RESEARCH COMMUNICATION

Association of a CYP17 Gene Polymorphism with Development of Breast Cancer in India

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Abstract

The human CYP17 gene, located on chromosome 10q24.3, plays a key role in sex steroid synthesis, mainly related to estrogen. A 5' UTR polymorphism involving a single base pair change in the promoter region results in increased transcriptional activity. In the present study of 250 breast cancer cases and 250 ma tched controls, the A1 genotype frequency was elevated in the disease group, while the A2 genotype frequency demonstrated no association. When data were stratified by risk conferring group, however, the A2 genotype frequency was increased in postmenopausal breast cancer cases (4.2%), patients positive for a family history of breast cancer (5.5%), high BMI, estrogen receptor (6.2%) and progesterone receptor negative (5.0%) status, HER2/neu positive (7.7%) status, positive node status (5.0%) as well as advanced stage of the disease. The A1A1 genotype linked with increased production of androgens might impact on onset of breast cancer while the A2 allele showed associations with respect to important risk conferring parameters.

Keywords: CYP17 - breast cancer - PCR-RFLP - receptor status - India

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Introduction

CPY17 plays key role in sex steroid synthesis and codes for enzyme which has bifunctional catalytic activity. One catalytic center performs the 17α-hydroxylation of pregnenolone and progesterone and another the 17, 20-lyase activity, is responsible for the conversions of 17 α-hydroxypregnenolone to dihydroepiandrosterone and 17α-hydroxyprogesterone to androstenedione, precursors of testosterone and estrogens (Weston et al., 1998). Recent studies had shown that estrogen metabolites could bind to DNA and trigger damage (Zhu and Conney., 1998). It was suggested that estrogen might be a complete carcinogen capable of causing genetic alterations and tumor initiation (Service, 1998). Since cytochrome P450c17 α is important in estrogen biosynthesis, increased or decreased activities might influence susceptibility to breast cancer.

The human CYP17 gene is located on chromosome 10q24.3 (Fan et al., 1992). Carey et al., (1994) first identified the MspA1 polymorphism of the CYP17 gene and revealed a significant association with polycystic ovarian disease and male pattern baldness in which androgen play major role. Single nucleotide polymorphism (T to C substitution) in 5'-promotor region creates an additional (CCACT-CCACC) Sp1 promoter site at 34 base pair upstream of the initiation of translation but downstream from the transcription start site. The T allele and C allele were reported as A1 and A2 alleles in the literature respectively. The A2 allele corresponds to an additional Sp-1 type promoter site in the 5' untranslated region of CYP17. Since it was thought that the number of 5' promoter elements correlates with promoter activity, it might be expected that an additional CCACC site might influence promoter activity, thereby up-regulating transcription. Inter-individuals differences in susceptibility to breast cancer are partially mediated through the levels of endogenous and exogenous steroid hormones (Feigelson et al., 1996). Recent invitro data suggested that the 5' Sp1-type site resulting from the T to C substitution does not actually bind transcription factor Sp-1 (Haung et al., 1999), but there was still some evidence to indicate that this polymorphism might influence endogenous steroid hormone levels (Bergman-Jungestrome et al., 1999). Feigelson et al., (1998) found that the A2 allele was associated with higher serum estrogen and progesterone levels compared with the A1 allele. Another study reported that the women with A2 allele had elevated levels of steroid and dehydroepiandrosterone (Haiman et al., 1999).

In the present study, we have examined a series of breast cancer cases as well as controls to determine whether CYP17 polymorphism influence the risk for the development of breast cancer.

Materials and Methods

A group of 250 breast cancer patients were selected

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for study. 250 healthy and age matched women without family history of breast cancer or any other cancers were selected to serve as control group. Cases were chosen from Nizam's Institute of Medical Sciences after confirmed diagnosis. The diagnosis of breast cancer was established by pathological examination, mammography, Fine needle aspiration (FNAC) and biopsy. Epidemiological history such as age at onset of breast caner, diet, socioeconomic status, occupation, reproductive history, family history and consanguinity were taken through personal interview with breast cancer patients using specific proforma. The patients were screened for receptor status of estrogen, progesterone and HER-2/neu by immunohisto chemical assay. Clinical history such as size of the tumor, presence of auxiliary nodes, extent of metastasis, stage and type of the breast cancer, chemotherapeutic drugs used and prognosis of the disease was collected with the help of oncologist. Informed consent was taken from all patients and controls included in the study.

