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CYP17 gene polymorphism and its association with high-risk north Indian breast cancer patients

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Abstract A single T > C change at the 5′ promoter region of the CYP17 gene is reported to be associated with increased risk of breast cancer. This study evaluates the influence of genetic polymorphism of CYP17 on breast cancer susceptibility. Two hundred and forty-two patients with histopathologically confirmed breast cancer and 212 age-matched controls were included in the present study. Information relating to age at onset of the disease, family history and estrogen receptor status was elicited. Investigation for CYP17 polymorphism was carried out in 106 early onset, 80 late onset and 56 familial cases. The frequencies of two CYP17 alleles were also analyzed in 116 (47.9%) cases with known estrogen receptor (ER) status confirmed immunohistochemically. A polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method was used to detect the polymorphism, and the genotypes identified were assigned as homozygous wild type (A1A1), heterozygous variant (A1A2), and homozygous variant (A2A2). Associations between the various genotypes in patients and controls were investigated with Fisher's exact test. All the tests were two tailed. The results showed that the frequency of heterozygous and homozygous CYP17 genotype was higher in early onset breast cancer patients (94.3%) than in controls (80.3%), and the difference was significant ($P = 0.001$). A highly statistically significant increased risk in carriers of homozygous A2 allele was found in young patients ($P \leq 0.001$) in comparison with patients having late onset condition ($P = 0.260$). However, no significant association between the genotype and breast cancer risk was observed among women with strong family history. Further, data had showed that patients (80.6%) with at least one A2 allele tended to exhibit ER-independent cell proliferation, although statistical significance could not be established ($P = 0.160$). The present findings suggest that CYP17 A2 allele gene polymorphism might play a significant role in breast cancer development in young Indian women.

Keywords CYP17 gene · Polymorphism in north Indian population · Early onset breast cancer · Familial breast cancer · Correlation with ER status

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Introduction

The breast is the second most common site of cancer in Indian women after cervix uteri. The average age of Indian breast cancer patients has been reported to be 50–53 years (National Cancer Registry Programme [NCRP] 2001) in various population-based registries located in the different parts of the country, whereas, in US white women, the average age of occurrence has been reported to be 61 years (Parkin et al. 2002). This reveals that the disease occurs a decade earlier in the

Indian population than in western countries. Thus, the reasons for the early age of occurrence amongst Indian women need to be studied.

The etiology of breast cancer in younger and older women differs in terms of inheritance, carcinogenesis and prognosis (Nixon et al. 1994; Bonnier et al. 1995). Hormonal risk is one of the non-genetic factors that may contribute to the disease's etiology. Several clinical, epidemiological and experimental studies have proved that estrogen and progesterone play a major role in the growth of normal breast tissue (Lupulescu 1995; Berstein and Press 1998), and breast cancer risk is strongly related to exposure to these endogenous steroid hormone levels (Pike et al. 2004), specifically estrogen (Feigelson and Henderson 1996). Although the exact mechanisms remain to be fully elucidated, the alkylation of cellular molecules and the generation of active radicals that can damage DNA (Nandi et al. 1995), together with the potential genotoxicity of estrogen and some of its metabolites (e.g. the catechol estrogens) (Nandi et al. 1995; Yager and Liehr 1996), have been implicated.

According to some studies, genetic polymorphism in genes related to the biosynthesis and metabolism of hormones and polycyclic aromatic hydrocarbons is associated with an altered risk of breast cancer and other types of cancer (Zhong et al. 1993; Abrosone et al. 1995; Taioli et al. 1995; Feigelson et al. 1997; Lavigne et al. 1997; Thompson et al. 1998). It is assumed that, among ethnic groups, genetic differences exist that effect steroid hormone metabolism and transport. Because cumulative exposure to circulating estrogen has been linked to increased risk of breast cancer, interest has been focused on the enzymes regulating the biosynthetic and metabolic pathways of these hormones.

