Association of Trp53 arg72pro polymorphic variants with breast cancer – a case control study in south Indian population

*Suresh K1, Venkatesan R1, Chandirasekar R1, Kumar BL2, Sasikala K1
1 Unit of Human Genetics, Department of Zoology, Bharathiar University, Coimbatore, India.
2 Department of Biotechnology, Kongunadu Arts and Science College, Coimbatore, India.

*Corresponding Author: ksureshbio@live.com

Abstract
The common single nucleotide polymorphism at Trp53 codon 72 is extensively analysed for its association with breast cancer, but conflicting evidences were obtained. We tested the hypothesis that the SNP at Trp53 codon 72 contributes to the development of breast cancer. Using the polymerase chain reaction-restriction fragment length polymorphism method, the genotype and allele distribution of the Trp53 codon 72 was determined among breast cancer patients (n=35) and healthy normal controls (n=37). Genotypic analysis revealed that the heterozygous (arg/pro) individuals were higher among breast cancer patients than in the controls. Overall, although there was no statistically significant difference in the allelic and genotypic distribution, higher number of arg allele was observed in breast cancer patients when compared to the controls suggesting that the arg allele may be associated with predisposing women to breast cancer.

Keywords: Breast cancer; First-degree female relatives; Single nucleotide polymorphism; Trp53 codon 72 polymorphism; Trp53 gene mutation.

Introduction
Breast cancer is the most frequent cancer in woman worldwide with 1.05 million new cases every year and represents over 20% of all malignancies among females (Parkin et al., 2001). It is also the primary cause of death among women globally, and in 2001, there were approximately 80,000 new breast cancer cases in India (Siddiqi et al., 2001). It is believed that oral contraceptive use (Kahlenborn et al., 2006), obesity (Carmichael, 2006) and hyperinsulinemia (Gunter et al., 2009) are probable factors increasing risks of developing breast carcinoma. In addition, DNA damage caused by carcinogenic agents, such as IR, ROS and estrogen metabolites, may contribute to genetic alterations that are critical in breast carcinogenesis (Smith et al., 2003). Although exposure to these risk factors causes the development of breast cancer only in a small group of exposed people, implying that genetic factors might contribute to the carcinogenic mechanisms and complex interactions between many genetic and environmental factors might be the major cause of breast cancer.

The genetic variants (arg, pro) of Trp53 have received attention as possible modifiers of cancer risk due to their critical role in cell cycle control, DNA repair and apoptosis and possible interaction with the breast cancer susceptibility genes BRCA1 and BRCA2. The Trp53 arg72pro polymorphism refers to the Ex4+199 G>C variant rs1042522, which results in the non-synonymous amino acid substitution of an arginine (72arg) amino acid at codon 72 with a proline (72pro) amino acid. Extensive studies have been carried out to reveal the association of arg72pro variants with breast cancer susceptibility (Wang-Gohrke et al., 1998; Papadakis et al., 2000; Suspit et al., 2003; Damin et al., 2006; Khaliq et al., 2000), however, inconsistent results were obtained and the association remains largely uncertain. The aim of the present work was to evaluate the association of the Trp53 arg72pro polymorphism with breast cancer, through assessing the genotype and subsequently, comparing the frequency of Trp53 arg72pro genotypes between breast cancer patients and controls.

Materials and Methods
The study consisted of 35 clinically confirmed breast cancer patients and 37 controls. The cancer patients included in the study were from various territory cancer care hospitals at Coimbatore and Erode districts of Tamilnadu State, South India. The controls were from general population employed in various professions. The objectives of the study were explained to the participants. They have answered for an interviewer-administered questionnaire covering standard demographic
questions as well as questions pertaining to their medical history and smoking habit. All the participants gave their written consent prior to inclusion in the study. DNA was extracted from the peripheral blood cells using standard procedure, involving SDS / Proteinase K digestion followed by ethanol precipitation.

