RESEARCH COMMUNICATION

CYP17 (T-34C), CYP19 (Trp39Arg), and FGFR2 (C-906T) Polymorphisms and the Risk of Breast Cancer in South Indian Women

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Abstract

Breast cancer is initiated by exposure to endogenous and exogenous estrogens. A case-control (n=250-500) study was undertaken to investigate the role of Single Nucleotide Polymorphisms (SNP's) in CYP17 (T-34C), CYP19 (Trp39Arg) and FGFR2(C-906T). Genotyping was done using the Taqman allelic discrimination assay for CYP17 (T-34C) and FGFR2 (T-906C) and PCR-CTPP for CYP19 (Trp39Arg). There was a significant protective association of the (TT/CC) genotype of the CYP17 gene against the risk of developing breast cancer (OR=0.68, 95% CI: 0.49-0.96), which was more significant in postmenopausal women (OR=0.56, 95% CI: 0.35-0.89) (p=0.015). CYP19 (Trp39Arg) is a rare polymorphism and all the cases were homozygous for the wild type Trp allele (100%); this was also the case for 99.2% of the controls. We were unable to detect any variant form of the CYP19 gene in south Indian women. There was no significant association between the risk of breast cancer and FGFR2 (C-906T). These results suggest that the CYP17 TT/CC genotype is associated with decreased risk for breast cancer, especially in post menopausal women.

Key Words: SNPs - CYP17 - FGFR2 - CYP19 - breast cancer

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Introduction

Genes involved in the metabolism of estrogens and growth factor receptors play an important role in breast cancer development (Dunning et al., 1999). The established risk factors known to modify lifetime exposure to estrogen and progesterone include, age at menarche, obesity, age at menopause, parity, and hormone replacement therapy. New susceptible loci for breast cancer have been identified in the recent genome wide scans. Polymorphism in low penetrance gene account for more than 70% of breast cancer, and its association with environmental and life style factors plays a vital role in developing the disease (Thompson and Easton., 2004; Dunning et al., 1999). These low risk genes are not only involved in development of the disease but can also predict the treatment outcome of the disease (Rajkumar et al., 2008).

CYP17 plays a key role in estrogen biosynthesis. Substitution of T-34C in 5'untranslated region of CYP17 gene contributes to high transcriptional activation of the variant allele (Feigelson et al., 1997, Huang et al., 1999). Studies done by Kristensen et al (1999) could not, however, verify this hypothesis. Variant CC allele of CYP17 gene already has been shown to be associated with increased risk of breast cancer among Indian postmenopausal women (Chacko et al., 2004; Chakraborthy et al., 2007). Premenopausal women with the CC alleles have increased levels of serum estradiol than those with the TT allele (Feigelson et al., 1998). This result also suggests that nulliparous women with CC/CC genotype have a higher risk of breast cancer. Although CYP17 polymorphism has been considered as a risk factor for breast cancer, contradicting results have been obtained. The CYP19 encodes P450 aromatase, which catalyzes three consecutive hydroxylation reactions converting C19 androgens to aromatic C18 estrogenic steroids. Genetic variation at this locus may alter aromatase activity which involves estrogen biosynthesis. Increased breast cancer risk was associated with the homozygous and heterozygous carrier variant (Arg/Arg) of CYP19 gene in premenopausal Japanese women (Hirose et al., 2004). FGFR2 which encodes a receptor tyrosine kinase is amplified or over expressed in some breast cancers (Grose & Dickson., 2005). Recent genome wide scans (Easton et al., 2007; Hunter et al., 2007) have reported a positive association of FGFR2 (C906-T) polymorphism with breast cancer.

We have undertaken a case control study to assess the association of CYP17 (T-34C) CYP19 (Trp39Arg) and

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FGFR2 (C906T) polymorphisms with breast cancer risk in South Indian women. We also explored potential interaction between the genotype and known risk factors for breast cancer.

Materials and Methods

Subjects

The participants of the study were 250 histologically proven breast cancer cases and 500 age matched controls. Details regarding sample collection and DNA extraction have been previously mentioned (Samson et al., 2007).

