

# Association of CYP19 polymorphisms with breast cancer risk: A case-control study

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## Abstract

**Background:** The CYP19 gene is located on chromosome 15 and it plays an important role in aromatization, which results in production of estrogen from androgens. The mutation in this gene can result in either increased or decreased aromatase activity. **Materials and Methods:** A case-control study was designed to compare 250 breast cancer cases with 250 age-matched healthy controls. The frequency distribution of CYP19 polymorphism was assessed by polymerase chain reaction confronting two pair primers (PCR-CTPP). **Results:** CYP19 polymorphism at codon 39 Trp/Arg (W39R) results in three genotypes TT, TC, and CC, but in the present study CC genotype was not found in breast cancer cases as well as in controls. The TT genotype was significantly elevated in disease (90.8%) as compared to controls (68.5%). The frequency of TC was found to be increased in premenopausal women with breast cancer (12.2%) and the frequency of TT genotype was increased in patients who were postmenopausal (94.1%). The increased frequency of heterozygotes was found in cases with familial incidences of cancer (10.8%), estrogen and progesterone receptor positive status, node positive status (9.8%), and occupied in agriculture (14.8%). Higher frequencies of both TT and TC genotype were increased in patients with high body mass index (BMI). The frequency of TT genotype was found to be increased in advanced stage of the disease. **Conclusion:** Hence, we conclude that W39 with increased aromatase activity confers greater risk to develop breast cancer especially in postmenopausal women and might also contribute to advanced stage.

**Key words:** Aromatization, breast cancer, CYP19, polymorphisms, receptor status

## INTRODUCTION

Aromatase (CYP19A1) was a cytochrome P450 enzyme (P450<sub>arom</sub>) that plays a crucial role in the biosynthesis of estrogens (C18 steroids) from androgens (C19 steroids) in all vertebrate species.<sup>[1]</sup> In humans, aromatase (CYP19) expression was regulated by different tissue-specific promoters in the placenta, ovary, breast, bone, adipose tissue, vascular endothelium, and brain; resulting in systemic (gonadal/ovarian) and local (extragonadal)

estrogen production in these tissues.<sup>[2]</sup> Aromatase exhibits important biological effects at different stages of development. For example, aromatase expression in the fetal-derived placenta protects the mother from the potentially androgenizing (virilizing) effects of fetal adrenal androgens during pregnancy, whereas ovarian (endocrine) and breast (paracrine/intracrine) aromatase expression was necessary for estrogen-dependent breast development at puberty. In addition, aromatase mediates uterine growth and bone maturation during adolescence and influences bone mineralization, lipid metabolism, and cardiovascular risk in the adult life.<sup>[3]</sup>

CYP19 protein localizes to the endoplasmic reticulum and catalyzes the last steps of estrogen biosynthesis, three successive hydroxylations of the A ring of androgens. Mutations in this gene can result in either increased or decreased aromatase activity the associated phenotypes suggested that estrogen functions both as

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a sex steroid hormone and as a promoter of growth or differentiation. CYP19 gene was localized to 15q21.1 by *in situ* hybridization<sup>[4]</sup> and spans at least 70 kb of genomic DNA and contains 10 exons.

Genetic variation at this locus might alter aromatase activity and thereby affect hormone levels. Miyoshi *et al.*,<sup>[5]</sup> had identified two novel polymorphisms in the CYP19 gene and showed that one of them (codon 39 Trp/Arg) was significantly associated with breast cancer risk among Japanese. The study of the codon 39 Trp/Arg polymorphism of CYP19 gene showed no association with breast cancer risk in the study group as a whole, but homozygous and heterozygous carriers of the variant Arg allele showed a significantly increased risk of breast cancer among premenopausal women with a late age at first full-term pregnancy.<sup>[6]</sup>

Genotypes of aromatase polymorphisms might influence the prognosis for breast cancer patients not only by affecting the extent of estrogen exposure, but also through an alteration in tumor characteristics.<sup>[7]</sup> No statistically significant differences were found between cases and controls in the genotype distributions of the 19 individual single nucleotide polymorphisms (SNPs) and the (TTTA)<sub>n</sub> repeat polymorphism evaluated in the study. No overall association of breast cancer risk with common CYP19A1 gene variants among Chinese women was observed.<sup>[8]</sup>

In the present study, we have examined a series of breast cancer cases as well as controls to determine whether CYP19 polymorphism influence the risk for the development of breast cancer.