**PCR amplification and polymorphism analysis of Trp53 arg72pro**

The primer sequences were obtained commercially (1st base, Singapore) and verified using UCSC In-silico PCR (http://genome-mirror.duhs.duke.edu/cgi-bin/hgPcr) to eradicate the possibility of amplification of any non-specific DNA sequences. Purified genomic DNA was amplified by PCR for exon 4 codon 72 of Trp53 gene. The PCR contained a quantity of 200ng of genomic DNA, 1µM of each forward 5' - TTG CCG TCC CAA GCA ATG GAT GA - 3' and reverse 5' - TCT GGG AAG GGA CAG AAG ATG AC - 3' primers, 10 mM of Tris-HCl, 50 mM of KCl, 2 mM MgCl2, 0.2 mM of each dNTPs (Fermentas, Germany) and 1.25 U of Taq DNA polymerase (Fermentas, Germany) in a final reaction volume of 50µl. The PCR amplification involved an initial denaturation at 94°C for 4 min, 30 cycles of denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec, extension at 72°C for 1 min and a final cycle of extension at 72°C for 5 min. The polymorphisms were identified by digesting the PCR products (199bp long) with 5 U of Bsh12361 (Fermentas) for 4-16 hours. The digested PCR product was resolved on 8% polyacrylamide gel (29:1) electrophoresis for 2½ hours at 65V/cm in 1X TBE buffer. Electrophoresis of the digestion product revealed that, the appearances of two bands correspond to arg/arg (113bp and 86bp long), three bands correspond to arg/pro (199bp, 113bp, and 86bp long) and one undigested band corresponds to pro/pro (199bp long).

**Statistical analysis**

Chi-square analysis ($\chi^2$) was applied to test the association between the genotypes and alleles between patients and controls. The odds ratio (OR) and their confidence intervals (CI) were calculated to estimate the strength of the association between polymorphism genotype alleles of patients and controls (Martin Bland and Douglas, 2000).

**Results**

The mean age, height and weight of the breast cancer patients was 54.05±5.89, 154.57±4.86, 52.94±6.76, respectively. Breast cancer was seen among 11.42% of first-degree relatives (FDFRs). The prevalence of breast cancer was high among the postmenopausal women (74.28%, mean age 53.61±7.25) and low among the premenopausal women (25.71%, mean age 51.00±4.94). Active smoking did not exist among breast cancer patients, but 25.71% of breast cancer patients were exposed to passive smoking. Meanwhile, tobacco-chewing habit was seen among 22.85% of breast cancer patients. Diabetes, high blood pressure, jaundice, epilepsy and anemia were seen among 11.42%, 5.71%, 2.85%, 2.85% and 2.85% of breast cancer patients respectively.

**Characteristics of breast cancer patients**

The mean age, height and weight of controls was 53.48±7.42, 155.70±5.81, 53.08±5.89 respectively. Regarding the menopausal status of the controls, 35.13% were premenopausal woman and 64.86% were postmenopausal woman. Active smoking was not seen, whereas passive smoking and tobacco chewing habit was seen among 21.62% and 13.51% of controls respectively. Diabetes in 8.10%, high blood pressure in 2.70%, migraine in 2.70% and arthritis in 8.10% of controls, were observed.

**Trp53 arg72pro analysis**

The distribution of three genotypes namely, arg/arg, arg/pro and pro/pro, observed in the breast cancer patients were 28.57%, 62.85% and 8.57% respectively. The controls showed 29.72%, 51.35% and 18.91% 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 ($\chi^2$=1.81, df=2, $P=0.40$). The allele frequencies of breast cancer patients and controls were fitted in the Hardy-Weinberg Equilibrium with an allele frequencies of 0.56 (Controls) and 0.60 (breast cancer patients) for arg-coding alleles and 0.44 (Controls) and 0.4 (breast cancer patients) for pro-coding alleles. No significant difference in allele frequencies between breast cancer patients and controls were observed ($\chi^2=0.31$, df=1, $P=0.57$). The combined analysis of arg/arg and arg/pro genotypes in breast cancer patients and controls revealed no significant association with cancer, but showed an increased breast cancer risk
Odd ratio (OR) = 1.27, 95% Confidence Interval (CI) 0.44-3.65, P=0.65]. Meanwhile, the combined analysis of *arg/pro* and *pro/pro* genotypes showed no significant association with breast cancer risk (OR=0.37, CI=0.08-1.63, P=0.17) (Table 1, Figure 1).