Five milliliters of blood was collected in an EDTA vaccutainer from patients as well as controls. DNA was isolated (Nuremberg et al., 1991) and used for amplification of CYP17 gene polymorphisms.

Statistical analysis

The results were analyzed using appropriate statistical tests by SPSS Version 14. Odds ratio was estimated to calculate the relative risk for each genotype to develop disease. Differences in genotype frequency distribution between disease and control groups was done using 2* $2\chi^2$ and χ^2 test for heterogeneity. Multivariate analysis was done for both polymorphisms with respect to the clinical variables.

CYP17 polymorphism: PCR-RFLP was done for identification of CYP17 polymorphism using specific primers (Roberta McKean-Cowdin et al., 2001). The amplified product was digested with 5 units of MspA1I enzyme (Fermentars) at 37°C for overnight and electrophoresed on 2% agorose gel. The A1 genotype is identified by the presence of 414 bp, A1/A2 genotype by 414, 290, 124 bp and the A2 genotype by 290 and124 bp. When BMI is considered, higher frequency of A2 genotype was found in breast cancer patients with overweight and obesity (5.8% & 2.2%) (Figure 1).

Results

CYP17 polymorphism showed a significant association with breast cancer (Table 1). The A1 genotype frequency was increased in breast cancer patients (68.7%) when compared to controls (55.4%) while A2 genotype frequency was not associated with breast cancer. The frequency of A2 genotype showed increasing but non significant trend in postmenopausal breast cancer women

Table 1. *CYP17* Polymorphism with Respect to Breast Cancer and Epidemiological Factors

Parameters	A1A1		A1A2		A2A2		Allele Frequency	
	n	%	n	%	n	%	A	
Disease (249)	171	68.7	69	27.7	9	3.6	0.83	0.17
Controls (249)	138	55.4	95	38.2	16	6.4	0.74	0.26
	χ^2=9.606			P=0.008*				
Hardy Weinberg disease	χ^2=0.38			Control χ^2=0				
Odds Ratio (CI 95 %)	A1A1 Vs A1A2: 1.706 (1.1636-2.5013)							
	A1A2 vs A1A2: 1.2912(0.539-3.0929)							
	A1A1 vs A2A2: 2.2029(0.9445-5.1381)							
Menopausal Status								
Premenopausal (131)	88	67.2	39	29.8	4	3.1	0.82	0.18
Postmenopausal (118)	83	70.3	30	25.4	5	4.2	0.83	0.17
	χ^2=0.755			P=0.69				
Odds Ratio (CI 95 %)	A1A1 vs A1A2: 0.816 (0.4647-1.4315)							
	A1A2 vs A1A2: 1.625 (0.4014-6.5786)							
	A1A1 vs A2A2: 1.325 (0.3441-5.105)							
Familial History								
Familial (73)	46	63.0	23	31.5	4	5.5	0.79	0.21
Non-Familial (176)	125	71.0	46	26.1	5	2.8	0.84	0.16
	χ^2=2.01	P=0.365						
Odds Ratio (CI 95 %)	A1A1 vs A1A2: 0.736 (0.4023-1.3464)							
	A1A2 vs A1A2: 0.625 (0.1531-2.552)							
	A1A1 vs A2A2: 0.46 (0.1184-1.7879)							
BMI								
<20 (14)	6	57.1	6	42.9	0	0	0.79	0.21
20-26.4 (27)	17	62.9	10	37.0	0	0	0.81	0.19
26.4-30 (104)	71	68.3	27	25.9	6	5.8	0.81	0.19
>30 (45)	32	71.1	12	26.7	1	2.2	0.84	0.16
	χ^2=5.347			P=0.5				
Occupation								
Housewives (173)	121	69.6	46	26.6	5	2.9	0.83	0.17
Agriculture (27)	19	70.4	8	29.6	0	0	0.85	0.15
White-Collar job (42)	27	64.3	13	31.0	2	4.8	0.80	0.20
Other (4)	4	57.1	2	28.6	1	14.3	0.71	0.29
	χ^2=3.925			P=0.687				

