RESEARCH COMMUNICATION

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

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Abstract

The present study was undertaken to examine the frequencies of GSTM1 (Null/Present), GSTP1 (Ile105Val) and p53 (Arg72Pro) genotypes and their relations to breast cancer susceptibility in South Indian women. This case - control study involved 250 consecutive breast cancer cases and 500 healthy controls matched in five-year age categories in the ratio of 1:2. Genotyping was performed by PCR for GSTM1, Real-Time Allelic discrimination assay for GSTP1 and PCR-CTPP for p53. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using conditional logistic regression after adjusting for the known risk factors for breast cancer. The frequencies for the GSTM1 Null genotype were 26% in the cases and 22% in the controls; for GSTP1 Ile/Ile, Ile/Val, Val/Val the frequencies were 46.6%, 41.9% and 11.5%, respectively, in cases and 46.0%, 43.8% and 10.2% in controls; for p53 Arg/Arg, Arg/Pro & Pro/Pro the frequencies were 26.4%, 50.0% and 23.6% in cases and 27.0%, 44.8% and 28.2% in controls. A nonsignificant elevation in breast cancer risk was observed among women who had the GSTM1 Null genotype (OR=1.24; 95% CI=0.83-1.84), the p53 Arg/Arg genotype (OR=1.28; 95% CI=0.81-2.03) and the Pro/Arg genotype (OR=1.49; 95% CI=0.99-2.25), and the GSTP1 Val/Val genotype (OR=1.1; 95% CI=0.64-1.91).

Key Words: Genetic polymorphisms - GSTM1 - GSTP1- p53 - breast cancer - SNPs

Asian Pacific J Cancer Prev, **8**, 253-257

Introduction

Breast cancer is the second most common cancer among Indian women and the commonest among women in urban India. Hereditary breast cancer may account for around 10% of the breast cancer risk, of these 5% are likely to be associated with mutation in high risk genes like BRCA1/ 2 (Futreal et al., 1994). Human breast cancer is a result of genetic and environmental interaction, and there are likely to be other low risk genes that are distributed more frequently in the population, which interact with environment carcinogens or lifestyle factors. It has been postulated that polymorphisms in genes involved in DNA repair, carcinogen metabolism and growth factor receptors increase the risk of cancer in some individuals (Dunning et al., 1999).

Glutathione S-transferase (GST) is a phase II drug metabolizing enzyme, which acts in coordination with phase I metabolizing enzymes in carcinogen metabolism. Members of the GST family are important candidates for involvement in susceptibility to commonly occurring forms of cancer because they may regulate an individual's ability to metabolize environmental carcinogens (Seidegard et al., 1998).

The GSTM1 gene is frequently associated with absence of one or both of its allele resulting in a null genotype. About 50% of Caucasians and 30.4% of South Indians lack the GSTM1 gene due to inherited homozygous deletion of both alleles (Rebbeck. 1997; Naveen et al., 2004). GSTM1 null genotypes results in the lack of enzyme activity and have been associated with increased risk of breast cancer in post-menopausal women (Ambrosone et al., 1995). The null GSTM1 genotype is also associated with high levels of DNA adducts, suggesting its role in carcinogenesis (Nazar et al., 1999). The GSTP1 Ile105Val polymorphisms reside at the substrate - binding site of the enzyme and the variant is common in Caucasians. This substitution results in a lower enzymatic activity and is associated with higher hydrophobic adduct levels and higher levels of polycyclic aromatic hydrocarbon-DNA adducts in human lymphocytes (Zimniak et al.,1994; Butkiewicz et al., 2000).

p53 tumor suppressor gene exhibits a common polymorphism (Birgander et al., 1996) at amino acid 72 resulting in either Proline ("C" allele) or Arginine ("G"

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allele) form, whose functional significance in carcinogenesis is controversial. Significant association between codon 72 and the risk of breast cancer has been reported in several studies but the results remain inconclusive (Sjalander et al., 1996). It has been suggested that Arginine form of p53 has a 15 -fold enhanced ability to induce apoptosis, compared with proline form. Mutations in the p53 gene have also been associated with altered repair and enhanced cytotoxicity because of DNA damage by benzo(a)pyrene diol epoxide adducts (Wani et al., 2000)

It has been postulated that the decrease in GST enzyme activity could result in higher frequency and a specific pattern of mutation in cancer and p53 is the most frequently mutated gene in human cancer and Arg 72 Pro is a common polymorphisms that exists in the population. In the present study, we investigated the presence of any interaction between the genotypes of these three genes. This is the first study to our knowledge, to give the genotype frequency of GSTP1 and p53 genes and its association toward breast cancer in South Indian Women. There exits little knowledge about the etiology of sporadic breast cancer in South Indian Women. Hence the present study was undertaken to determine the distribution genotype frequencies for GSTM1 (Present/Null), GSTP1 (Ile105Val) and p53 (Arg72Pro) polymorphisms among patients with breast cancer and age-matched controls, and to look for association of the polymorphisms with the risk of breast cancer.

