken from Wright and Manos (13). Blood specimens of the control group were collected in tubes containing EDTA, and DNA was prepared from leukocyte pellets by SDS lysis, ammonium acetate extraction and ethanol precipitation (14).

Genotyping method for CYP17 and CYP19. For genotyping, the DNA extracted from the blood of the controls and from the paraffin-embedded samples from non-tumoral neighboring breast tissue was used. *CYP17* genotypes were determined following polymerase chain reaction (PCR) according to the method of Ambrosone *et al*. (15). Genotyping for the *CYP19* polymorphism was conducted by a new method, PCR with confronting two-pair primers, according to the method of Hirose *et al*. (12).

Table I. *Allelic variant frequencies of CYP17 and CYP19 polymorphisms among breast cancer patients and controls.*

	Genotype	Controls		Breast cancer	
		N	%	N	%
CYP17	A1A1	38	41.8	18	32.7
	A1A2	44	48.4	27	49.1
	A2A2	9	9.9	10	18.2
	A1	-	66.0	-	57.0
	A2	-	34.0	-	43.0
CYP19	TT	27	29.7	3	5.5
	TC	64	70.3	52	94.5
	CC	-	-	-	-
	T	-	65.0	-	53.0
	C	-	35.0	-	47.0

Statistical analysis. Statistical analyses were performed using SPSS version 7.5 (SPSS Inc. Chicago, USA) including the Chi-square (κ^2) test, Fisher's exact test and the Pearson correlation test, odds ratios (ORs) and 95% confidence intervals (95% CI) were calculated.

Results

The allelic varient frequencies of *CYP17* and *CYP19* polymorphisms are shown on Table I. For *CYP17*, the frequencies showed no significant association between the patient and control group, the A1A1 genotype was slighlty higher in the control group and the frequency of the A2A2 genotype was double in the patient group. Carrying the A2 allele increased the breast cancer risk 1.4-fold ($p>0.05$, OR: 1.47, 95% CI: 0.73-2.96). The frequency of the heterozygote genotype was almost the same in both the patient and control groups. When the groups were statistically compared for the *CYP19* polymorphism, TC variance in the patient group for *CYP19* the gene was found to be very highly significant ($p<0.001$; $\kappa^2=12.31$; OR=7.30; 95% CI=2.29-25.64). The TC genotype was 20% higher in the patient group. None of the participants had the CC genotype. Also the frequency of the TT genotype was six-fold increased in the controls. Therefore the T allele of *CYP19* might be the protective allele.

Combined analysis was conducted to assess the cumulative effects of possible risk and protective attributes of the alleles. First, the higher risk genotypes, carriers of the C alleles of both genes, were evaluated together, and statistical significance was observed ($p=0.006$, $\kappa^2=7.01$, OR: 2.53, 95% CI: 1.26-5.07) (Figure 1). This finding demonstrated that possession of the A2 allele of *CYP17* supported the risk

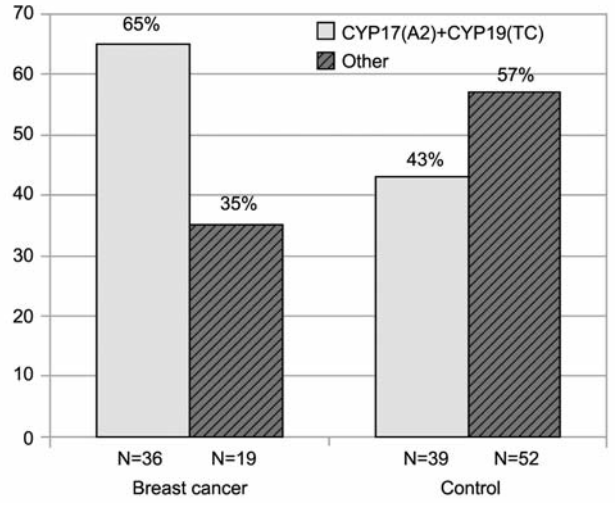

Figure 1. *Comparison of individuals carrying the A2 allele of CYP17 the TC genotype of CYP19 with the other haplotypes (p=0.006, κ²: 7.01; OR: 2.53, 95% CI: 1.26-5.07).*

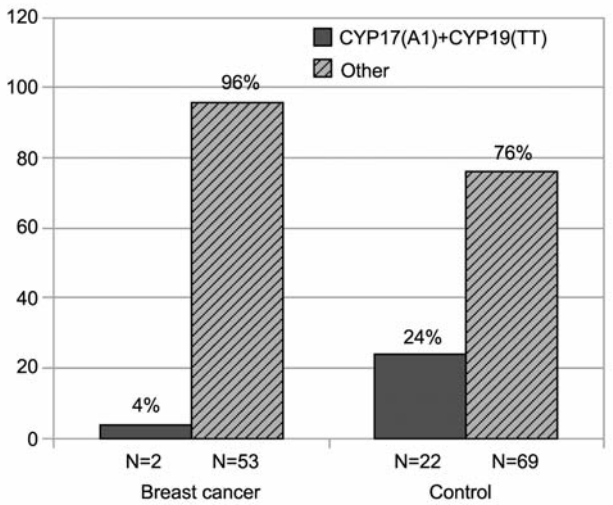

Figure 2. *Comparison of individuals carrying the A1 allele of CYP17 and the TT genotype of CYP19 with the other haplotypes (p=0.082; OR: 1.47, 95% CI: 0.73-2.96).*