Table 2. CYP17 Polymorphism with Respect to Breast Cancer Clinical Parameters

Parameters	A1A1		A1A2		A2A2		Allele	
	n	%	n	%	n	%	A	
Estrogen receptor								
Positive (90)	70	77.8	18	20	2	2.2	0.89	0.11
Negative (97)	59	60.8	32	32.9	6	6.2	0.77	0.23
	χ^2=6.605			P=0.037				
Odds Ratio (CI 95 %)	A1A1 Vs A1A2: 2.1092 (1.0756-4.1359)							
	A1A2 vs A1A2: 1.6875(0.3079-9.2496)							
	A1A1 vs A2A2: 3.5593(0.6922-18.301)							
Progesterone receptor								
Positive (87)	64	73.6	20	22.9	3	3.4	0.85	0.15
Negative (100)	65	65	30	30	5	5	0.80	0.20
	χ^2=1.627 P=0.45							
Odds Ratio (CI 95 %)	A1A1 Vs A1A2 1.4769 (0.7612-2.8654)							
	A1A2 vs A1A2: 1.1111(0.2384-5.1784)							
	A1A1 vs A2A2: 1.641(0.37645-7.1543)							
HER2/neu								
Positive (26)	16	61.5	8	30.7	2	7.7	0.77	0.23
Negative (27)	14	51.9	12	44.4	1	3.7	0.74	0.26
	χ^2=1.248			P=0.536				
Odds Ratio (CI 95 %)	A1A1 Vs A1A2: 1.7143 (0.5446-5.396)							
	A1A2 vs A1A2: 0.3333(0.0257-4.3192)							
	A1A1 vs A2A2: 0.5714(0.0467-6.9986)							
Node Status								
Positive (121)	85	70.2	30	24.8	6	5.0	0.83	0.17
Negative (75)	54	72.0	19	25.3	2	2.7	0.78	0.22
	χ^2=0.621			P=0.733				
Odds Ratio (CI 95 %)	A1A1 Vs A1A2: 0.9969 (0.511-1.9448)							
	A1A2 vs A1A2: 0.5263(0.0961-2.882)							
	A1A1 vs A2A2: 0.5247(0.1022-2.6949)							
Stage								
I (11)	8	72.7	3	27.3	0	0	0.86	0.14
II (96)	75	78.1	18	18.8	3	3.1	0.88	0.12
III (72)	49	98.1	21	29.2	2	2.8	0.83	0.17
IV (48)	26	54.2	19	39.6	3	6.25	0.74	0.26
	χ^2=9.63			P=0.141				

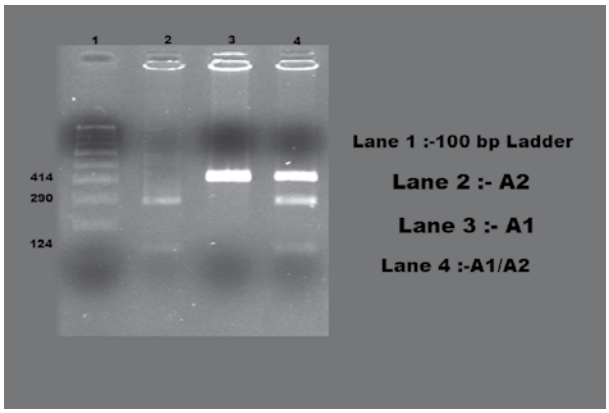

Figure 1. CYP17 Gene Polymorphism Gel Electrophoresis

(4.2%) when compared to premenopausal cases (3.1%). The frequency of A2 genotype showed association with positive family history of patients (5.5%) when compared to non-familial cases (2.8%). A2 genotype frequency was increased in estrogen receptor (6.2%) and progesterone receptor negative (5.0%) status and HER2/neu positive (7.7%) status (Table 2). When the node status and the stage of the breast cancer were observed, the frequency of A2 genotype was increased in patients with positive node status (5.0%) as well as advanced stage of the disease (2.8% & 6.25%).