The estrogen-forming ovary expresses the CYP17 gene (Hanukoglu 1992), which codes for the cytochrome P450c17a enzyme and is located on chromosome 10, spans 6,569 bp, and is divided into eight exons (Picado-Leonard and Miller et al. 1987). The cytochrome P450c17a enzyme mediates both steroid 17a-hydroxylase and 17,20 lyase activities and functions at key branch points in human steroid genesis (Brentano et al. 1990). In steroidogenesis, cholesterol is first converted to pregnenolone, by an enzyme encoded by CYP11a, and then to progesterone by 3β-hydroxysteroid dehydrogenase; 17a-hydroxylase converts pregnenolone and 17-hydroxyprogesterone, respectively. A single polymorphism, T- > C, in the CYP17 gene, which codes for the above enzyme involved in estrogen synthesis, creates an additional Sp I- type promoter site (CCACC) 34 bp upstream of the initiation of translation but downstream of the transcription start site (Carey et al. 1994), which is associated with increased estrogen levels. The base pair change also creates a recognition site for the MspA1 digestion of a polymerase chain reaction (PCR) product that has been used to designate arbitrarily two alleles, A1 and A2. It has been reported that women who are heterozygous or homozygous for the CYP 17 A2 allele show comparatively higher serum levels of estradiol than do women with an A1/A1 genotype (Feigelson et al. 1998).

To investigate the role of CYP17 gene polymorphism in high-risk Indian breast cancer patients, and mainly targeting early onset breast cancer and familial cases, we studied a total of 242 cases for CYP 17 gene polymorphism. We analyzed frequencies of the two CYP 17 alleles in (a) a group of young breast cancer patients (≤40 years) and late-onset cancer patients and (b) familial breast cancer patients. We additionally investigated whether expression of steroid receptor has any association with CYP17 polymorphism.

Materials and method

Study population

Two hundred and forty-two patients with histologically confirmed breast cancer, referred from the departments of Surgery and Cancer Surgery of Safdarjang Hospital, New Delhi, and LLRM Medical College, Meerut, during 1999–2003, were selected for the study.

Of the 242 breast cancer patients, 106 were young (below 40 years), 80 (above 40 years) had a late-onset condition, and 56 had a family history of cancer of the breast, or ovary, or both. The patient's ages ranged from 13 years to 78 years, with a mean age of 41.9 years (SD ± 11.8 years).

Informed consent was obtained from all the participating patients, and the study was carried out with the approval of the Ethics Review Committee of Safdarjang Hospital, New Delhi.

A series of 212 age-matched (±2 years) controls, matched for early (106) and late (80) age breast cancer, was selected from the women attending antenatal checkups and from blood bank donors in Delhi. For some of the cases more than one control was selected. The mean age of controls was 39.9 years (SD ± 11.5 years). While selecting controls we took care to see that no differences existed with regard to the ethnicity of the breast cancer patients. No separate controls with a family history were selected for the patients with a family history of breast cancer, and the comparison was

carried out with the same group of control subjects selected earlier according to early and late onset. The mean ages between breast cancer patients with a family history and the control series were almost similar. Here, it can be said, it was more similar to frequency matching.

Estrogen receptor (ER) status was estimated immuno-histochemically in 116 cases; 93 (80.1%) ER negative and 23 (19.8%) ER positive cases were included in the present study to find out the association between estrogen receptor status and CYP17 genotype.

Blood sample collection

Peripheral blood samples (ca.10 ml) from all patients and controls were collected into EDTA vials. The buffy coat was separated and frozen at –70°C for further use.

DNA extraction

Genomic DNA was extracted from peripheral blood lymphocytes by the standard phenol–chloroform extraction method. Blood was first digested with lyses buffer I (30 mM Tris, 5 mM EDTA and 50 mM NaCl) and lyses buffer II (20% SDS, 100 μg/ml Prot.K), followed by extraction with Tris-saturated phenol and chloroform-isoamyl alcohol (24:1), and finally recovered by ethanol precipitation.

PCR reaction

Briefly, a PCR fragment containing the base pair change was generated using the following primers: CYP17 F-5′CATTCGCACTCTGGAGTC 3′ and CYP17 R-5′AGGCTCTTGGGGGTACTTG 3′.

The PCR reactions were carried out in 25 μl aliquots containing approximately 50 ng genomic DNA, 20 pmole of each primer, 1 × reaction buffer, 100 μM deoxynucleotide triphosphates, and one unit of Taq polymerase. The reaction was performed in an Eppendorf thermal cycler, and the DNA was denatured at 94°C for 5 min and amplified during 30 cycles of 94°C for 1 min, annealing at 57°C for 1 min, extension at 72°C for 1 min, with final extension at 72°C for 5 min.

