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Polymorphism of Hormone Synthesis and Metabolizing Genes and Breast Cancer Risk: A Multigenic Case-control Study

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Endogenous and exogenous hormones influence breast cancer risk including estrogen biosynthesis pathway, vitamin D receptor pathway, and the androgen receptor pathway. Genes involved in these pathways are CYP17, which encodes an enzyme involved in estradiol and testosterone synthesis, androgen receptor (AR), which binds testosterone and DHT and regulates breast cell growth and the vitamin D receptor (VDR), which binds vitamin D and down-regulates breast growth.The current study was proposed to determine whether polymorphisms in the CAG repeat in exon 1 of AR, *Msp*AI T > C substitution of CYP17, and *Apa*I, *Taq*I, poly-A repeat in the VDR gene contribute to breast cancer risk. Logistic regression models were used to evaluate individual and joint contributions of genotypes to breast cancer risk.Seventy (70) breast cancer patients and eighty healthy women (80) were recruited for the study. PCR based RFLP and fragment analysis assays were used to determine genotypes of hormone metabolizing genes. Considering CYP17 A2 allele, VDR Poly-(A) L, and AR ≥ 20 CAG repeats as high risk alleles, a multigenic model of breast cancer susceptibility was developed to identify women who carry a combination of alleles to put them at relatively higher risk to develop breast cancer. All the high-risk genotypes were positively associated with risk. The risk among women carrying three high-risk alleles was OR:4.68 [95% confidence interval (CI), 0.77–28.0; *p* for trend = 0.10] compared with those who carried none. The conditional logistic regression analysis revealed that the heterozygous TC genotype for CYP17 and AR1AR2 of AR, imparted significantly fourfold risk for the breast cancer risk, in comparison to the referent genotype TT and AR1AR1 [adjusted ORs:3.705(1.236,11.106), *p* = 0.019] and [4.391(1.324,14.557), *p* = 0.016], respectively. Gene X Gene interaction showed that the combinations TC*AA, TC*Aa, TC*aa and CC*Aa imparted significantly four to fifteen fold more risk for the breast cancer [(4.377 (1.159, 16.520), *p* = 0.029); 4.041(1.092, 14.956), *p* = 0.036); (15.071(0.975, 232.81), *p* = 0.052); (4.151(1.053, 16.371), *p* = 0.042), respectively)]. Genes involved in hormone synthesis and metabolizing pathway may play a role in breast cancer development as supported by the multigenic model of breast cancer susceptibility.

Key words: Breast Cancer, CYP17 gene, Vitamin D receptor gene, Androgen receptor gene, polymorphism, Multigenic model.

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INTRODUCTION

In biological systems, DNA, RNA, protein, and metabolites frequently interact with each other to perform biological functions, and respond to environmental factors. The complex interactions among genes and between genes and environment may partially explain the role of "lost heritability" in complex diseases like breast cancer. Through high-throughput screening of large breast cancer case-control subjects several high and low penetrance alleles have now been identified in more than 20 genes involved in DNA damage signaling and repair, and about 70 low-penetrance loci have been discovered through recent genome-wide association studies. Interaction among these genetic factors may play an important role through gene–gene interaction common in such complex disease.

Despite vast literature reported on the involvement of steroid hormones in breast cancer, the exact role of the hormones in breast cancer and endometrial physiology is unclear. Based on the existing epidemiological and experimental evidences that implicates hormones in the etiology of human breast cancer (Aguas *et al*., 2005; Henderson and Feigelson, 2000), the present study proposes a multigenic model of breast cancer predisposition that included genes involved in hormone biosynthesis and metabolism pathways. The research hypothesized that functionally relevant polymorphisms in such genes would exhibit small, but additive effects on individual susceptibility to breast cancer, and that specific combinations could result in a high-risk profile by influencing lifetime levels of hormones.

CYP17, VDR and AR are key susceptibility genes participating in major pathways of estrogen biosynthesis, vitamin D receptor and androgen receptor. Hence,to determine whether the profiles of these genes is associated with breast cancer, we examined breast cancer risk associated with combinations of high-risk genotypes using women with all three putative low-risk genotypes as the reference groups.