Discussion

The frequency distribution of CYP17 genotype showed a significant association between CYP17 polymorphism and breast cancer in the present study. The A1 genotype frequency was elevated in disease group as compared to controls while A2 genotype frequency was not associated with breast cancer. The CYP17 genotype distribution in disease and control did not deviate from Hardy–Weinberg equilibrium. Earlier studies had reported the conflicting results on CYP17 polymorphism. The first study by Feigelson et al., (1996) had showed the significant association of A2 allele with increased breast cancer susceptibility. The increased risk for A2 allele was also related to other risk confounding factors such as age, family history etc (Bergman-Jungestrom et al., 1999; Spurdle et al., 2000). Two studies had found that women carrying A2 allele were less likely to use hormonal replacement therapy, due to high levels of circulating estrogens in the body (Haiman et al., 1999; Feigelson et al., 1996). No significant association with CYP17 polymorphism was found with respect to breast

cancer in other studies (Dunning et al., 1999; Mitrunen et al., 2000). The study from Indian population had also reported significant association of A2 with breast cancer in young women (Chakraborty et al., 2007). In general the studies showing A2 association with breast cancer have based their explanation that additional Sp1 site created by mutation resulted in promoter hyper activation and increased transcription leading to elevated estrogen level conferring risk to breast cancer. Experimental analysis on testing the binding ability of this site to the Sp1 have proved that this site does not bind with Sp1. A significant association of A1A1 genotype with prostate cancer was reported (Wadelius et al., 1999) which was attributed to increased circular androgen levels possibly by CYP17A1 encoded enzyme, thus making it important susceptible factor for prostate cancer. Two other studies also claimed that the A1A1 genotype might increase the risk of developing prostate cancer and rheumatoid arthritis (Habuchi et al., 2000; Sui-Foon Lo et al., 2004).

The pathway of estrogen metabolism is mediated by the activities of multiple genes, such as CYP17, CYP1A1, COMT, HSD17B1 (Weber et al., 2000). The failure to demonstrate an association between the CYP17 MspA1 polymorphism and breast cancer risk might imply that variation of any single gene might have only a limited impact on estrogen metabolism. It is conceivable that breast cancer risk related to any one locus will be small because gene-gene interactions are likely to operate. Therefore the effect of these polymorphisms on susceptibility to breast cancer could be minor. Our study indicated the association of A1 allele with breast cancer, when data was not classified with respect to other variables.

When menopausal status of the breast cancer was observed, the frequency of A2 genotype showed increasing but nonsignificant trend in postmenopausal breast cancer women (4.2%) when compared to premenopausal cases (3.1%). Several studies had reported the conflicting results with respect to menopausal status and CYP17 polymorphism. Dunning et al., (1998) reported the association of premenopausal breast cancer with increased A2 genotype. Feigelson et al., (1997) showed increased frequency of postmenopausal breast cancer patients with A2 genotype. No association of A2 genotype with postmenopausal breast cancer women was observed by another study (Mitrunen et al., 2000).

The frequency of A2 genotype showed association with positive family history of patients (5.5%), in accordance with the previous study, suggesting that the female relatives with A2 genotypes were at an increased risk compared to A1 and A1A2 genotypes. These findings imply that A2 genotype might modify the effect of other familial risk factors for breast cancer (Spurdle et al., 2000).

Higher frequency of A2 genotype was found in breast cancer patients with obesity. Circulating estrogens are produced from conversion of androstenedione and testosterone by the aromatase enzyme in the adipose tissue. Increased production of androgens would result in increased levels of estrogens in women with obesity. No significant association of A2 genotype with occupation was observed.

Patients with estrogen receptor (6.2%) and progesterone receptor negative (5.0%) status as well as HER2/neu positive (7.7%) status were found to be with increased frequency of A2 genotype. When the node status and the stage of the breast cancer were observed, the frequency of A2 genotype was increased in patients with positive node status (5.0%) as well as advanced stage of the disease. Our results were similar with the study of Feigelson et al., (2002) who had reported that the A2 allele was significantly associated with advanced stage of breast cancer. Elevated estrogen levels throughout the premenopausal period and aromatization in the fatty tissues were suggested as explanation for the observed association between A2 and a higher risk of advanced breast cancer. In conclusion in the present study, A1A1 genotype showed a significant association with breast cancer, when data was not stratified with respect to different variables. The A2 allele showed increased frequency with respect to postmenopausal status, positive family history, high BMI, estrogen and progesterone negative status, HER2/neu positive status, node positive and with advanced stage of the disease but they did not show any significance statistically. The lack of significant association with A2 could be due to small sample with this genotype in both disease and controls.

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References

Bergman-Jungestrom M, Gentile M, Lundin AC, et al (1999). Association between CYP17 polymorphism and risk of breast cancer in young women. *Int J cancer*, **84**, 350-3.