## MATERIALS AND METHODS

A group of 250 breast cancer patients were selected for study and 250 healthy and age matched women without family history of breast cancer or any other cancers were selected to serve as control group. The diagnosis of breast cancer was established by pathological examination, mammography, fine needle aspiration cytology (FNAC), and biopsy. Epidemiological history such as age at onset of breast cancer, 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 immunohistochemical 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 ethylenediaminetetraacetic acid (EDTA) vacutainer from patients as well as controls. DNA was isolated<sup>[9]</sup> and used for amplification of CYP19 gene polymorphisms.

## Statistical analysis

The results were analyzed using appropriate statistical tests by Statistical Package for Social Sciences (SPSS) version 14. Odds ratio (OR) 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  $2 \times 2 \chi^2$  and  $\chi^2$  test for heterogeneity.

## CYP19 polymorphism

Polymerase chain reaction confronting two pair primers (PCR-CTPP) was done for identification of CYP19 polymorphism using specific primers.<sup>[6]</sup> Four primers are added to the same PCR mix. After PCR amplification, genotypes are identified directly on 2% agarose gel. Arginine allele (CC) was identified by the presence of 427 and 264 bp fragments. Tryptophan allele (TT) was identified by 427 and 200 bp fragments and heterozygotes by 427, 264, and 200 bp fragments [Figure 1].

## RESULTS

The polymorphism of CYP19 at codon 39 Trp/Arg (W39R) results in three genotypes TT, TC, and CC, respectively; but in the present study CC genotype was not found in breast cancer cases as well as in controls. The TT genotype was significantly elevated in disease (90.8%) as compared to controls (68.5%). The frequency of TC was found to be increased in premenopausal women with breast cancer (12.2%) and the frequency of TT genotype was increased in patients who were postmenopausal (94.1%). The increased frequency of heterozygotes was found in cases

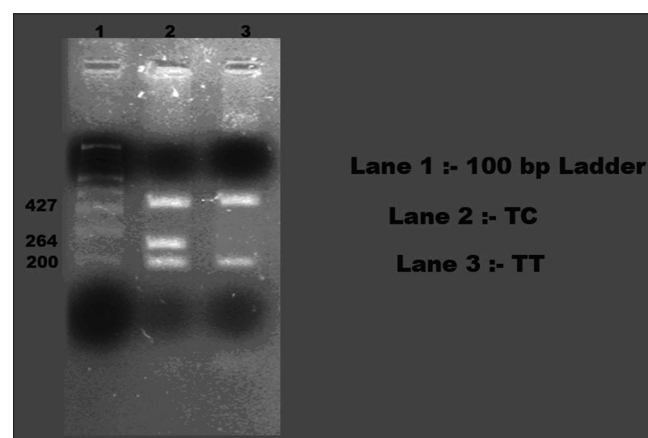


Figure 1: CYP19 gene polymorphism gel electrophoresis

with familial incidences of cancer (10.8%) when compared to nonfamilial cases. Higher frequencies of both TT and TC genotype were increased in patients with high body mass index [Table 1]. The elevated frequencies of patients with estrogen and progesterone receptor positive status and occupied in agriculture (14.8%) were observed with TC genotype. The heterozygote frequency was increased in breast cancer patients with node positive status (9.8%) and association was not found with respect to stage of the breast cancer. But the frequency of TT genotype was found to be increased in advanced stage of the disease [Table 2].

## DISCUSSION

The CYP19 gene, located on chromosome 15, encodes the enzyme P450 aromatase, which catalyzes three consecutive hydroxylation reactions converting C19 androgens to aromatic C18 estrogenic steroids. This enzymatic complex belongs to the class of mammalian endoplasmic reticulum cytochrome P450, anchored with the N-terminal membrane domain extending from A20 to W39. The deletion of first 53 residues of aromatase protein resulted in complete loss of enzymatic action. Genetic variation at this locus may alter aromatase activity and thereby affect hormone levels. Several point mutations in CYP19 were also associated with CYP19 loss of function. However, the mutation R264C did not influence the function but failed to associate with the onset of breast cancer. A tetranucleotide repeat polymorphism (TTTA)<sub>n</sub> in intron 4 of the CYP19 gene<sup>[10]</sup> and C826T variation in exon 7 (Arg264Cys) were studied by several groups.<sup>[11,12]</sup> Kristensen *et al.*,<sup>[13]</sup> found that the (TTTA)<sub>12</sub> allele frequency was increased significantly in breast cancer cases. Miyoshi *et al.*,<sup>[5]</sup> had identified a polymorphism in the CYP19 gene at promoter region and showed that codon 39 Trp/Arg was significantly decreased the breast cancer risk among Japanese.