CYP17 encodes the cytochrome p450c17 enzyme, which mediates both steroid 17-hydroxylase and 17, 20-lyase activities, and functions at key branch points in human steroidogenesis (Brentano *et al*., 1990). A single basepair polymorphism (T27C) in the 5' untranslated region of CYP17, 34 bp upstream from the initiation of

translation and 27 bp downstream from the transcription start site creates a recognition site for the *MspA*I restriction enzyme and has been used to designate alleles A1 and A2. According to several studies endogenous hormone levels are associated with this polymorphism (Chako *et al*., 2004; Haiman *et al*., 1999). In addition, several studies have examined the association CYP17 and breast cancer with mixed results (Chakraborty *et al*., 2007; Kristensen *et al*., 1999; Lin *et al*., 2001; Surekha *et al*., 2010).

The second important candidate gene in this multigenic model is *VDR* gene, a steroid hormone receptor expressed in breast tissue and known to modulate the rates of cell proliferation. Common allelic variants identified in human *VDR* gene have been extensively studied with respect to risk for a variety of diseases including breast cancer. The best-studied VDR polymorphisms include a start codon polymorphism FokI (T/C) in exon II, BsmI (A/G) and ApaI (C/A) in an intronic region between exon VII and IX, and a TaqI (T/C) variant in exon IX. Although not functionally known, SNPs *Bsm*I, *Apa*I and *Taq*I are strongly linked with a singlet (A) repeat in the 30-untranslated region of the gene (Ingles *et al*., 1997; Slatter *et al*., 2001) that may influence VDR mRNA stability. Several published reports examined the relationship between one or more VDR polymorphisms and breast cancer risk or progression (Bretherton-Watt *et al*., 2001; Chakraborty *et al*., 2009; Hou *et al*., 2002; Ingles *et al*., 2000; McCullough *et al*., 2007; McKay *et al*., 2009; Newcomb *et al*., 2002).

The androgen receptor gene, a member of nuclear receptor family, is widely expressed in breast tissue implying that it may have a significant biological and clinical relevance. The exon 1 of AR gene contains tri-nucleotide repeat polymorphism, CAG (encoding poly-glutamine) which flank the N-terminal domain of the AR protein, where the transactivation activity resides. Recently some studies have suggested an association of glutamine tract with degree of difference in breast cancer risk. The relationship has been examined in several case-control studies in different populations, with longer CAG repeats associated with an increase in breast cancer risk (Giguère *et al*., 2001; Kadouri *et al*., 2001; Liede *et al*., 2003; Mao *et al*., 2015); whereas others demonstrated limited impact of AR-CAG repeat on breast cancer (Dagan *et al*.,

2002; Chintamani *et al*., 2010; Menin *et al*., 2001).

The purpose of the present study was to investigate the association among genes involved in key steps of the biosynthesis and metabolism of hormones with breast cancer risk in women in a multigenic case-control study; and develop a breast cancer risk model to distinguish women at higher risk of breast cancer.

MATERIALS AND METHODS

Study Population

In the case-control study, 70 histologically confirmed breast carcinoma patients (study group) referred to Institute of Pathology from the department(s) of Surgery and Cancer Surgery of Safdarjung Hospital, New Delhi, India, and 80 healthy controls were selected. Mean age of patients was 40.9 ± 10.7 years and controls were 39.3 ± 11.9years. The patients constituted 50 (71.4%) early onset cases (≤ 40 years), 20 (28.5%) late onset and 11(15.7%) cases with family history of breast/ovarian cancer. Histopathology examination showed infiltrating ductal carcinoma in 74.2% cases and infiltrating lobular carcinoma in 7% cases. Twenty three patients presented with stage I and IIa and 39 patients with stage IIb and III (locally advanced) and 8 in stage IV.

Control samples were collected from women attending antenatal check-ups and blood bank donors in Delhi. Informed consent was obtained from all participating patients and the study was carried out with the approval of Ethicsl Review Committee of Safdarjung Hospital, New Delhi.

Genotype Assay

DNA was purified from buffy coats of peripheral blood samples. The CYP17 assay has been described previously (Chakraborty *et al*., 2007). A PCR fragment containing the bp change was generated using the following forward (F) and reverse (R) primers: CYP17-F, 5'-CATTCGCACTCTGGAGTC-3'; and CYP17-R, 5'-AGGCTCTTGGGGTACT TG-3'. The PCR products were digested for 3 h at 37°C using *MspA*I, separated by 2% agarose gel electrophoresis, and stained with ethidium bromide to identify the bp change.