Materials and Methods

Samples

This study included 250 breast cancer patients and 500 healthy controls matched on five-year age category in the ratio of 1:2, who fulfilled the inclusion criteria. Inclusion criteria for cases: (a) histological confirmation of breast cancer, (b) no previous cancer treatment and (c) informed consent was mandatory. Inclusion criteria for controls: (a) no prior diagnosis of benign breast disease (b) no history of hysterectomy or mastectomy or oophorectomy (c) no relatives with breast or ovarian or endometrial or prostrate cancer (d) no physical or mental disability which would preclude their participation in the study. About 15ml of heparanized blood samples and a brief questionnaire about their personnal history and food habits were collected from the cases and age-matched controls subjects after obtaining informed consent.

Genotyping

DNA was extracted from buffy coat by QIAmp DNA blood kit (QIAGEN Inc., Valencia, CA, USA) according to the manufacturer's instructions. The quality and integrity of the DNA extracted were quantitated by spectrophotometer and checked for amplification for ABL gene by PCR. Apart from this, DNA was quantitated by real-time PCR for RnaseP gene (Applied Biosystems, Foster City, CA), which is a single copy gene. RnaseP quantitation was done to know the exact quantity of amplifiable DNA.

Genotyping for GSTM1 was done by the PCR method proposed by Stucker et al (1999) with modification, using exon-specific primers, (Forward 5'-CTGCCCTACTTGATTGATGGG-3'and Reverse 5'CTGGATTGTAGCAGATCA TGC-3'). PCR reactions were carried out in 20ml volume containing 50ng of genomic DNA, 10 pmoles of each primers, 100mm of dNTPs, 10x reaction buffer (GENECRAFT, Germany) and 0.5 unit of Taq polymerase (GENECRAFT, Germany). Amplification was done for 30 cycles with initial denaturation at 95∞C for 10 min, followed by 1min each for denaturation, annealing, extension at 95∞C, 64∞C, 72 ∞C and final extension at 72∞C for 5 min. The presence of the gene was determined by the amplification of 273-bp band, in 2% agarose gel electrophoresis stained with ethidium bromide. While the absence of the band denoted null genotype.

Genotyping for GSTP1 Ile105Val was carried out by the Taqman Allelic Discrimination method using the ABI 7000 Sequence Detection System (Applied Biosystems, Foster City, CA).The primer and probe sequence for genotyping was obtained from Cancer Genome Anatomy Project SNP500 Cancer Database and subsequently checked by Primer express software (Applied Biosystems, Foster City,CA).

Table 1. Distribution of Socio Demographic & Reproductive Factors among Breast Cancer Cases and Controls

Variables	Cases	%	Controls	%	p value
Median age	46 years		47 years		
Religion					
Hindu	205	82.0	443	88.6	0.06
Christian	26	10.4	35	7.0	
Muslim	18	7.2	19	3.8	
Others	1	0.4	3	0.6	
Marital status					
Ever married	244	97.6	487	97.4	0.86
Unmarried	6	2.4	13	2.6	
Parity (among married women)					
Parity	228	93.4	461	94.7	0.65
Nulliparity	15	6.6	26	5.3	
Age at menarche*					
9-12	47	18.9	62	12.4	0.06
13	58	23.3	126	25.2	
14	49	19.7	127	25.4	
15-20	95	38.1	185	37.0	
Age at first child birth (among parious women)					
< = 20	95	41.7	212	46.0	0.02
20-29	116	50.9	235	51.0	
30+	17	7.4	14	3.0	
Menopause					
Pre	115	46.2	303	60.6	<0.001
Post	134	53.8	197	39.4	
Abortions					
No	160	66.0	340	69.8	0.28
Yes	83	34.0	147	30.2	
Consanguineous marriage					
No	191	78.3	416	85.4	0.01
Yes	53	21.7	71	14.6	
Obesity					
No	174	69.9	356	71.2	0.65
Yes	76	30.6	144	28.8	