Genotyping

Genotyping for CYP17 (T34C) was carried out by the Taqman Allelic Discrimination method using the ABI 7000 Sequence Detection System (Applied Biosystems, Foster City, CA). DNA sequencing was done to identify each allele of the SNP's and the heterozygous conditions and subsequently these were used as controls in all the runs. A 93 base pair product was amplified which includes the T-C polymorphisms using the following primers and fluorescently labeled allele specific probes: Forward primer 5'-GCCTCCTTGTGCCCTAGAGGT-3', Reverse primer 5'-CCACGAGCTCCCACATGGT-3', Probe "T" allele 5' FAM- TCCACTGCTGTCTAT-MGB 3'and Probe "C" allele 5' VIC- CTCCACCGCTGTC-MGB 3'. 30-50ng of DNA was used for amplification along with 900nm of each primer and 100nm of each probe and 2 x Taqman universal PCR master mix (Applied Biosystems). Amplification condition were 2 min at 50°C, 10min at 95°C and 40 cycles of 30 sec at 92°C and 1min at 60°C. Genotyping analysis for CYP 19 (Trp39Arg) was performed by PCR with confronting two-paired primers (PCR-CTPP) as described previously by Hirose K et al (2004). FGFR2 (C-906T) genotyping analysis was performed by the Taqman Allelic Discrimination method (Applied Biosystems, Foster City, CA). Primers and probes mix were obtained directly from Applied Biosystems Assays-on-Demand™. In addition, random samples were sequenced to check the accuracy of the genotyping.

Detection of Progesterone Receptors

Semi quantitative detection of progesterone receptor was done by immunohistochemistry using Anti-Human Progesterone Receptor, monoclonal antibody (clone PgR 636) from DAKO Denmark as described previously (Press et al., 2002).

Statistical Analysis

Descriptive statistics were used to present the distributions of case and control subjects with respect to the factors studied. Chi-squared test was used to test for statistical significance in the difference in the proportion of subjects between cases and control groups, for the factors measured on a nominal scale. Analysis was conducted as matched and unmatched case control study. Logistic regression was performed to calculate the odds ratio (OR) always accompanied by 95% confidence intervals and p values. In the multivariate model, factors that were adjusted included religion, age at menarche, age at first child birth, menopausal status, and consanguineous marriage.

Table 1. Effects of Gene Factors on Breast Cancer Risk by Univariate and Multivariate Conditional Logistic Regression Analysis

Variables#	Cases	Controls	OR 95% CI	OR 95% CI
CYP 17			Univariate	Multivariate
TT/TT	127 (50.8)	220 (44.0)	1.00*	1.00*
CC/CC	32 (12.8)	54 (10.8)	1.02 (0.63-1.64)	0.96 (0.57-1.62)
TT/CC	91 (36.4)	226 (45.2)	0.68 (0.43-0.96)	0.61 (0.43-0.88)
FGFR2				
CC/CC	127 (50.8)	261 (52.2)	1.00*	1.00*
TT/TT	35 (14.0)	63 (12.6)	1.14 (0.71-1.84)	1.09 (0.66-1.81)
CC/TT	88 (35.2)	176 (35.2)	1.00 (0.75-1.41)	1.00 (0.71-1.42)

* Reference Category; # Adjusted for religion, age at menarche, age at first child birth, menopausal status, consanguineous marriage

Table 2. Association Between CYP17 Polymorphisms and Breast Cancer in Pre- and Post-Menopausal Women - Univariate

	Pre- Cases	Controls	OR 95% CI	Post- Cases	Controls	OR 95% CI
TT/TT	59	145	1.00*	68	75	1.00*
CC/CC	16	31	1.27 (0.65-2.49)	16	23	0.77 (0.37-1.57)
CC/TT	40	127	0.77 (0.49-1.23)	50	99	0.56 (0.35-0.89)$

*Reference; $P=0.015

Results

Allelic frequencies for CYP17 (T-34C) and FGFR2 (C906T) have been given in Table 1. Sequencing was done randomly to check for the presence of the alleles, and the sequencing results were always concordant with the

Table 3. Association Between Combinations of CYP17 and FGFR2 Genotypes and Breast Cancer Risk