The DNA segment surrounding VDR *Apa*I site was amplified by PCR using primers: (VDR-F) 5'-GTGGGATTGAGC AGTGAG-3' and (VDR-R) 5'-ATCATCT TGGCATAGAG-3'. For *Taq*I site,(*Taq*-F) 5'-CAGAGCATGGACAGGGAGCAAG

-3' and (*Taq*-R) 5'-TGGATCATCTTGGC ATAGAGCAGG-3' primers were used. The digested fragments of *Apa*I were subjected to run on polyacrylamide gel electrophoresis(PAGE) at 100 V and visualized with ethidium bromide; whereas the digested fragments of *Taq*I were resolved in 3% agarose gel stained with ethidium bromide to identify the bp change.

A 413 bp fragment VDR 3'-UTR poly-Amicrosatellite was amplified using forward primer 5'-GTGTAGTGAAAAG GACACCGG-3'labeled with ABI-FAM (Applied Biosystems, USA) and reverse primer 5'-GACAGAGGAGGGCGTGAC TC-3'.Similarly, an approximate 288 bp fragment of AR gene spanning CAG repeat was amplified using forward primer 5'-TCCAGAATCTGTTCCAGAG CGTGC-3' labeled with ABI-FAM and reverse primer 5'-GCTGTGAAGGTTGC TGTTCCTCAT-3'. Fluorescent-amplified DNA of both VDR (poly-A) and AR (CAG) genes along with LIZ standard and formamide were heat denatured at 95ºC for 5 min, chilled on ice and loaded on 3130xl sequencer. The sequencing data was analyzed using ABI Gene Mapper software package.

Statistical Analysis

The test for the difference between the proportion of various genotypes for the considered polymorphisms (*CYP17*, *VDR Taq*I, *Apa*I, Poly-A and *AR)* with the diseased state (0: control, 1: breast cancer) was tested by Chi Square test, along with the test for trend presented in Table 1. The results of the Hardy Weinberg (HW) test of equilibrium are depicted at the foot note of the Table 1. The conditional logistic regression analysis was applied to estimate the odds ratio (OR) for each genotype of the various polymorphisms, by considering wild type as the referent category. The results of the conditional logistic regression analysis are illustrated (Table 1). The results of the gene-gene multiplicative interactions are depicted (Table 2). The data enclose results of the CYP**Apa*I and CYP*AR only, as the other second order interaction were statistically insignificant and the assessment of higher order interaction, yielded unstable parameter estimates under the conditional logistic regression analysis. In relation to the poly-morphisms under study, the results were interpreted in terms of adjusted odds ratio (estimate of corresponding

Table 1: Odds ratios for breast cancer by polymorphisms in the hormone metabolizing and receptor genes! (Row %, column %)

Polymorphisms		Association				Conditional logistic regression analysis			
		Case (70) n (%)[!]	Control (80)	χ^2, df, *p*	Trend	Unadjusted OR (95%CI)	*p*	Unadjusted OR(95%CI)	*p*
CYP17									
	TT	9 (27.3, 12.9)	24 (72.7, 30.0)			1.000		1.000	
	TC	35 (54.7, 50.0)	29 (45.3, 36.3)	6.732, 2, 0.034	0.093	3.364 (1.316, 8.598)	0.011	3.705 (1.236, 11.106)	0.019
	CC	26 (49.1, 37.1)	27 (50.9, 33.8)			2.353 (0.900, 6.157)	0.081	2.531 (0.811, 7.897)	0.110
Taq-I									
	TT	35 (47.3, 50.0)	39 (52.7, 48.8)			1.000		1.000	
	Tt	32 (46.4, 45.7)	37 (53.6, 46.3)	0.055, 2, 0.973	0.838	1.119 (0.559, 2.240)	0.751	1.142 (0.493, 2.642)	0.757
	tt	3 (42.9, 4.3)	4 (57.1, 5.0)			0.721 (0.114, 4.563)	0.729	0.432 (0.035, 5.412)	0.515
Apa-I									
	AA	34 (41.0, 48.6)	49 (59.0, 61.3)			1.000		1.000	
	Aa	32 (52.5, 45.7)	29 (47.5, 36.3)	2.871, 2, 0.238	0.092	1.519 (0.767, 3.009)	0.231	1.046 (0.468, 2.339)	0.912
	aa	4 (66.7, 5.7)	2 (33.3, 2.5)			3.581 (0.576, 22.249)	0.171	3.260 (0.472, 22.506)	0.231
VDR POLY-A									
	SS	29 (45.3, 41.4)	35 (54.7, 43.8)			1.000		1.000	
	SL	24 (36.9, 34.3)	41 (63.1, 51.3)	12.445, 2, 0.002	0.059	0.601 (0.286, 1.261)	0.178	0.611 (0.259, 1.439)	0.259
	LL	17 (81.0, 24.3)	4 (19.0, 5.0)			4.281 (1.250, 14.658)	0.021	5.536 (1.385, 22.123)	0.015
AR									
	AR1AR1	30 (40.0, 42.9)	45 (60.0, 56.3)			1.000		1.000	
	AR1AR2	15 (68.2, 21.4)	7 (31.8, 8.8)	5.436, 2, 0.066	0.346	2.869 (1.026, 8.028)	0.045	4.391 (1.324, 14.557)	0.016
	AR2AR2	25 (47.2, 35.7)	28 (52.8, 35.0)			1.119 (0.501, 2.501)	0.784	1.050 (0.391, 2.819)	0.923