The polymorphism of CYP19 codon 39 Trp/Arg (W39R) results in three genotypes TT, TC, and CC respectively; but in the present study CC genotype was not found in breast cancer cases as well as in controls. The TT genotype was significantly elevated in disease (90.8%) as compared to controls (68.5%). Miyoshi *et al.*,<sup>[5]</sup> reported that homozygous (CC) and heterozygous (TC) carriers of the variant allele Arg at codon 39 showed a significantly decreased risk of breast cancer (OR = 0.39, 95% confidence interval (CI) = 0.17-0.89). However, Hirose *et al.*,<sup>[6]</sup> had reported 0.7% of CC genotype in breast cancer cases and could not find any significant association of CYP19 polymorphism with breast cancer.

The aromatase activity positively promotes the risk to develop breast cancer due to its role in estrogen synthesis

**Table 1: CYP19 polymorphism with respect to breast cancer and epidemiology**

Parameters	TT		TC	
	n	%	n	%
Disease (250)	227	90.8	23	9.2
Control (248)	170	68.5	78	31.5
$\chi^2$ : 38.13 (P=0.001)*				
Hardy-Weinberg for disease $\chi^2$ =0.58, for control $\chi^2$ =8.64*				
OR (CI 95%) TT vs TC: 4.5284 (2.7308-7.5092)				
Premenopausal (131)	115	87.5	16	12.2
Postmenopausal (119)	112	94.1	7	5.9
$\chi^2$ : 2.992 (P=0.08)				
OR (CI 95%) TT vs TC: 0.4492 (0.178-1.1334)				
Familial (74)	66	89.2	8	10.8
Nonfamilial (176)	161	91.5	15	8.5
$\chi^2$ : 0.326 (P=0.568)				
OR (CI 95%) TT vs TC: 0.7686 (0.311-1.8992)				
BMI				
<20 (14)	13	92.9	1	7.1
20-26.4 (27)	26	96.3	1	3.7
26.4-30 (104)	91	87.5	13	12.5
>30 (45)	41	91.1	4	8.9
$\chi^2$ : 2.1 (P=0.55)				
Occupation				
Housewives (173)	159	91.9	14	8.1
Agriculture (27)	23	85.2	4	14.8
White-collar job (43)	38	88.4	5	11.6
Other (7)	7	100	0	0

$\chi^2$ =Chi-square, OR=Odds ratio, CI=Confidence interval, BMI=Body mass index, TT=Tryptophan allele, TC=Tryptophane/arginine allele, CYP19=Cytochrome P450 19 gene

**Table 2: CYP19 Polymorphism with respect to breast cancer clinical parameters**

Parameters	TT		TC	
	n	%	n	%
Estrogen receptor status				
Positive (90)	80	88.9	10	11.1
Negative (98)	90	91.8	8	8.2
$\chi^2$ : 0.471 (P=0.49)				
OR (CI 95%) TT vs TC: 0.7111 (0.2676-1.8895)				
Progesterone receptor status				
Positive (87)	75	86.2	12	13.8
Negative (101)	95	94.1	6	5.6
$\chi^2$ : 3.329 (P=0.07)				
OR (CI 95%) TT vs TC: 0.3947 (0.1415-0.1008)				
HER-2/neu				
Positive (26)	25	96.2	1	3.8
Negative (27)	27	100	0	0
$\chi^2$ : 1.058 (0.304)				
Node status				
Positive (122)	110	90.2	12	9.8
Negative (75)	69	92.0	6	8.0
$\chi^2$ : 0.189 (P=0.664)				
OR (CI 95%) TT vs TC: 0.7971 (0.2859-2.222)				
Stage				
I (11)	8	72.7	3	27.3
II (96)	86	89.6	10	10.4
III (73)	67	91.8	6	8.2
IV (46)	46	95.8	2	4.2
$\chi^2$ : 2.1 (P=0.55)				