Variables#	FGFR2	CYP17	Cases	Controls	Univariate OR 95% CI	Multivariate OR 95% CI
	CC/CC	TT/TT	66	115	1.00*	1.00*
	CC/CC	CC/CC	20	25	1.42 (0.73-2.73)	1.29 (0.63-2.66)
	CC/CC	TT/CC	41	121	0.59 (0.36-0.94)+	0.57 (0.34-0.96)+
	TT/TT	TT/TT	16	28	0.99 (0.49-1.97)	1.06 (0.50-2.25)
	TT/TT	CC/CC	2	8	0.47 (0.99-2.25)	0.43 (0.08-2.22)
	TT/TT	TT/CC	17	27	1.12 (0.55-2.30)	0.90 (0.42-1.92)
	CC/TT	TT/TT	45	77	1.01 (0.63-1.62)	1.12 (0.67-1.87)
	CC/TT	CC/CC	10	21	0.78 (0.34-1.78)	0.82 (0.35-1.94)
	CC/TT	TT/CC	33	78	0.72 (0.43-1.20)	0.59 (0.34-1.03)

*Reference; #Adjusted for religion, age at menarche, age at first child birth, menopausal status, consanguineous marriage; +p=0.03

Table 4. Association Between Combinations of FGFR2 Genotypes and Progesterone Receptor Status

	PR-Positive	PR-Negative	
CC/CC	32	73	
TT/TT	10	18	
CC/TT	32	30	p = 0.02

genotyping results.

The heterozygous genotype of CYP17 (TT/CC) was found to be significantly associated with risk for breast cancer both in univariate and multivariate analysis, irrespective of the menopausal status (OR 0.61; CI, 0.43-0.88), the effect being greater in postmenopausal women (OR: 0.56; 95% CI: 0.35-0.89) (Table 2). In the present study population all the cases were homozygous for CYP19 Trp/Trp allele (100%); in the controls 99.2% were homozygous for Trp/Trp allele and 0.8% were heterozygous (Trp/Arg). No significant association was seen between FGFR2 (C906T) and breast cancer risk in south Indian women. In the cases there was an association between FGFR2 (C906T) and progesterone receptor (PR) status (P<0.02) but not with estrogen receptor (ER) status.

Combined analysis was done to study the gene-gene interaction among CYP17 (T34C) and FGFR2 (C906T). CYP17 TT/TT and FGFR2 (CC/CC) was taken as the reference category. CYP17 (TT/CC) and FGFR2 (CC/CC) genotypes were associated with a decreased risk in Univariate (OR-0.59; 95% CI: 0.36-0.94), and multivariate analysis (OR-0.57; 95% CI: 0.34-0.96).

Discussion

To the best of our knowledge, this study examines for the first time, the association between the polymorphism in CYP19 (Trp39Arg) and FGFR2 (C906T) and breast cancer risk in South Indian women.

Variant CC allele of CYP17 is associated with increased enzyme activity and higher life time exposure of estrogens. Women carrying the CC allele were reported to be associated with an increased risk of advanced breast cancer (Feigelson et al., 1997, Bergman-Jungestrom et al., 1999, Huang et al., 1999, Spurdle et al., 2000, Chacko et al., 2004); while other studies failed to show this association (Hamajima et al., 2000; Ambrosone et al., 2003; Mitrunen et al., 2000; Wu et al., 2003; Ahsan et al., 2005; Helzlsouer et al., 1998). The proportion of CC alleles among South Indian women in this study (10.8%) is similar to that of the Caucasian population (8-17%) (Weston et al., 1998; Cui et al., 2003; Hefler et al., 2004). Among Asian population, the frequency of CC/CC was 28% for Taiwanese (Huang et al., 1999) and 16-21% for Japanese (Hamajima et al., 2000; Miyoshi et al., 2000). We found a significant association with the TT/CC genotype of CYP17 and a lowered breast cancer risk. Postmenopausal women with TT/CC genotype had a greater decrease in the risk. Zhang et al (2008) reported an increased risk associated, in postmenopausal women carriers of TT/CC genotype (OR = 1.77, 95% CI = 1.11-2.83), which is contradictory to our result. In another study, increased risk of breast cancer was observed in nulliparous women who were carriers of TT/CC genotype (OR = 1.31; 95% CI: 0.74 - 2.32) (Verla-Tebit et al., 2005).