[!] Test for Hardy Weinberg Equilibrium [cases: (CYP17(T:0.38, C:0.62, *p* = 0.60; Taq-I (T:0.72, t:0.28, *p* = 0.19); Apa-I (A:0.72, a:0.28, *p* = 0.32); VDR-PolyA (S:0.59, L:0.41, *p* = 0.01); AR (AR1:0.59, AR2:0.41, *p* < 0.001); controls: (CYP17 (T:0.48, C:0.0.62, *p* = 0.0.06; Taq-I (T:0.72, 0.28, *p* = 0.20); Apa-I (A:0.80, a:0.20, *p* = 0.34); VDR-PolyA (S:0.70, L:0.30, *p* = 0.07); AR (AR1:0.59, AR2:0.41, *p* < 0.001)]

* (Row %, column %)

polymorphism adjusted for all other polymorphism under investigation) and corresponding 95% Confidence Interval (CI). However the results of the Gene X Gene interaction are interpreted in terms of unadjusted OR with 95% CI. A two sided $p \leq 0.05$ was considered statistically significant. The data analysis for the present study was performed on STATA 8.0 version.

RESULTS

The association of CYP17 and VDR Poly-A was significantly associated with breast cancer and marginally significant with AR (p = 0.034, 0.002 and 0.066, respectively). The cases were found to have significantly greater proportion of CYP17 CC genotype (37.1%) than controls (33.8%) (Table 1). The VDR gene polymorphism showed significant association with Poly-A repeats with 24.3% of cases with LL genotype, than controls (5.0%). The associated test for trend for CYP17 and VDR Poly-A repeat was marginally significant (p = 0.093 and p = 0.059). In relation to the AR polymorphism, the cases demonstrated AR1AR2 in greater percentage (21.4%) than controls (8.8%) with marginal differences. The association of the other polymorphisms with the breast cancer risk was statistically insignificant ($p >$ 0.05) (Table 1).

The conditional logistic regression analysis (Table 1) revealed that the heterozygous TC genotype for CYP17 and AR1AR2 of AR, imparted significantly increased (fourfold) risk for the breast cancer risk, in comparison to the referent genotype TT and AR1AR1 [adjusted OR:3.705 (1.236 – 11.106), p = 0.019] and [adjusted OR:4.391 (1.324 – 14.557), p = 0.016, respectively)]. Moreover, in relation to the homozygous reference category SS, the risk of breast cancer associated with the LL genotype for the VDR-Poly-A repeat polymorphism was about six fold (adjusted OR: 5.536 (1.385 – 22.123), p = 0.015).

The result of gene X gene interaction (Table 2) revealed that in comparison to the homozygous referent combination CC*AA of CYP17 and *ApaI* polymorphism, the combinations TC*AA, TC*Aa, TC*aa and CC*Aa imparted significantly four to fifteen fold more risk for the breast cancer [{4.377 (1.159 – 16.520), p = 0.029}; {4.041 (1.092 – 14.956), p = 0.036}; {15.071 (0.975 – 232.81), p = 0.052}; {4.151

Table 2: Result of Gene-Gene Multiplicative Interaction for CYP with Apa-I and AR