*excludes one case which had not attained menarche

Table 2. Multifactorial Analysis of Socio-Demographic & Reproductive Factors to Elicit the Relative Risk of Breast Cancer by Conditional Logistic Regression

Factors[+]	Odds ratio	95% CI
Religion		
Hindu	1.00*	-
Christian	2.11	1.00 - 4.43
Muslim	1.39	0.77 - 2.51
Others	0.89	0.08 - 9.29
Age at menarche[$]		
9-12 years	1.00*	-
13	0.56	0.34 - 0.94[#]
14	0.50	0.29 - 0.86[#]
15+ years	0.65	0.40 - 1.06
Age at first child birth (among parous women)		
20 years	1.00*	-
20-29	1.21	0.84 - 1.73
30+ years	2.98	1.35 - 6.58[#]
Menopausal status		
Pre-menopausal	1.00*	-
Post-menopausal	5.18	2.87 - 9.34[#]
Cosanguineous marriage		
No	1.00*	-
Yes	1.66	1.09 - 2.54[#]

*reference category; [$]excludes one case not attained menarche; [#]p=0.05; [+]every factor adjusted for the others in the table; CI, Confidence interval

Genotyping analysis for p53 was carried out by PCR with confronting two-paired primers (PCR-CTPP) as described previously by Hamajima et al (2001). Sequencing was done to confirm the presence of individual gene polymorphisms and subsequently these samples were used as controls in all the runs.

Statistical Analysis

The distribution of subjects with respect to the factors studied is presented using descriptive statistics. The difference in the proportion of subjects between the cases and control groups, of factors measured on a nominal scale is tested for statistical significance using Chi-squared test. Odds ratios for exposure factors compared to appropriate reference categories were calculated by conditional logistic regression analysis to elicit the magnitude of risk of breast cancer, always accompanied by the 95% confidence interval.

Results

The distribution of selected demographic characteristics and known major risk factors for breast cancer are shown in Table 1. Median ages for cases and controls were 46 and 47 years respectively. The distribution of women born of consanguineous marriage was higher in cases than controls (p=0.01). Women having their first child birth after 30 years of age, post-menopausal women and women attained menarche between 9 and 12 years of age were significantly higher among cases than controls.

Table 2 gives the relative risk of socio-demographic and reproductive factors for getting breast cancer based on multifactorial conditional logistic regression analysis. The effect of each factor is obtained after adjusting for the other factors in the table. A two-fold increase in odds ratio among Christians compared to Hindus was observed. The odds ratio increased with increasing age categories at the first child birth: it was higher by 21% for 20-29 years and 3-folds for 30+ years compared to less than 20 years of age. A 66% elevated risk was forthcoming among women who were born of consanguineous marriage while the risk was five folds more for post-menopausal than pre-menopausal state. The odds ratio was decreased among those attained menarche at the age of 13 years (44%), 14 years (50%) and 15 years and more (35%) compared to 9-12 years of age. The results were statistically significant.

The distribution of GSTM1, GSTP1 and p53 alleles and genotype frequency among the breast cancer cases and controls are shown in Table 3. Among the cases, GSTM1 null genotype was seen in 65 (26.0%) and in controls it was seen in 110 (22.0%). There was an increased risk associated with the null genotype, but was not statistically significant. GSTP1 (Ile105 Val) allelic distribution for cases was 118 (47.2%) for homozygous wild type (Ile/Ile), 29 (11.6%) for homozygous variant (Val/Val) and 103 (41.2%) for heterozygous; in controls 230 (46.0%), 51 (10.2%) and 219 (43.8%) respectively, suggesting GSTP1 Val/ Val genotype was associated with an increased risk for breast cancer, but statistically not significant.

Frequencies of Arg/Arg, Arg/Pro and Pro/Pro for p53

Table 3. Distribution of GSTM1, GSTP1 and P53 Genotypes and the Risk of Breast Cancer by Conditional Logistic Regression Analysis

Variables#	Cases	Controls	Univariate Odds Ratio	95% CI	Multivariate[#] Odds Ratio	95% CI
GSTM						
++ / ++	185	390	1.00*		1.00*	
-- / --	65	110	1.26	(0.88 - 1.80)	1.24	(0.83 - 1.84)
P53						
Pro / Pro	59	141	1.00*		1.00*	
Arg / Arg	66	135	1.17	(0.77 - 1.81)	1.28	(0.81 - 2.03)
Pro / Arg	125	224	1.33	(0.92 - 1.95)	1.49	(0.99 - 2.25)
GSTP1						
Ile / Ile	118	230	1.00*		1.00*	
Val / Val	29	51	1.11	(0.67 - 1.83)	1.10	(0.64 - 1.91)
Ile / Val	106	219	0.92	(0.67 - 1.26)	0.90	(0.64 - 1.26)

