



Research Paper

To study association of trp 53 arg 72 pro polymorphic variant with breast cancer-a case control study in north Indian population

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Abstract

Objectives: The single nucleotide polymorphism (SNP) at Trp53arg72 pro genotypes is found to be associated with breast cancer, with variable results, the aim of this study is to evaluate the frequency of SNP at Trp 53 codon 72 and its association with breast cancer in north Indian population.

Methods: 30 patients with breast cancer were compared with 30 controls. Genotype and allele distribution of Trp53 codon 72 were determined using polymerase chain reaction- restriction fragment length polymorphism method.

Results: The genotypic distribution of arg/arg, arg/pro, pro/pro among cancer patients vs controls were 53.33%, 33.33%, 13.33% and 56.7%, 26.7%, 16.7% respectively ($p=0.83$).

Conclusion: though the difference in genotypic distribution was not significant, we found in the higher number of heterozygous genotypes (arg/pro) in cancer tissue suggesting, heterozygous (arg/pro) genotype may predispose women to breast cancer. This necessitates conducting the study including more patients.

Introduction

Breast cancer trend in world

Breast cancer is most common cancer in women worldwide, accounting for 25% of all cases.

Mutations can disrupt normal growth control or can dismantle cell cycle checkpoints that otherwise control cell division or induce cell apoptosis as a response to DNA damage or oncogene activation. P53 and RB gene play a pivotal role in this aspect.

P53 gene

P53 is a tumor suppressor gene which primarily functions as to:- cell cycle inhibitor, Apoptosis

regulators, DNA repair, Inhibition of angiogenesis and metastasis^[1]. Mutation in p53 gene lead to inactivation of function of p53 gene predisposing to carcinoma formation. One such mutation is Trp53 arg 72 pro polymorphism.

Association of Trp 53 arg 72 pro polymorphic variant with breast cancer

The genetic variants of Trp53 (arg, pro) have received attention as possible modifiers of cancer risk. This is due to their role in cell cycle control, DNA repair, apoptosis and possible interaction

with the breast cancer susceptibility genes BRCA1 and BRCA2.^[2,3]

TP53 rs1042522 (Arg72Pro) polymorphism is one of the extensively studied non synonymous polymorphism located in exon 4. In codon 72, substitution of guanine (G) to cytosine (C) lead to alteration of arginine (Arg) to proline (Pro) in the protein structure of p53. Changing of Arg to Pro leads to altering the targeting capacity of p53 to proteasome (p53-mediated apoptosis) and also alters the stimulation of p73, another important tumor suppressor protein, transcription. In a study by Dumont et al. (2003)^[4] Arg form stimulates apoptosis better by at least five times compared to Pro form. This improved apoptosis in Arg variant is due to better localization to mitochondria which in turn is related to enhanced binding and ubiquitination of P53 through E3 ubiquitin ligase MDM2.^[5,6] P53 inactivation is responsible for many other cancer including breast cancer, hence it is important to study polymorphisms in p53 gene as a risk factor for breast cancer.

Methodology

The study was conducted in the department of Surgery and pharmacology at KGMU. Mutation analysis of p53 gene was done on diseased tissue samples of 30 patients, who were confirmed to have breast cancer by routine protocol (ultrasound+mammography+histopathology).

Their tissue samples were assessed for P53 Mutation by real-time PCR. Normal Tissue of same patients (confirmed non malignant by histopathological examination) was taken as control. Patients who received chemotherapy before study and recurrent case of breast cancer after chemotherapy and radiotherapy were excluded. Targeted gene specific probes and primers was used for real-time PCR for identification of mutation (ABI-step one) using master mix. Genomic DNA was extracted from fresh tumor tissue using a QIAamp DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

DNA quality assessment

The quality and quantity of DNA was checked through Quawell spectrophotometer (Quawell Technology Inc. San Jose, CA 95161-2738)) and QbitBR fluorimeter (Agilent, Santa Clara, CA, USA).

DNA having absorption ratio A260/280 greater 1.8 was considered for further analysis.

Genotyping Protocol: For this we designed unlabeled PCR primers and TaqMan® MGB probes (FAM™ and VIC® dye-labeled in 40X assay mix (Assays-by-Design SM Service for SNP Genotyping Assays) for genotyping rs1042522. The alleles were scored in each well using, TaqMan® Genotyping Master Mix with 20ng of specific genomic DNA (Table 1) following the universal thermal cycling parameters as described in Table 2. Each sample was processed in triplicate and negative control was also processed for real time analysis with every 96 well format assay.

