**eISSN: 09748369, www.biolmedonline.com**

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

***Suresh K[1], Venkatesan R[1], Chandirasekar R[1], Kumar BL[2], Sasikala K[1]**
[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 arg*72*pro* polymorphism refers to the Ex4+199 G>C variant rs1042522, which results in the non-synonymous amino acid substitution of an arginine (72*arg*) amino acid at codon 72 with a proline (72*pro*) amino acid. Extensive studies have been carried out to reveal the association of *arg*72*pro* variants with breast cancer susceptibility (Wang-Gohrke et al., 1998; Papadakis et al., 2000; Suspitsin 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 arg*72*pro* 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 ($I^{st}$ 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 $MgCl_2$, 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$^oC$ for 4 min, 30 cycles of denaturation at 94$^oC$ for 30 sec, annealing at 60$^oC$ for 30 sec, extension at 72$^oC$ for 1 min and a final cycle of extension at 72$^oC$ for 5 min. The polymorphisms were identified by digesting the PCR products (199bp long) with 5 U of *Bsh*12361 (Fermentas) for 4-16 hours. The digested PCR product was resolved on 8% polyacrylamide gel (29:1) electrophoresis for $2^{1/2}$ 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**

*Characteristics of breast cancer patients*

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 controls*

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 0.40 (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

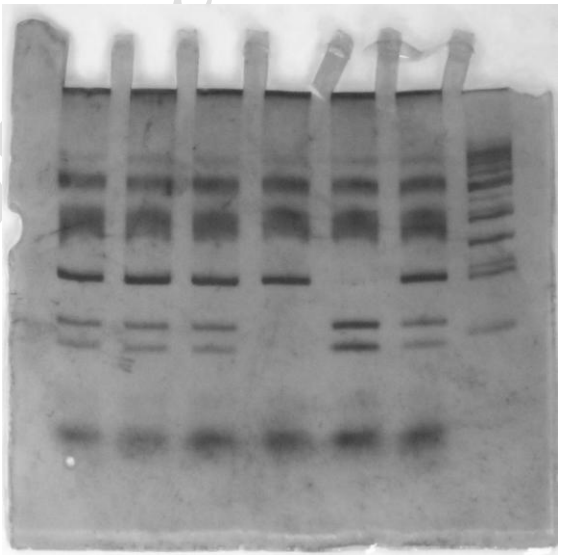
[Odd ratio (OR) = 1.27, 95% Confidence Interval (CI) 0.44-3.65, P=0.65]. Meanwhile, the combined analysis of *arg/pro* and *pro/pro* genotypes showed no significant association with breast cancer risk (OR=0.37, CI=0.08 - 1.63, P=0.17) (Table 1, Figure 1).

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

| Genotypes | Controls (%) n=37 | Breast cancer patients (%) n=35 | P ($X^2$) | OR (95% CI) P |
|---|---|---|---|---|
| arg/arg | 11 (29.72) | 10 (28.57) | 0.40 * (1.81) | |
| arg/pro | 19 (51.35) | 22 (62.85) | | 1.27 (0.44-3.65) 0.65 * |
| pro/pro | 7 (18.91) | 3 (8.57) | | 0.37 (0.08-1.63) 0.17 * |
| arg allele frequency | 0.56 | 0.60 | 0.57 * (0.31) | |
| pro allele frequency | 0.44 | 0.40 | | 0.82 (0.42-1.60) |

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 arg*72*pro* 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 (Sjalander et al., 1996), American (Weston et al., 1997), German (Wang-Gohrke et al., 1998, 2002), Russian (Suspitsin 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 arg*72*pro* polymorphism with breast cancer risk was reported in Japanese (Kawajiri et al., 1993), Pakistani (Khaliq et al., 2000), Tunisian (Mabrouk et al., 2003), Finnish (Tommiska et al., 2005) and Iranian populations (Khadang et al., 2007).

The present work investigated the association of *Trp53 arg*72*pro* 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 *pro*72 allele shows a North-South gradient, from 0.17 [Swedish Saamis] to 0.63 [African Blacks-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 *arg*72, 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 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 arg* is only slightly less efficient than *Trp53 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 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 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 arg/pro*) tend to preferentially express the *Trp53 arg* allele at the RNA level (Siddique et al., 2005). By contrast, the *Trp53 arg* allele was preferentially expressed in most heterozygote breast cancer patients, suggesting that *Trp53 arg* expression correlates with breast cancer development (Siddique et al., 2005). These findings together with many other reports indicating that the *Trp53 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 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 arg/pro*), moreover the *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 *arg* alleles in breast cancer patients made it reasonable to suspect the role of *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 *arg* allele was associated with breast cancer predisposition in woman. Keshava et al. (2002) observed a high prevalence of the *Trp53 arg* genotype in breast cancer patients among Caucasian women of New York. In this context, it was shown that Caucasians are primarily *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 arg* homozygosity (55.5%) compared with the healthy control group (33.3%), who also suggested that it is possible that *Trp53 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 *arg/arg* (61%) in breast cancer patients than the controls (20%). It is suggested that *Trp53 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 arg* allele in a Norwegian population. Ohayon et al. (2005) reported that the *arg* allele was significantly associated with breast cancer in non-Ashkenazi-Jews. Similarly, Ma et al. (2006) found a significant association of *arg* allele with breast cancer risk in Chinese population.

We found that the *Trp53 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 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 *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 arg* expressers in the Chinese breast cancer cohort, indicating that there is a strong correlation between carcinogenesis and the expression of the *Trp53 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 arg*72*pro* 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 ethnics with large number of cases versus controls should examine the expression status of the *Trp53* gene, in addition to determining the genotype.