increased by possession of the TC variant of *CYP19*. The risk of the TC variant of *CYP19* was also reduced when evaluated together with the A1 allele carriers of *CYP17* (p=0.082). The cumulative effect of the protective allele (A1) of *CYP17* and TT allele of *CYP19* was evaluated and was ascertained to be significantly less in the patient group (p=0.001, κ^2: 10.53, OR: 8.47, 95% CI: 1.9-37.04) (Figure 2).

Discussion

CYP17 can play an important role, especially in breast cancer, polycystic ovary syndrome, endometrial cancer and prostate cancer. Genetic variation of *CYP17* causes differences in circulating hormone levels (16, 17). Moreover, *CYP17* may also affect androgen levels, either directly or by means of its effects on estrogen levels (16). Recent studies showed a possible protective association with the A1 allele and a risk association with the A2 allele of *CYP17*. For the first time, Fiegelson *et al*. showed higher serum estrogen and progesterone levels in A2 allele-carrying premenoposal women (6). Haiman *et al*. measured serum hormone fractions in 462 postmenopausal women and the A2A2 subjects had slightly higher levels of estrone (17). Morever, Fiegelson *et al*. showed that women with the A2A2 genotype required less hormone replacement therapy than women with the A1A1 genotype (18). The age at menarche is also partly hormonally mediated (5). Although having a later age menarche is considered as a risk-reducing factor for breast cancer, this effect disappears in women with the A2A2 genotype. The A2 allele of *CYP17* has been associated with an increased risk of breast cancer due to increased lifetime estrogen exposure in sub-groups such as premenopausal, nulliparous women (5). Here no significant relationship between the A2 allele of *CYP17* and breast cancer was found. The A2A2 genotype frequency of the control group (9.9%) was similar to that in the Caucasian population (8-17%) (16), while in the patient group, the A2A2 allele frequency was 18.2 %. Significance might have been reached with a larger population. The A2 allele frequency differs in Asian and Caucasian populations and is higher than that of the A1 allele in Asian populations. Thus the *CYP17* polymorphism cannot be directly associated with breast cancer (16). Few studies have investigated the relationship of *CYP17* polymorphisms with other breast cancer and estrogen metabolism related genes. However, when compared cumulatively with *CYP19* polymorphisms in the present study, significance was reached, suggesting cooperation between *CYP17* and *CYP19* in breast cancer development. It is plausible that both risky and protective traits of *CYP17* and *CYP19* occur in breast cancer.

The *CYP19* T/C is a rare polymorphism, only a few Hawaiians (2.1%) and Japanese (2.9) have the homozygote mutant genotype (19). Thus, with a Caucasian population, the CC genotype of *CYP19* was not observed in the present study. Miyoshi *et al*. (11) and Hirose *et al*. (12) found an association between C allele-bearing and breast cancer risk for premenopausal Japanese women and the present results also corroborate that data. In Figure 1, the TT haplotype appeared to be the protective haplotype. Bearing the C allele increased the breast cancer risk markedly.

This study for the first time examined the association between the *CYP17 Msp*A1 polymorphism and the *CYP19* T/C polymorphism and breast cancer risk. In combined analysis, the protective and risky attributes of the alleles were obvious.

When the TC genotype of *CYP19* together with the *CYP17* A2 allele was evaluated, it was significantly more frequent in the patient group (p=0.006, κ^2: 7.01, OR: 2.53, % CI: 1.26-5.07). This means that carrying the A2 allele of *CYP17* supported the increased risk in the C allele carriers of *CYP19*. Another interesting finding was that the risk constituted by carrying the C allele of *CYP19* was reduced in the carriers of the A1 allele of *CYP17* (p=0.082). The frequency of both the A1 variant of *CYP17* and the TT genotype of *CYP19* combination was statistically lower in the patient group (p=0.001, κ^2: 10.53, OR: 8.47, 95% CI: 1.9-37.04), thus these haplotypes might be protective against breast cancer.

In conclusion, the results were consistent with the individual studies of *CYP17* and *CYP19* in the literature, however, in combined analysis, the frequency of the higher risk alleles was higher and the frequencies of the low risk alleles lower in the patient groups. The enhanced risk and enhanced protectivity of the variants suggest that these low penetrance genes may act together. Therefore, more studies including other low penetrance genes in the same individuals should be studied with larger groups. Studies in the literature have been conducted in hospital groups, therefore it is not reliable to regard these data as population frequencies. Population studies of both polymorphisms should be conducted.

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