*Reference category [#]Each factor was adjusted for the other two factors in the table +factors like religion, age at menarche, age at first child birth,menopausal status, consanguineous marriage CI: Confidence interval

among cases were 66(26.4%), 125 (50%) and 59 (23.6%) respectively; in controls it was 135 (27.0%), 224 (44.8%) and 141 (28.2%) respectively. There was an increased risk associated with the p53 Arg/Arg and Arg/Pro genotype, but statistically not significant.

To evaluate the interaction between the genotypes, we examined the combination effect of GSTM1, GSTP1 and p53 genotypes. GSTM1 (Present/Present), GSTP1 (Ile/Ile) and p53 (Pro/Pro) were taken as reference category. Combination analysis was carried out using two markers at a time and the odds ratio was calculated. The analysis did not reveal any statistically significant difference among cases and controls in a) GSTM1 and GSTP1, b) GSTM1 and p53 and c) GSTP1 and p53 combinations. Combined analysis of GSTM1, GSTP1 and p53 with menopausal status revealed no statistically significant association.

Discussion

Variation or deletion of GST enzyme activity in individuals results in impaired removal of DNA damaging electrophiles, which leads to increased risk of somatic mutation leading to tumor formation (Rebbeck.,1997).The results of our study indicate that the GSTM1 (Null/Present), GSTP1 (Ile105Val) and p53 (Arg72Pro) pose a non-significant elevation in breast cancer risk.

Prevalence of GSTM1 null genotype differs among different population; It is 22-35% in the Africans, 38-67% in Caucasians, 33-63% in East Asian population (Rebbeck., 1997) and 26% in Indian population (Buch et al., 2001).However in our study the frequency of homozygous deletion was 26% among cases and 22% in controls and there was no statistically significant association between GSTM1 null genotype and breast cancer. The lack of statistically significant association of GSTM1 homozygous null genotype with breast cancer in our study was consistent with several recent studies (Vogl et al., 2004; Egan et al., 2004; Chacko et al., 2005).

The GSTP1 Val/Val genotype is uncommon and it exists in 5% of Caucasians (Garte et al., 2001). GSTP1 Valine form was recently found to be more active than the Isoleucine form in conjugation reactions involving bulky diol epoxides of PAH (Sundberg et al., 1998). Helzlsouer et al (1998) observed a positive association for GSTP1 Val/Val genotype in post-menopausal women, whereas studies conducted by Milkan et al (2000) and Zhao et al (2001) did not report any positive association of GSTP1 with breast cancer.

The p53 Arg72Pro polymorphism was well characterized in both functional analyses and association studies, with the arginine variant appearing to be more susceptible to the degradation induced by human papillomavirus E6 protein (Storey et al., 1998). 40% of Caucasians while only 10% of African Americans are homozygous for Arginine72 (Tenti et al., 2000). There exist a controversy about the relation between the risk of tumorigenisis and p53 Arg72Pro polymorphism. There are only few reports assessing the p53 codon 72 polymorphism in breast cancer, with inconsistent results (Buyru et al., 2003; Mabrouk et al., 2003; Ohayon et al., 2005).

In our study the GSTM1, GSTP1, p53 polymorphisms did not show any association with the menopausal status for breast cancer risk. Our results are very similar to those by Garcia-Closas et al (1999); Bailey et al (1998); and Milikan et al (2000) who reported no association for GSTM1 null genotype in pre or postmenopausal women. Similarly Egan et al (2004) reported no association of GSTP1 Ile105Val with pre-or postmenopausal status. As far as p53 Arg72Pro is concerned, Noma et al (2004) revealed a significant association with ER positive breast cancer, especially, in post-menopausal women.

In conclusion, our study has shown that women born of consanguineous marriage, post-menopausal women and women who had their first child birth after 30 years of age, have a significantly high risk and women who attained menarche after 12 years of age have decreased risk for breast cancer. All the three polymorphisms studied showed a nonsignificant increased elevation in breast cancer risk.

Acknowledgement

We thank the Chennai Willingdon Corporate Foundation for funding the study and Ms.Parimala for her help in collecting samples and the administration of the questionnaire.

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