$\chi^2$ =Chi-square, OR=Odds ratio, CI=Confidence interval, TT=Tryptophan allele, TC=Tryptophane/arginine allele, CYP19=Cytochrome P450 19 gene

especially in older women. The mutation at 39 codon abolished or reduces the protein function irrespective of the substrate used,<sup>[14]</sup> suggesting that this codon position might play critical role in aromatase activity. W39 (tryptophan at codon 39) has been reported to be highly conserved and is hydrophobic amino acid in contrast to arginine which is a positively charged residue, hence the latter residue could participate in the ionic interaction with E42 (glutamic acid) according to structural model. Several ionic interactions between arginine and glutamic acid have been reported in other P450 cytochromes favoring the formation secondary structures. In support of this Carani *et al.*,<sup>[15]</sup> identified homozygous mutant R365Q associated with low activity. Keeping in view the role of arginine in ionic interactions, it was speculated that W39R of CYP19 could destabilize the structure of aromatase by forming salt bridge with E42 leading to inactivation. Hence, our study gains support from the above that TT genotype (W39) confers higher risk to breast cancer than TC (we did not find any homozygous CC in our data). TT genotype with normal activity might predispose to breast cancer in the presence of other confounding factors, whereas CC genotype (abolish aromatase) might reduce the risk to breast cancer development.

The frequency of TC was found to be increased in premenopausal women with breast cancer (12.2%) when compared to postmenopausal women (5.9%). The present study is in accordance with Japanese study,<sup>[6]</sup> who has suggested that homozygous and heterozygous carriers of the variant allele Arg had significantly increased risk of breast cancer among premenopausal women with a later age at full-term pregnancy. The frequency of TT genotype was increased in patients who were postmenopausal (94.1%). In general, estrogen synthesis in postmenopausal women occurs principally in the adipose tissue, variation in aromatase activity in this subset of women might be of greater importance to breast cancer risk.

The higher frequency of heterozygotes was found in cases with familial incidences of cancer (10.8%) when compared to nonfamilial cases. Higher frequency of both TT and TC genotype were increased in patients with high body mass index, which was the risk confounding factor for breast cancer. Agarwal *et al.*,<sup>[6]</sup> suggested that breast cancer patients might have an inherently higher aromatase expression in breast adipose tissue when compared to healthy women. After menopause, estrogen in the circulation is predominantly estrone (E1), derived from the peripheral aromatization of androstenedione. Plasma estrogen production is directly correlated with body weight, indicating that most of the postmenopausal, extra glandular aromatization of plasma androstenedione takes place in adipose tissue.<sup>[17]</sup>

When occupation of the patients was considered, higher frequency of agricultural laborers (14.8%) with TC genotype was observed, suggesting the role of xenoestrogens, which are estrogen like products present in the pesticides. These estrogens will be stored in the fatty tissues like breast tissue and increases the level of estrogens along with estrogens produced due to aromatization by CYP19 genes leading to cumulative load of estrogen. The present study indicates that agricultural laborers with TC genotype are at higher risk to develop breast cancer.

The frequency of TC genotype was also increased in patients with estrogen and progesterone receptor positive status. The heterozygote frequency was increased in breast cancer patients with node positive status (9.8%) and association was not found with respect to stage of the breast cancer. But the frequency of TT genotype was found to be increased in advanced stage of the disease, which suggests that this genotype might lead to bad prognosis of breast cancer.

Genetic polymorphism in CYP19 might be involved in mechanism other than protein structure such as mRNA folding, stability, modulation of transcription and translational regulation. In support of this Kristensen *et al.*,<sup>[13]</sup> had observed variation in mRNA levels in relation to C/T polymorphism of exon 10 of CYP19. Introduction of polar residue into hydrophobic anchor domain of CYP450 might influence cellular localization or trafficking. An immunoblot analysis of W39R mutation performed on subcellular fraction detected aromatase in microsomal fraction and not in cytosol. This inactivation can be presumed to be due to decreased expression or structural modification of enzyme.

## CONCLUSION

Hence, we conclude that W39 with increased aromatase activity confers greater risk to develop breast cancer especially in postmenopausal women and might also contribute to advanced stage.

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