Carey AH, Waterworth D, Patel K, et al (1994). Polycystic ovaries and premature male pattern baldness are associated with one allele of the steroid metabolism gene CYP17. *Hum Mol Genet*, **3**, 1873-6.

Chakraborty A, Murthy NS, Chintamani C, et al (2007). Cyp17 gene polymorphism and its association with high-risk north Indian breast cancer patients. *Hum Gent*, **52**, 159-65.

Dunning AM, Healey CS, Pharoah PDP, et al (1998). No association between a polymorphism in the steroid metabolism gene CYP17 and risk of breast cancer. *Br J Cancer*, **77**, 2045-7.

Dunning AM, Healey CS, Pharoah PDP, et al (1998). A systematic review of genetic polymorphisms and breast cancer risk. *Cancer Epidemiol Biomarkers Prev*, **8**, 843-54.

Fan YS, Sasi R, Lee C, et al (1992). Localization of the human CYP17 gene to 10q24.3 by fluorescence insitu hybridization and simultaneous chromosome banding. *Genomics*, **14**, 1110-1.

Feigelson HS, Henderson BE (1996). Estrogen and breast cancer. Carcinogenesis, **17**, 2279-84.

Feigelson HS, Coetzee GA, Kolonel LN, et al (1997). A polymorphism in the CYP17 gene increases the risk of breast cancer. *Cancer Res*, **57**, 1063-5.

Feigelson HS, Mc Kean-Cowdin R, Pike MC, et al (1998). Cytochrome P450c17α (CYP17) polymorphism is associate with serum estrogen and progesterone concentration. *Cancer Res*, **58**, 585-7.

Feigelson HS, Mc Kean-Cowdin, Henderson BE (2002).

Concerning the CYP17 MspA1 polymorphism and breast cancer risk: a meta analysis. *Mutagenesis*, **17**, 445-6.
Habuchi T, Liging Z, Suzuki T, et al (2000). In creased risk of prostrate cancer and benign prostratic hyperplasia associated with CYP17 gene polymorphism with a gene dosage effect. *Cancer Res*, **60**, 5710-3.
Haiman CA, Hankinson SE, Spieglman D, et al (1999). The relationship between a polymorphism in CYP17 with plasma hormone levels and breast cancer. *Cancer Res*, **59**, 1015-20.
Huang CS, Chern HD, Chang KJ, et al (1999). Breast cancer risk association with genotype polymorphism of the estrogen -metabolizing genes CYP17, CYP1A1 and COMT: A multigenic study on cancer susceptibility. *Cancer Res*, **59**, 4870-5.
Lahiri DK, Nurnberger JI Jr (1991). A rapid non-enzymatic method for the preparation of HMW from blood RFLP studies. *Nucleic Acid Research*, **19**, 5444.
Lo SF, Huang CM, Lin HC (2004). Association of CYP17 gene polymorphism and rheumatoid arthritis in chinese patients in central Taiwan. *Rheumatol Int*, **25**, 580-4.
Miteunen K, Jourenkova N, Kataja V, et al (2000). Steriod metabolism gene CYP17 polymorphism and the development of breast cancer. *Cancer Epidemiol Biomarkers Prev*, **9**, 1343-8.
McKean-Cowdin R, Feigelson HS, Pike MC, et al (2001). Risk of endometrial cancer and estrogen replacement therapy history by cyp17 genotype. *Cancer Research*, **61**, 848-9.
Service RF (1998). New role for estrogen in cancer? *Science (Wash)*, **279**, 631-3.
Spurdle AB, Hopper JL, Dite GS, et al (2000). CYP17 promoter polymorphism and breast cancer in Australian women under age fourty years. *J National Cancer Ins*, **92**, 1674-81.
Wadelius M, Andersson AO, Johansson JE, et al (1999). Prostrate cancer associated with CYP17 genotype. *Pharmacogenetics*, **9**, 635-9.
Weber BL, Nathanson KL (2000). Low penetrance genes associated with increased risk for breast cancer. *Eur J Cancer*, **36**, 1193-9.
Weston A, Pan CF, Bleiweiss IJ, et al (1998). CYP17 genotype and breast cancer risk. *Cancer Epidemiol Biomarl Prev*, **7**, 941-5.
Zhu BT, Conney AH (1998). Is 2-methoxyestradiol an endogenous estrogen metabolite that inhibits mammary carcinogenesis? *Cancer Res*, **58**, 2269-77.