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

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Controls (%) n=37</th>
<th>Breast cancer patients (%) n=35</th>
<th>P (X²)</th>
<th>OR (95% CI) P</th>
</tr>
</thead>
<tbody>
<tr>
<td>arg/arg</td>
<td>11 (29.72)</td>
<td>10 (28.57)</td>
<td>0.40</td>
<td>1.27 (0.44-3.65) 0.65</td>
</tr>
<tr>
<td>arg/pro</td>
<td>19 (51.35)</td>
<td>22 (62.85)</td>
<td>0.57</td>
<td>0.37 (0.08-1.63) 0.17</td>
</tr>
<tr>
<td>pro/pro</td>
<td>7 (18.91)</td>
<td>3 (8.57)</td>
<td></td>
<td>0.82 (0.42-1.60)</td>
</tr>
</tbody>
</table>

OR – Odds Ratio; CI – Confidence Interval  
* P > 0.05

**Figure 1. Digested PCR products with the restriction enzyme Bsh12361.**

8% Polyacrylamide gel, Electrophoretic conditions: 2:15 hours in 1X TBE buffer, current 65V/cm.  
From left to right: Lane 1, 2, 3 and 6: arg/pro; Lane 4: pro/pro, Lane 5: arg/arg; Lane 7: 20bp marker.
Discussion
In our study, about 11.42% of breast cancer patients were FDFRs. Family history is a well established risk factor for breast cancer, with the familial relative risk (FRR) being approximately two-fold for first degree relatives of breast cancer patients compared with controls from the general population (Familial Breast Cancer, 2001). Rajeswari et al. (2000) found that the DNA damage significantly increased from controls to FDFRs and from FDFRs to breast cancer patients. Who further observed that the FDFRs showed ~ 2.5 times higher DNA damage as compared with the controls. Decreased DNA repair synthesis in leukocytes has also been reported in breast cancer patients (Jaloszynski et al., 1997) and healthy FDFRs of breast cancer patients (Rao et al., 1998).

In the general population, reproductive factors including age at menarche, late age at first full-term pregnancy, null and low parity and late age at menopause are established risk factors for an increased risk of breast cancer (Key et al., 2001). The patterns of risk associated with reproductive history suggest that the lifetime estrogen exposure (Steiner and Klubert, 2008) and late age at menopause (Kelsey et al., 1993) are associated with increased rates of breast cancer. According to Meshram et al. (2009) menopause (≥50 years of age) was observed to be associated with increased risk and the risk was 7.9 times more among women who had menopause at or after 50 years of age compared to women who had menopause before 45 years. The present study is consistent with the previous studies; we found that breast cancer was prevailed among 74.28% (Mean age = 53.61±7.25) of postmenopausal woman than the 25.71% (Mean age = 51.00±4.94) of premenopausal woman.

The study of genetic polymorphisms in genes associated with the repair of DNA damage and those involved in cell cycle control are particularly attractive targets, since an inability to tightly regulate either of these two processes is likely to result in less than the most favorable outcome. Even though studies correlated Trp53 arg72pro variants with breast cancer, conflicting evidences are available over the influence of this genetic variation on manifestation of breast cancer.