Interaction	Association				Conditional logistic regression analysis	
	Case (70)	Control (80)	χ^2, df, p	p (Trend)	Unadjusted OR(95%CI)	p
CYP*Apa I	n(%)[!]					
TT*AA	5 (22.7, 7.1)	17 (77.3, 21.3)	9.108, 7, 0.245	0.044	1.000	
TT*Aa	4 (36.4, 5.7)	7 (63.6, 8.8)			1.882 (0.350, 10.119)	0.461
TT*aa	0 (0.0, 0.0)	0 (0.0, 0.0)			-	-
TC*AA	16 (53.3, 22.9)	14 (46.7, 17.5)			4.377 (1.159, 16.520)	0.029
TC*Aa	17 (54.8, 24.3)	14 (45.2, 17.5)			4.041 (1.092, 14.956)	0.036
TC*aa	2 (66.7, 2.9)	1 (33.3, 1.3)			15.070 (0.975, 232.81)	0.052
CC*AA	13 (41.9, 18.6)	18 (58.1, 22.5)			2.225 (0.590, 8.390)	0.238
CC*Aa	11 (57.9, 15.7)	8 (42.1, 10.0)			4.151 (1.053, 16.371)	0.042
CC*aa	2 (66.7, 2.9)	1 (33.3, 1.3)			6.283 (0.453, 87.100)	0.171
CYP*AR						
TT*AR1AR1	5 (35.7, 7.1)	9 (64.3, 11.3)	19.889, 8, 0.011	0.044	1.000	
TT*AR1AR2	1 (16.7, 1.4)	5 (83.3, 6.3)			0.264 (0.219, 3.189)	0.295
TT*AR2AR2	3 (23.1, 4.3)	10 (76.9, 12.5)			0.400 (0.069, 2.310)	0.306
TC*AR1AR1	14 (48.3, 20.0)	15 (51.7, 18.8)			2.378 (0.533, 10.621)	0.256
TC*AR1AR2	7 (87.5, 10.0)	1 (12.5, 1.3)			17.763 (1.500, 211.329)	0.023
TC*AR2AR2	14 (51.9, 20.0)	13 (48.1, 16.3)			1.397 (0.343, 5.688)	0.641
CC*AR1AR1	11 (34.4, 15.7)	21 (65.6, 26.3)			0.782 (0.191, 3.205)	0.732
CC*AR1AR2	7(87.5, 10.0)	1 (12.5, 1.3)			9.993 (0.864, 115.630)	0.065
CC*AR2AR2	8 (61.5, 11.4)	5 (38.5, 6.3)			2.657 (0.441, 16.000)	0.280

[!] (Row %, column %)