Restriction fragment length polymorphism analysis

The PCR products were digested with MspA1 at 37°C for 3 h. The reaction mixture was as follows: 15 μl of PCR product, 2.5 μl of digestion buffer, 0.25 μl of BSA and 0.5 μl of restriction enzyme MspA1. The digested

fragments were visualized on 2% agarose gel with ethidium bromide staining to identify the base pair change.

Statistical analysis

Fisher's exact test was applied to find out the association between breast cancer and CYP 17 genotype for all cases combined together and by onset condition and family history.

Furthermore, the allelic frequencies in the control population were calculated by the χ^2 test to find out the consistency with the Hardy–Weinberg equilibrium.

Results

We examined the T > C polymorphism of the CYP17 gene in both sporadic and familial breast cancer and in a suitable control population. Analysis was conducted for 242 cases, including 106 cases of early onset, 80 of late onset, and 56 familial cases, together with 212 age-matched controls.

Patterns of homozygosity and heterozygosity of the A2 allele and homozygosity of the A1 allele are illustrated in Figs. 1 and 2. Significance was calculated to evaluate the risk of breast cancer for A2 allele carriers (A2/A2, A1A2 and A1A2 + A2A2). Considering the early onset cases, when the allele frequencies of the CYP17 polymorphism were compared between the affected and control population, we observed a significant difference ($P = 0.001$) (Table 1). Young women heterozygous and homozygous for the A2 allele were four times more likely to have breast cancer. When breast carcinoma cases with A2A2 was considered alone, the P value was found to be highly statistically significant ($P \leq 0.001$). The data showed that 40.5% of the affected population had the A2A2 genotype compared with 25.1% of the control population. Only
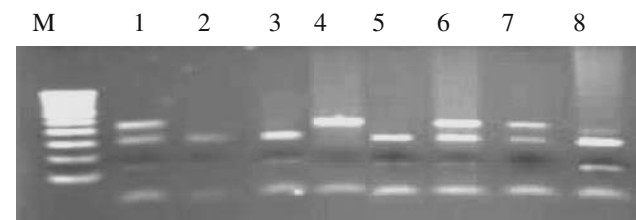

Fig. 1 MspA1 restriction fragments of CYP17 alleles separated by agarose gel electrophoresis. *Lane M* contains a m.w. marker (100-bp ladder) and the homozygous A1 genotype is shown in *lane 4*; the heterozygous pattern in *lanes 1*, *6* and *7* and the homozygous A2 genotype in *lanes 2*, *3*, *5* and *8*
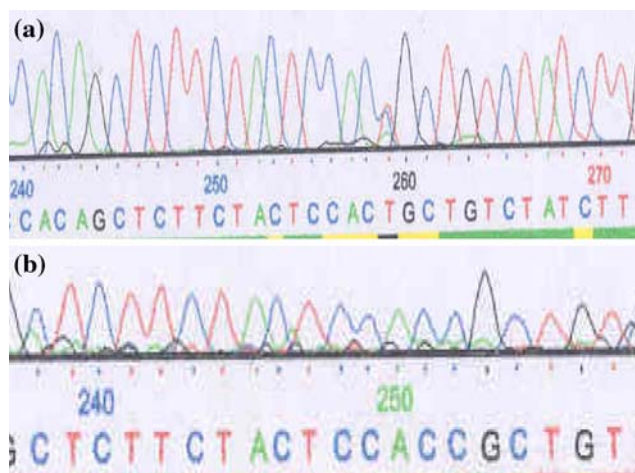
Fig. 2 Electrofluorogram representing (**a**) heterozygous and (**b**) homozygous condition of the CYP17 A2 allele

5.6% of the affected population had the A1 allele, compared with 19.6% of the controls.

The distribution of A2 and A1 alleles and the association with late-onset condition of cancer are shown in (Table 1). Individuals heterozygous for the A2 allele had a P value of 0.382, whereas women homozygous for the A2 allele expressed a slightly higher risk ($P = 0.260$), although statistical significance could not be established.