Several case control studies suggested that pro allele was associated with breast cancer risk in Swedish (Sjaland er et al., 1996), American (Weston et al., 1997), German (Wang-Gohrke et al., 1998, 2002), Russian (Supsitsin et al., 2003), Japanese (Noma et al., 2004) and Slovakian (Franekova et al., 2007) populations. A few investigations supported that arg allele was associated with breast cancer risk in Greek (Papadakis et al., 2000), Turkish (Buyru et al., 2003), Italian (Bonafe et al., 2003), Israeli (Ohayon et al., 2005), Chinese (Ma et al., 2006), and Brazilian (Damin et al., 2006) populations. No association of Trp53 arg72pro polymorphism with breast cancer risk was reported in Japanese (Kawajiri et al., 1993), Pakistani (Khaliq et al., 2000), Tunisian (Mabrouk et al., 2003), Finnish (Tomniska et al., 2005) and Iranian populations (Khadang et al., 2007).

The present work investigated the association of Trp53 arg72pro polymorphism with breast cancer outcome and found that neither arg nor pro was significantly associated with breast cancer outcome. However, in our study arg allele frequency was found to be higher (0.63) in breast cancer patients than the controls (0.53). Sharp ethnic differences in the arg and pro allele frequencies have been observed. In the Northern hemisphere, the pro72 allele shows a North-South gradient, from 0.17 [Swedish Saamis] to 0.63 [African Black-Nigerians] (Beckman et al., 1994). In Western Europe (France, Sweden, and Norway), North America (USA), Central and South America (Mexico, Costa-Rica and Peru) and Japan, the most common allele is arg72, with frequencies ranging from 0.60 to 0.83. (IARC, 2010).

The two polymorphic variants of the wild type Trp53 have been shown to have different biochemical properties; Trp53 pro form was suggested to activate transcription much more efficiently than the Trp53 arg variant (Thomas et al., 1999), the Trp53 arg variant is more efficiently induces cell death than the Trp53 pro variant (Dumont et al., 2003), the Trp53 pro variant was shown to induce cell cycle arrest better than the Trp53 arg form (Pim and Banks, 2004). These data highlight that both the polymorphic variants of Trp53 might have involved for selectively regulating specific cellular functions. Besides these functions, there is emerging evidence for Trp53 role in regulating the various DNA-repair processes (Sengupta and Harris, 2005) among other functions.

Using several cellular systems, Siddique et al. (2005) reported that cells expressing Trp53 pro form are able to repair DNA-damage much more efficiently than the Trp53 arg- expressing cells, thus it is inferred that Trp53 pro variant
plays critical role [preferentially by inducing $Trp53$ dependent DNA-repair target gene promoters (p53R2)] in the $Trp53$ dependent DNA-repair process, which may influence cancer risk. These data indicated that $Trp53 \text{ arg}$ might be a predisposing allele to cancer, probably owing to its reduced ability to repair damaged DNA, a process that has been shown to be important in cancer formation. $Trp53 \text{ arg}$ is only slightly less efficient than $Trp53 \text{ pro}$ in its ability to repair damaged DNA. Subtle differences manifested owing to combination of polymorphic effects in several gene loci may result in the overall sensitivity to cancer development. In this respect, it was recently shown that the polymorphism in the $Mdm2$ promoter (SNP309) was involved in cancer susceptibility (Bond et al., 2004). Further, Siddique et al. (2005) reported that $Trp53 \text{ arg}$-expressing cells were less effective in removing micronuclei (small nuclei, arise from acentric chromatids or chromosome fragments induced by radiation or other DNA damaging agents), suggesting that $Trp53 \text{ arg}$ might be less potent in reducing genomic instability and perhaps cancer predispositions.