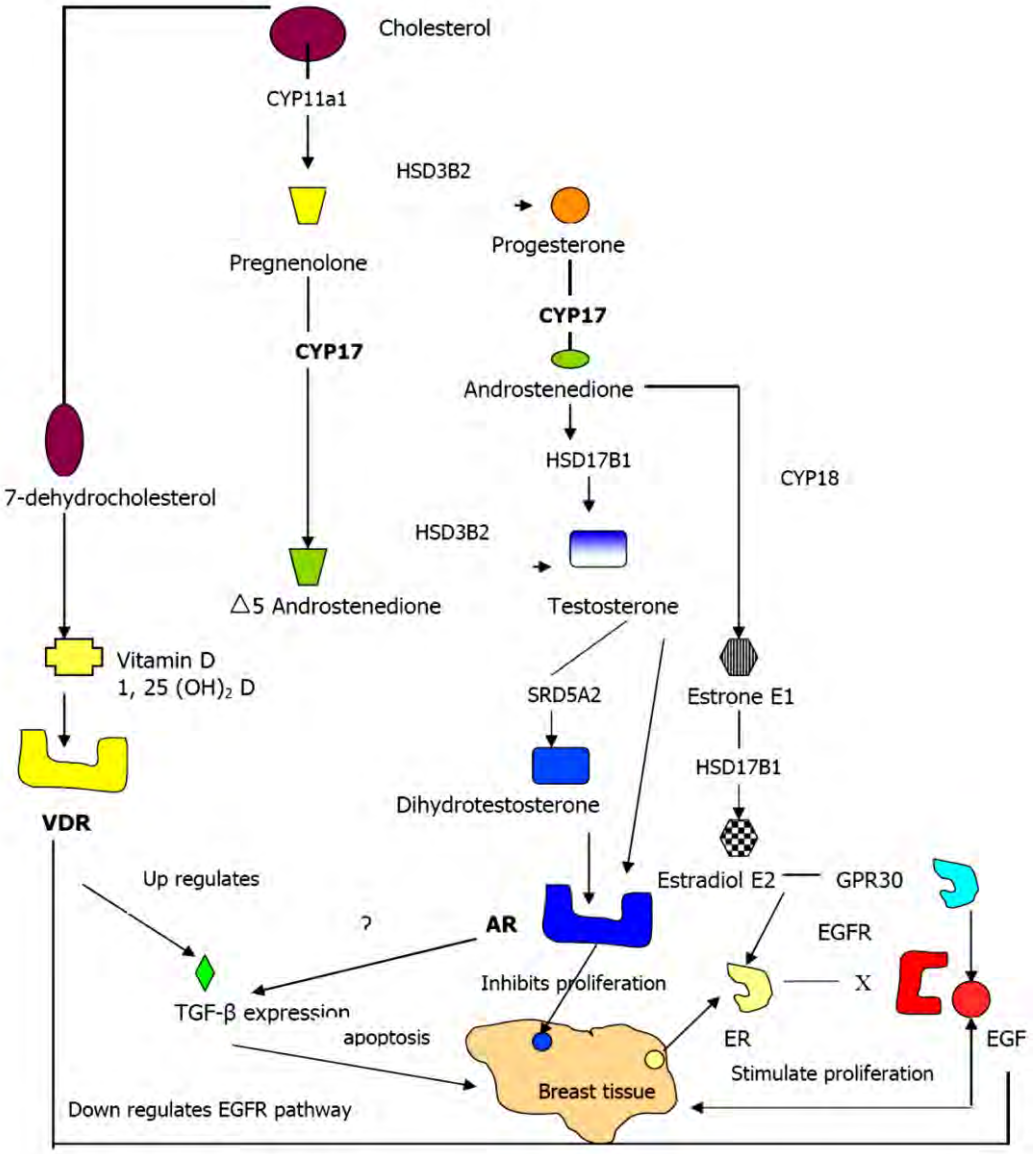

Figure 1: Genetic polymorphisms along the hormonal pathway and BC development.

(1.053 – 16.371), $p = 0.042$}, respectively]. Considering the contributory effect of CYP17 with AR, it was detected that the combination TC*AR1AR2 gave significantly more risk [OR: 17.763 (1.50–211.3)] for the breast cancer, in comparison to the referent category TT*AR1AR2.

Discussion

Our observations and previous reports on hormonal carcinogenesis suggest that sequence variants in functionally relevant genes associated with estrogen and androgen metabolism and targeting receptors outlined in Fig. 1 may define high-risk or low-risk profiles for breast hyperplasia. Considering the common

polymorphism of the CYP17 gene, Carey *et al*. (1994) hypothesized that the number of recognition sites for the SpI transcription factor may influence promoter activity and thereby up-regulate CYP17 gene transcription leading to overexpression of the enzyme and subsequent synthesis of estrogen. The major function of estrogen is to stimulate cell growth and proliferation via ER as a transcription activator. Women with heterozygous or homozygous CYP17 polymorphism have demonstrated high serum estradiol concentrations (Haiman *et al*., 1999; Chacko *et al*., 2004), although the use of an extra binding site has not been confirmed experimentally (Kristensen *et al*., 1999). Our result suggests that the T > C polymorphism in the CYP17 gene is associated with breast cancer risk. The data showed that 50% of affected population had the TC genotype compared with 36.3 % of control population. Breast carcinoma cases with A2 (C) allele when considered alone, expressed a slightly higher risk ($p = 0.01$) when compared with TT as a reference group.

In the estrogen biosynthesis pathway, CYP17 converts 17-hydroxyprogesterone to androstenedione which converts to testosterone. Therefore, CYP17 is a key enzyme for androgen biosynthesis as well. Data from eight prospective cohort studies have reported association between endogenous testosterone levels and breast cancer risk, using testosterone measured from blood samples gathered at baseline from postmenopausal women (Lillie *et al*., 2003). The main receptor for testosterone is the AR. By binding to a subset of EREs, the AR can prevent activation of target genes that mediate the stimulatory effects of 17β-estradiol on breast cancer cells (Peters *et al*., 2009). Therefore, the present study further investigated the influence of CAG repeat length and its association with Breast Cancer risk. The analysis was done defining short allele as CAGn< 20 (AR1) and the long as CAGn ≥ 20 (AR2). The cut-off point was chosen as 20 repeats because the mode of CAGn in cases and controls was approximately 20 repeats. Marginally significant difference was observed in women with average of both CAG repeat alleles did not exceed 20 (CAGn< 20) compared with women with CAG repeats > 20 ($p = 0.09$). However women with single long allele AR genotypes (AR1AR2) were at significantly higher risk of developing the disease compared with those bearing both short allele AR genotypes