Table 1: Allelic Determination PCR protocol

Reaction component	Volume/well (25µl reaction)	Final Concentration
TaqMan® Genotyping Master Mix	12.5	1X
40X Assay Master Mix	0.625	1X
Genomic DNA diluted in H ₂ O	11.875	-
Total	25	

Table 2: Thermal Cycler Conditions

Time and Temperature		
Initial Steps	Each of 40 cycles	
	Denature	Anneal/Extend
Hold	Cycle	
10 min 95 ⁰ C	15 sec 92 ⁰ C	1 min 60 ⁰ C

Statistical Analysis

Groups of continuous variables were compared by student 'T' test or analysis of variance (ANOVA), and discrete data was analysed with Fischer exact test or chi-square test.

Results

Thirty patients enrolled in my study, cancer tissue

and normal breast tissue (internal controls) obtained after mastectomy, normal breast tissue confirmed by histopathological examination in department of pathology, KGMU.

Allele A1 – arg –G nucleotide – labelled as VIC
 Allele A2 - pro –C nucleotide - labelled as FAM
 The raw data obtained from genotyping experiment using ABI Step OnePlus Real Time PCR System, Center for Advance Research, KGMU was analyzed through TaqManGenotyper software. The genotype call was evaluated through threshold quality value=0.94. All the samples were of high quality value 0.99. Detailed results as depicted in Table 1

Table 1: showing the genotypic distribution of Trp53 arg72pro in breast cancer cases and control subjects

Genotype	Cases (n=30)		Control (n=30)	
	No.	%	No.	%
Arg/Arg	16	53.33	17	56.7
Arg/Pro	10	33.33	8	26.7
Pro/Pro	4	13.33	5	16.7

$\chi^2=0.364$ (df=1); p=0.834(NS)

The distribution of three genotypes namely, arg/arg, arg/pro and pro/pro, observed in the

breast cancer patients were 53.33%, 33.33% and 13.33% respectively. The controls showed 56.7%, 26.7 % and 16.7% of arg/arg, arg/pro and pro/pro respectively. There was no significant difference in the distribution of genotypes between breast cancer patients and controls ($\chi^2=0.364$, df=1, P=0.834). In both cases and controls genotype, homozygous genotype Arg/Arg was most common whereas homozygous genotype Pro/Pro was least common.

Table 2: Allele frequency

	Cases(n=30)	Controls (n=30)
Arg allele frequency	0.70	0.70
Pro allele frequency	0.30	0.30

The allele frequencies of breast cancer patients and controls: Allele frequencies of 0.70 (Controls) and 0.70 (breast cancer patients) for arg-coding alleles and 0.30 (Controls) and 0.30 (breast cancer patients) for pro-coding alleles. No significant difference in allele frequencies between breast cancer patients and controls were observed ($\chi^2=0.00$, df=1, P=1.00).