In this context, recent evidences have shown that healthy Asian heterozygous individuals ($Trp53 \text{ arg/pro}$) tend to preferentially express the $Trp53 \text{ arg}$ allele at the RNA level (Siddique et al., 2005). By contrast, the $Trp53 \text{ arg}$ allele was preferentially expressed in most heterozygote breast cancer patients, suggesting that $Trp53 \text{ arg}$ expression correlates with breast cancer development (Siddique et al., 2005). These findings together with many other reports indicating that the $Trp53 \text{ arg}$ was associated with cancer predisposition (Sjalander et al., 1996; Weston and Godbold, 1997; Papadakis et al., 2000; Bergamaschi et al., 2003), suggested that even if the $Trp53 \text{ arg}$ form might be capable of inducing apoptosis better, it might not be efficient in preventing cancer formation. In our study, analysis of genotypic distributions revealed that about 62.5% of breast cancer patients and 51.35% of controls were heterozygous ($Trp53 \text{ arg/pro}$), moreover the $\text{arg}$ allele frequency was slightly higher in breast cancer patients 0.63 when compared to controls 0.53. Even though the distribution of genotypes and allele frequency between breast cancer patients and controls were statistically insignificant, the presence of more number of heterozygous individuals and $\text{arg}$ alleles in breast cancer patients made it reasonable to suspect the role of $\text{arg}$ allele in predisposing woman to breast cancer in our study. To add more strength to our result, several experimental evidences support the notion that $\text{arg}$ allele was associated with breast cancer predisposition in woman. Keshava et al. (2002) observed a high prevalence of the $Trp53 \text{ arg}$ genotype in breast cancer patients among Caucasian women of New York. In this context, it was shown that Caucasians are primarily $\text{arg}$-expressers (Siddique et al., 2005) and they are about 2-fold more prone to cancer than the Asians (Oliver et al., 2002). Aoki et al. (2009) reported that breast cancer patients presented a significantly over representation of $Trp53 \text{ arg}$ homozygosity (55.5%) compared with the healthy control group (33.3%), who also suggested that it is possible that $Trp53 \text{ arg}$ homozygosity is associated with breast cancer and may represent a potential risk factor for breast tumorigenesis. Papadakis et al. (2000) observed higher frequency of $\text{arg/arg}$ (61%) in breast cancer patients than the controls (20%). It is suggested that $Trp53 \text{ arg}$ homozygosity could represent a risk factor for the tumorigenesis of the breast. Langerod et al. (2002) reported a growth advantage of breast carcinoma cells carrying the $Trp53 \text{ arg}$ allele in a Norwegian population. Ohayon et al. (2005) reported that the $\text{arg}$ allele was significantly associated with breast cancer in non-Ashkenazi-Jews. Similarly, Ma et al. (2006) found a significant association of $\text{arg}$ allele with breast cancer risk in Chinese population.

We found that the $Trp53 \text{ pro}$ allele was less prevalent (8.57%) in the breast cancer patients when compared to controls (18.91%). Our results are in contrast with the previous reports (Sjalander et al., 1996; Weston et al., 1997; Wang-Gohrke et al., 1998, 2002; Suspitsin et al., 2003; Noma et al., 2004; Franekova et al., 2007). Our study suggested that the $Trp53 \text{ pro}$ allele was not associated or may confer a decreased risk to development of breast cancer. Alawadi et al. (2010) studied the $Trp53$ gene polymorphism with breast cancer risk in Arab women and found that the $\text{pro}$ homozygosity at codon 72 was associated with decreased breast cancer risk.

Siddique et al. (2005) showed that there is a significant increase in the number of $Trp53 \text{ arg}$ expressers in the Chinese breast cancer cohort, indicating that there is a strong correlation between carcinogenesis and the expression of the $Trp53 \text{ arg}$ allele in the Chinese population. However, there was no significant
increase in the numbers of Trp53 arg homozygotes between the Chinese healthy and cancer populations, supporting many previous studies and the present study which reports a lack a correlation between a particular genotype and cancer predisposition (Weston and Godbold, 1997). According to Siddique et al. (2005), it may be possible that the Trp53 arg allele may not be functionally involved, but its expression may simply relate to tumorigenesis.

**Conclusion**

In conclusion, our study suggests that an association may exist between the Trp53 arg72pro polymorphism and breast cancer development. However, the study’s sample size limits the possibility to confirm the association of Trp53 72 codon polymorphism with breast cancer. In order to obtain a more definitive conclusion concerning the association of this polymorphism with breast cancer, a careful study in different ethnic with large number of cases versus controls should examine the expression status of the Trp53 gene, in addition to determining the genotype.

**References**


development. Cancer Epidemiology Biomarkers and Prevention, 14:2245-2252.