(AR1AR1) (p = 0.02), although, a trend in risk was not observed with AR2AR2 genotype. Several studies have observed an association between increasing AR CAG repeat length and a linear decrease in AR transactivation activity (Beilin *et al*., 2000; Chamberlain *et al*., 1994; Choong *et al*., 1996). Shorter alleles of the AR gene may be associated with a better response to circulating androgens, resulting in better "repression" of breast cancer development and/or progression. However, the functional explanation for the observation is not clear.

The third gene in the multigenic model is the VDR gene. Identification of 1,25 (OH)2D3 and VDR as components of a signaling network that affects breast tissue proliferation and differentiation, indicates that Vitamin D may play a protective role against mammary transformation and the common VDR gene variants may be associated with the risk of breast cancer. It is possible that polymorphisms in the VDR gene alter the ability of 1, 25, D3 to interact with the VDR gene resulting in effective induction of transcription of genes via the VDR, even in a woman not deficient in 1, 25, D3.

We have investigated the association between VDR genotype and breast cancer risk in Indian population. The results indicate that VDR poly-A genotypic variants confers susceptibility to breast cancer risk, and the odds of a woman with LL genotype developing breast cancer were twice (OR = 2.49) than that for a woman with genotype SS. In view of the functional importance of poly-A polymorphism, the SNP marks the relevant locus for possible direct effect on mRNA function. On the other hand, variant *Apa*I and *Taq*I were not associated with breast cancer risk. As functional variants in genes of hormonal pathways may influence breast carcinogenesis, and the multicausal etiology of breast cancer, it is likely that multiple risk variants act simultaneously, synergistically or additively to influence breast cancer susceptibility.

Gene–gene interaction analysis revealed that combinations of unfavorable genotypes involving TC*AR1AR2 significantly increased breast cancer risk (17 fold) with TT*AR1AR2 as a reference genotype. Further, in a multigenic model, a significant interaction was observed between CYP17 and *Apa*I variant of VDR with a trend 0.04. Although no significant interaction was observed on combining VDR and AR variants in the

Table 3: Estimated OR of breast cancer development associated with number of high risk genotypes of low penetrance genes related to hormonal pathway

No. of high-risk genotypes					
	Code (CYP17, VDR, AR)	No. of cases (%) (n = 70)	No. of control (%) (n = 80)	OR (95% CI)	*p* value
No putative high risk genotype	000	2 (2.8)	3 (3.7)	Ref.	-
One putative high risk genotype	001, 002, 010, 020, 100, 200	13 (18.5)	27 (33.7)	0.72 (0.12–4.06)	1.0
Two putative high risk genotype	011, 012, 021, 022, 110, 120, 201, 202, 210, 220	30 (42.8)	42 (52.5)	1.07 (0.20–5.69)	1.0
Three putative high risk genotype	111, 112, 122, 211, 212, 221, 222	25 (37.5)	8 (10.0)	4.68 (0.77–28.0)	0.1

0 = A1A1, SS, < 20 < 20 CAG repeat

1 = A1A2, SL, < 20 ≥ 20 CAG repeat

2 = A2A2, LL, ≥ 20≥ 20 CAG repeat

present study, few reports suggest direct or indirect interaction of VDR and AR at receptor level in prostate, colon and rectal carcinoma (Slattery *et al*., 2006).

To evaluate possible cumulative effect of the defined risk alleles, age-adjusted multivariate logistic regression on combinations of at-risk alleles compared with the no-risk allele reference was analyzed.The data revealed a trend of increasing breast cancer risk with increase in number of high risk alleles. The high-risk genotype results indicated possibility of positive association with breast cancer risk. Compared with no high-risk alleles, genotypes carrying two putative high risk alleles showed a mild increase in risk for developing breast cancer (OR = 1.07). However, the risk further increased for genotypes with three putative high risk alleles (OR = 4.68) (Table 3). Thus, identification of SNPs in functional or regulatory genes may be critical in defining a panel of SNPs indicating association with increased or decreased risk to breast cancer.

CONFLICT OF INTEREST

The authors claim no conflict of interest.

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