Even though our results suggest that TT/CC allele of CYP17 might play a role in breast cancer development, we are unable to explain the biological mechanism of CYP17 heterozygous genotype lowering the risk for breast cancer. We did not observe any significant association between other risk factors for breast cancer and CYP17 genotypes.

CYP 19 Trp39Arg is a rare polymorphism. The Arg39 allele was only detected among the Hawaiians (2.1%) and the Japanese (2.9%). Only a few studies are available to report the association between CYP19 (Trp39Arg) with breast cancer (Hirose et al., 2004, Miyoshi et al., 2000, Nativelle-Serpentini et al., 2002).

FGFR2 (C906-T) is a newly identified gene polymorphism associated with breast cancer risk. In our study FGFR2 (C906-T) was not associated with breast cancer risk in south Indian women, which is in contrast to the data from the west (Hunter et al., 2007, Easton et al., 2007). In our cases FGFR2 (C906-T) was significantly associated with PR receptor status, however these results are contradictory to Garcia-Closas et al., (2008) results, which showed no association between FGFR2 and Progesterone receptor. Interactions between CYP17 and FGFR2, showed a statistically significant association with breast cancer risk. We are unable to find studies which have done combined genotype analysis for CYP17 and FGFR2 for us to compare our results with.

In conclusion, our study demonstrates an association between CYP17 and breast cancer risk especially in postmenopausal women. We are unable to explain the role of CYP17 heterozygous genotype and breast cancer risk; we speculate that this association is probably due to chance.

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References

Ahsan H, Whittemore AS, Chen Y, et al (2005). Variants in estrogen-biosynthesis genes CYP17 and CYP19 and breast cancer risk: a family-based genetic association study. *Breast Cancer Res*, **7**, 71-81.

Ambrosone CB, Moysich KB, Furberg H, et al (2003). CYP17 genetic polymorphism, breast cancer, and breast cancer risk factors. *Breast Cancer Res*, 5, 45-51.

Bergman-Jungeström M, Gentile M, Lundin A-C, the South-East Breast Cancer Group (1999). Association between CYP17 gene polymorphism and risk of breast cancer in young women. *Int J Cancer*, **84**, 350-3.

Chacko P, Rajan B, Mathew BS, Joseph T, Pillai MR (2004). CYP17 and SULT1A1 gene polymorphisms in Indian breast cancer. *Breast Cancer*, **11**, 380-8.

Chakraborty A, Murthy NS, Chintamani C, et al (2007). CYP17 gene polymorphism and its association with high-risk north Indian breast cancer patients. *J Hum Genet*, **52**, 159-65.

Cui JS, Spurdle AB, Southey MC, et al (2003). Regressive logistic and proportional hazards disease models for within-family analyses of measured genotypes, with application to a CYP17 polymorphism and breast cancer. *Genet Epidemiol*, **24**, 161-72.

Dunning AM, Healey CS, Pharoah PD, et al (1999). A systematic review of genetic polymorphisms and breast cancer risk. *Cancer Epidemiol Biomarkers Prev*, **8**, 843-54.

Easton DF, Pooley KA, Dunning AM, et al (2007). Genome-wide association study identifies novel breast cancer susceptibility loci. *Nature*, **447**, 1087-93.

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

Feigelson HS, Shames LS, Pike MC, et al (1998). Cytochrome P450c17alpha gene (CYP17) polymorphism is associated with serum estrogen and progesterone concentrations. *Cancer Res*, **58**, 585-7.

Garcia-Closas M, Hall P, Nevanlinna H, et al (2008). Heterogeneity of breast cancer associations with five susceptibility loci by clinical and pathological characteristics. *PLoS Genet*, **25**, e1000054.

Grose R, Dickson C (2005). Fibroblast growth factor signaling in tumorigenesis. *Cytokine Growth Factor Rev*, **16**, 179-86.

Hamajima N, Iwata H, Obata Y, et al (2000). No association of the 5' promoter region polymorphism of CYP17 with breast cancer risk in Japan. *Jpn J Cancer Res*, **91**, 880-5.