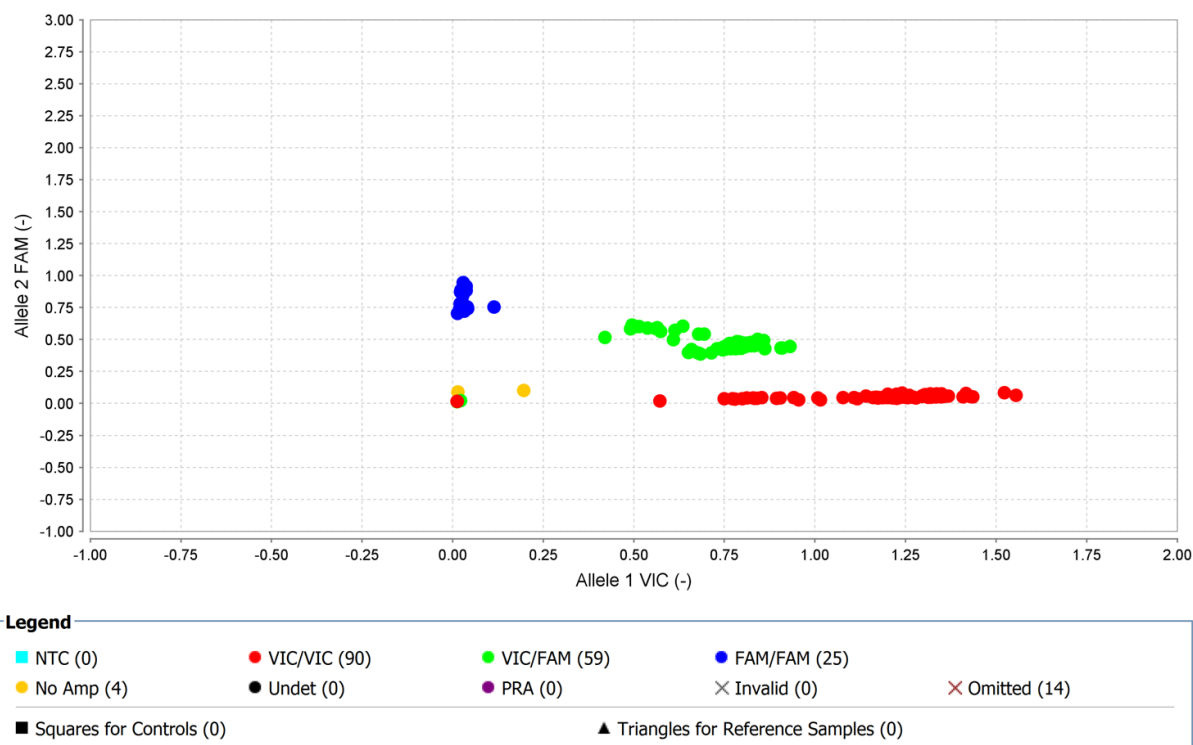


Figure 1: gene distribution chart of arg and pro allele.

Discussion

Breast cancer is multifactorial disorder in women. P53 gene have important role in multiple cellular functions, including DNA repair, gene transcription and apoptosis. Significant association between the p53 codon 72 polymorphism and breast cancer risk have been reported in various studies,^[7] no such association have been identified in other studies. Genetic variations and disease susceptibility have been investigated using Single nucleotide polymorphisms (SNPs). Pro allele has been associated with breast cancer risk in many populations.^{[8][9][10]} Some studies supported Arg allele (Trp53 arg72pro polymorphism) associated with breast cancer risk^{[11][12][13]} while other studies do not support such association.^{[14][15][16]}

Different geographical distribution and ethnicity may be the reason for observed inconsistency. Pro72 allele shows a North-South gradient, from 0.17 [Swedish Saamis] to 0.63 [African Blacks-Nigerians] in north hemisphere population^[17]. Arg72 is most common allele, with frequencies ranging from 0.60 to 0.83 in Western Europe (France, Sweden, and Norway), Central and South America (Mexico, Costa-Rica and Peru), North America (USA) and Japan [IARC, 2010]^[18].

In our study, distribution of three genotypes homozygous arg heterozygous arg/pro and homozygous pro, in breast cancer patients were 53.33% , 33.33% and 13.33% respectively and controls showed 56.7%, 26.7 % and 16.7% respectively. No significant difference in the distribution of genotypes between breast cancer patients and internal controls were found ($\chi^2=0.364$, $df=1$, $P=0.834$). Similar study conducted in southern India^[19], shows distribution of three genotypes in breast cancer patients were 28.57%, 62.85% and 8.57% respectively and Controls showed 29.72%, 51.35% and 18.91% respectively. Just similar to our studies, there was no significant difference in the distribution of genotypes between breast cancer patients and controls ($\chi=1.81$, $df=2$, $P=0.40$) where found in south India population.

We had found that neither pro nor arg allele was associated with breast cancer risk. Population size in this study was small as compared to other related studies, this study results may improve with investigating over bigger population and correlating genotype data and clinical features of breast cancer patients. A number of Metaanalysis where done for establishing association of Trp 53 arg 72 pro polymorphism variant with breast carcinoma risk, there result were similar to our result (Jing Hou et al)^[20] showing no relation between this mutation and breast carcinoma risk, but other metaanalysis (Meireluziagonclaves et. al)^[21] show significant association between Trp53 arg 72 pro polymorphism and breast cancer risk.

Conclusion

Our study shows distribution of three genotypes namely, arg/arg, arg/pro and pro/pro, observed in the breast cancer patients were 51.6%, 32.3% and 16.1% respectively. The controls showed 56.7%, 26.7 % and 16.7% of arg/arg, arg/pro and pro/pro respectively with P-value=0.834 suggesting that no association exist between the Trp53 arg72pro polymorphism and breast cancer development. Although there is no significant difference in the distribution of genotype between cases and controls (p value=0.834), but higher number of heterozygous (arg/pro) genotype in cancer tissue suggest, heterozygous (arg/pro) genotype predispose women to breast cancer.

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