The statistical significance of breast cancer associated with strong family history stratified by genotype and early or late onset is shown in Table 2. The average age of breast cancer patients with a family history

(33.2 years) and of control subjects (32.1 years) in the early onset group were found to be almost similar. Similarly, even in the late onset group, the mean ages of breast cancer patients (53.9 years, with family history) and controls (51.7 years) were also similar. No obvious association was observed between both early onset familial cases ($P = 0.768$) and late onset familial cases ($P = 0.675$) with breast cancer risk when compared with age-matched controls.

Because of the role of CYP17 in estrogen biosynthesis, we next determined whether the genotype was associated with estrogen receptor status. However, the genotype comparison was not carried out according to early and late onset as it resulted in very few subjects in some of the groups.

The association of the CYP17 genotype with steroid receptor expression in breast cancer tissues is shown in Table 3. Cases were analyzed for a possible connection between estrogen receptor status and an additional promoter site for the CYP17 gene in the malignant cells, but no apparent association was observed between ER status and A2 allele polymorphism, although the study has shown that women with negative ER expression were at twice the risk of breast cancer when associated with the C allele ($P = 0.160$).

Discussion

The response of an organ to the proliferative effects of a hormone may be a progression from normal growth

Table 1 Distribution of CYP17 genotype frequencies in early and late onset breast cancer patients with no family history and in age-matched controls, together with P values (Values in *parentheses* indicate percentages, n number of subjects studied)

Genotype	Early onset			Late onset		
	Breast cancer ($n = 106$)	Controls ($n = 127$)	P	Breast cancer ($n = 80$)	Controls ($n = 85$)	P
A1A2	57 (53.7)	70 (55.1)	0.013	41 (51.2)	40 (47.0)	0.382
A2A2	43 (40.5)	32 (25.1)	<0.001	16 (20.0)	13 (15.2)	0.260
A1A2 + A2A2	100 (94.3)	102 (80.3)	0.001	57 (71.2)	53 (62.3)	0.250
A1A1	6 (5.6)	25 (19.6)	–	23 (28.5)	32 (37.6)	–

Table 2 Distribution of CYP17 genotype frequencies in familial breast cancer patients and non-familial age-matched controls (≤40/>40 years) together with P values (Values in *parentheses* indicate percentages, n number of subjects studied)

Genotype	Early onset familial cases			Late onset familial cases		
	Breast cancer ($n = 22$)	Controls with no family history ($n = 127$)	P	Breast cancer ($n = 34$)	Controls with no family history ($n = 85$)	P
A1A2	12 (54.5)	70 (55.1)	0.756	18 (55.3)	40 (52.6)	0.657
A2A2	7 (31.8)	32 (25.1)	0.502	5 (35.7)	13 (31.7)	1.000
A1A2 + A2A2	19 (86.3)	102 (80.3)	0.768	23 (91.0)	53 (84.4)	0.675
A1A1	3 (13.6)	25 (19.6)	–	11 (8.9)	32 (15.5)	–

Table 3 Association between CYP17 genotype in breast cancer cases, together with tumor characteristics and *P* values (Values in *parentheses* indicate percentages, *n* number of subjects studied)

Estrogen status			
Genotype	Negative (n = 93)	Positive (n = 23)	P
A1A2	46 (49.4)	10 (43.4)	0.252
A2A2	29 (31.1)	5 (21.7)	0.206
A1A2 + A2A2	75 (80.6)	15 (65.2)	0.160
A1A1	18 (19.3)	8 (34.7)	–

to hyperplasia to neoplasia. The hypothesis that cumulative exposure of breast tissue to ovarian hormones is one of the major determinants of breast cancer has existed for at least 30 years. According to the polygenic model developed by Henderson and Feilgeson, there are functionally important polymorphisms in the genes encoding enzymes involved in hormone biosynthesis, which lead to differences in individual susceptibility to breast cancer.

Cytochrome CYP17 encoding P-450 17a-hydroxylase is involved in estrogen biosynthesis, and polymorphism of this gene has been identified in the general population. Carey et al. (1994) have described the common polymorphism of the CYP17 gene and have hypothesized that the number of recognition sites for the Sp I transcription factor may influence promoter activity and thereby up-regulate transcription of the CYP17 gene. These could, theoretically, lead to increased levels of the enzyme, which, in turn, might affect the synthesis of estrogen; the major function of estrogen is to stimulate cell growth and proliferation by expressing growth factors via the function of the ER as a transcription activator. Women who are heterozygous or homozygous for a cytochrome CYP17 polymorphism had also shown high serum estradiol concentrations (Haiman et al. 1999; Chako et al. 2004), although the use of an extra binding site has not been confirmed experimentally (Nedelcheva Kristensen et al. 1999).