Hefler LA, Tempfer CB, Grimm C, et al (2004). Estrogen-metabolizing gene polymorphisms in the assessment of breast carcinoma risk and fibroadenoma risk in Caucasian women. *Cancer*, **101**, 264-9.

Helzlsouer KJ, Huang HY, Strickland PT, et al (1998). Association between CYP17 polymorphisms and the development of breast cancer. *Cancer Epidemiol Biomarkers Prev*, **7**, 945-9.

Hirose K, Matsuo K, Toyama T, et al (2004) The CYP19 gene codon 39 Trp/Arg polymorphism increases breast cancer risk in subsets of premenopausal Japanese. *Cancer Epidemiol Biomarkers Prev*, **13**, 1047-11.

Huang CS, Chern HD, Chang KJ, et al (1999). Breast cancer risk associated with genotype polymorphism of the estrogen-metabolizing genes CYP17, CYP1A1, and COMT: a multigenic study on cancer susceptibility. *Cancer Res*, **59**, 4870-5.

Hunter DJ, Kraft P, Jacobs KB, et al (2007). A genome-wide association study identifies alleles in FGFR2 associated with risk of sporadic postmenopausal breast cancer. *Nat Genet*, **39**, 870-4.

Kristensen N, Haraldsen EK, Anderson KB, et al (1999). CYP17 and breast cancer risk: the polymorphism in the 5' flanking area of the gene does not influence binding to Sp-1. *Cancer Res*, **59**, 2825-8.

Mitrunen K, Jourenkova N, Kataja V, et al (2000) Steroid metabolism gene CYP17 polymorphism and the development of breast cancer. *Cancer Epidemiol Biomarkers Prev*, **12**, 1343-8.

Miyoshi Y, Iwao K, Ikeda N, Egawa C, Noguchi S (2000). Breast cancer risk associated with polymorphism in CYP19 in Japanese women. *Int J Cancer*, **89**, 325-8.

Nativelle-Serpentini C, Lambard S, Séralini GE, Sourdaine P (2002) Aromatase and breast cancer: W39R, an inactive protein. *Eur J Endocrinol*, **146**, 583-9.

Press M, Spaulding B, Groshen S, et al (2002). Comparison of different antibodies for detection of progesterone receptor in breast cancer. *Steroids*, **67**, 799-813.

Rajkumar T, Samson M, Rama R, et al (2008). TGFß1 (Leu10Pro), p53 (Arg72Pro) can predict for increased risk for breast cancer in South Indian women and TGFß1 (leu10pro) allele predicts response to concurrent neo-adjuvant chemo-radiotherapy. *Breast Cancer Res Treat*, **112**, 81-7.

Samson M, Swaminathan R, Rama R, et al (2007). Role of GSTM1 (Null/Present), GSTP1 (Ile105Val) and P53 (Arg72Pro) genetic polymorphisms and the risk of breast cancer: a case control study from South India. *Asian Pac J Cancer Prev*, **8**, 253-7.

Spurdle AB, Hopper JL, Dite GS, et al (2000). CYP17 promoter polymorphism and breast cancer in Australian women under age forty years. *J Natl Cancer Inst*, **92**, 1674-81.

Thompson D, Easton D(2004). The genetic epidemiology of breast cancer genes. *J Mammary Gland Biol*, **9**, 221-36.

Verla-Tebit E, Wang-Gohrke S, Chang-Claude J (2005) CYP17 5'-UTR MspA1 polymorphism and the risk of premenopausal breast cancer in a German population-based case-control study. *Breast Cancer Res*, **7**, 455-64.

Weston A, Pan CF, Bleiweiss IJ, et al (1998) CYP17 genotype and breast cancer risk. *Cancer Epidemiol Biomarkers Prev*, **7**, 941-4.

Wu AH, Seow A, Arakawa K, et al (2003). HSD17B1 and CYP17 polymorphisms and breast cancer risk among Chinese women in Singapore. *Int J Cancer*, **20**, 104, 450-7.

Zhang L, Gu L, Qian B, et al (2008). Association of genetic polymorphisms of ER-alpha and the estradiol-synthesizing enzyme genes CYP17 and CYP19 with breast cancer risk in Chinese women. *Breast Cancer Res Treat*, [Epub ahead of print].