Our result suggests that the T > C polymorphism in the CYP17 gene is associated with an early onset breast cancer risk. In a population of 43.8% individuals affected by breast cancer and compared with a control population of 60% individuals with no cancer history, the presence of the A2 allele was significantly associated with an increased risk for breast cancer development. A similar kind of association was evaluated in a case–control study on a Korean population, between the T > C polymorphism in the 5'UTR of the CYP17 gene and breast cancer risk (Myung et al. 2005). It was found that homozygosity for the 5′-UTR variant

(CYP17) is most common in East Asian (32%) and Japanese (22%) populations and is less common among White [mainly European and North American (14%)] and Black [mainly African-American (13%)] populations, but selection biases are likely to have affected these frequency estimates (Sharp et al. 2004).

Levels of circulating estrogens and androgens may be important in the etiology of premenopausal breast cancer (Eliassen et al. 2006). Analysis of the CYP17 genotype in relation to onset condition has revealed that the A2 allele mediates an increased risk of breast cancer in premenopausal women compared with postmenopausal women. A similar viewpoint has been put forward by a study conducted by the South-East Sweden Health Care Region, where it showed a statistically significant association between CYP17 genotype and early onset breast cancer (Malin et al. 1999). The data from a case–control study conducted on Australian women suggested that women with the CYP17 CC genotype are at an increased risk of early onset breast cancer (Spurdle et al. 2000). Several investigators have demonstrated that breast cancer in young women, compared with older counterparts, is different in terms of pathological features and prognosis, thus suggesting that early onset and late onset breast cancer may have different biological origins. Thus, it could be said that CYP17 gene polymorphism may play a different role in early onset breast cancer cases because the breast etiology may vary between premenopausal and postmenopausal women (Thompson et al. 1998). The criteria used for the selection of the control population may also have influenced the result. However, the genotype dispersal in our control subjects was within that expected and did not deviate from the Hardy–Weinberg equilibrium.

We have not found any significant effect of CYP17 polymorphism on familial breast carcinoma. It may be possible that the other genes involved in hormone biosynthesis pathway may be playing an important role at the genetic level in familial cases. This has raised a need for studies on the potential role of other genes encoding for estrogen metabolism and transport enzymes in individual breast cancer susceptibility (Coughlin and Piper 1999).

Analysis of tumor characteristics in relation to genotype revealed that breast tissue lacking estrogen receptor was most common among carriers of the A2 allele. Although overall statistical significance could not be established, the CYP17 polymorphism has been found significantly more often in breast cancer cases without estrogen receptor (80.6%) than in estrogen receptor positive cases (65.2%). Initiation of malignancy and subsequent clonal expansion may occur

among proliferating cells that lack hormone receptors but are stimulated by the increased level of estrogen caused by the A2 allele. The reason could be that, recently, it has been demonstrated that estrogen can also stimulate cell proliferation without expressing any molecules. The pathway, known as a non-genomic pathway, does not involve ER but requires a G-protein-coupled receptor called GPR30. Experiments suggest that estrogen may bind to GPR30, activating metalloproteinases to cleave heparin-bound epidermal growth factor (EGF). The released EGF can then stimulate cell proliferation by acting on its EGF receptor (Clevenger 2004; Gilbey et al. 2004).

In conclusion, our present study suggests that there exists a possible correlation between CYP17 genotype and breast cancer and that the polymorphism in the same gene influences early onset breast cancer by increasing the risk of the disease in individuals carrying the A2 allele. This provides an important early step in defining a model that can explain individual susceptibility in terms of genetic susceptibility and endogenous estrogen exposure. The A2 allele of the CYP17 gene is responsible for increased estrogen levels, and, more recently, the steroid hydroxylases themselves have been found to be promising new targets for drug development. Instead of the use of anti-hormones to inhibit the action of steroid hormones at the level of steroid hormone receptors CYPs may be used to help in the development of new inhibitors of hydroxylase enzymes (Hakki and Bernhardth 2006).

As a genetic marker, the CYP17 genotype may provide a tool for identifying high-risk groups of women, especially considering the early onset condition, for disease prevention and early treatment.

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