### References

Alawadi S, Ghabreau L, Alsaleh M, Abdulaziz M, Rafeek M, Akil N, Alkhalaf M, 2010. P53 gene polymorphisms and breast cancer risk in Arab women. Medical Oncology, In press (DOI: 10.1007/s12032-010-9505-4).

Aoki MN, Herrera AC, Amarante MK, Laino do Val Carneiro J, Pelegrinelli Fungaro MH, Ehara Watanabe MA, 2009. CCR5 and p53 codon 72 gene polymorphisms: Implications in breast cancer development. International Journal of Molecular Medicine, 23(3):429-435.

Beckman G, Birgander R, Sjalander A, Saha N, Holmberg PA, Kivela A, Beckman L, 1994. Is p53 polymorphism maintained by nature selection? Human Heredity, 44:266-270.

Bergamaschi D, Gasco M, Hiller L, Sullivan A, Syed N, Trigiante G, Yulug I, Merlano M, Numico G, Comino A, Attard M, Reelfs O, Gusterson B, Bell AK, Heath V, Tavassoli M, Farrell PJ, Smith P Lu X, Crook T, 2003. p53 polymorphism influences response in cancer chemotherapy via modulation of p73-dependent apoptosis. Cancer Cell, 3:387-402.

Bonafe M, Ceccarelli C, Farabegoli F, Santini D, Taffurelli M, Barbi C, Marzi E, Trapassi C, Storci G, Olivieri F, Franceschi C, 2003. Retention of the p53 codon 72 arginine allele is associated with a reduction of disease-free and overall survival in arginine/proline heterozygous breast cancer patients. Clinical Cancer Research, 9:4860-4864.

Bond GL, Hu W, Bond EE, Robins H, Lutzker SG, Arva NC, Bargonetti J, Bartel F, Taubert H, Wuerl P, Onel K, Yip L, Hwang SJ, Strong LC, Lozano G, Levine AJ, 2004. A single nucleotide polymorphism in the MDM2 promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans. Cell, 119:591-602.

Buyru N, Tigli H, Dalay N, 2003. P53 codon 72 polymorphism in breast cancer. Oncology Reports, 10:711-714.

Carmichael AR, 2006. Obesity and prognosis of breast cancer. Obesity Reviews, 7:333-340.

Damin AP, Frazzon AP, Damin DC, Roehe A, Hermes V, Zettler C, Alexandre CO, 2006. Evidence for an association of TP53 codon 72 polymorphism with breast cancer risk. Cancer Detection and Prevention, 30:523-529.

Dumont P, Leu JI, Della Pietra III AC, George DL, Murphy M, 2003. The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. Nature Genetics, 33:357–365.

Familial Breast Cancer, 2001. Collaborative reanalysis of individual data from 52 epidemiological studies including 58,209 women with breast cancer and 101,986 women without the disease. Lancet, 358:1389-1399.

Franekova M, Zubor P, Stanclova A, Dussan CA, Bohusová T, Galo S, Dobrota D, Kajo K, Péc M, Racay P, 2007. Association of p53 polymorphism with breast cancer: a case-control study in Slovak population. Neoplasma, 54:155-161.

Gunter MJ, Hoover DR, Yu H, Wassertheil-Smoller S, Rohan TE, Manson JE, Li J, Ho GY, Xue X, Anderson GL, Kaplan RC, Harris TG, Howard BV, Wylie-Rosett J, Burk RD, Strickler HD, 2009. Insulin, insulin like growth factor-I, and risk of breast cancer in postmenopausal women. Journal of the National Cancer Institute, 101:48-60.

IARC TP53 Database, 2010. http://www-p53.iarc.fr/Polymorphism.html

Jaloszynski P, Kujawski M, Czub-Swierczek M, Markowska J, Szyfter K, 1997. Bleomycin-induced DNA damage and its removal in lymphocytes of breast cancer patients studied by comet assay. Mutation Research, 385:223-233.

Kahlenborn C, Modugno F, Potter DM, Severs WB, 2006. Oral contraceptive use as a risk factor for

premenopausal breast cancer: a meta-analysis. Mayo Clinic Proceedings, 81:1290-1302.

Kawajiri K, Nakachi K, Imai K, Watanabe J, Hayashi S, 1993. Germline polymorphisms of p53 and CY1A1 genes involved in human lung cancer. Carcinogenesis, 14:1085-1089.

Kelsey JL, Gammon MD, John EM, 1993. Reproductive factors and breast cancer. Epidemiology Reviews, 15:36-47.

Keshava C, Frye BL, Wolff MS, McCanlies EC, Weston A, 2002. Waf-1 (p21) and p53 polymorphism in breast cancer. Cancer Epidemiology Biomarkers and Prevention, 11:127-130.

Key TJ, Verkasalo PK, Banks E, 2001. Epidemiology of breast cancer. Lancet, 2:133-140.

Khadang B, Fattahi MJ, Talei A, Dehaghani AS, Ghaderi A, 2007. Polymorphism of Tp53 codon 72 showed no association with breast cancer in Iranian women. Cancer Genetics and Cytogenetics, 173:38-42.

Khaliq S, Hameed A, Khaliq T, Ayub Q, Qamar R, Mohyuddin A, Mazhar K, Qasim-Mehdi S, 2000. P53 mutations, polymorphisms, and haplotypes in Pakistani ethnic groups and breast cancer patients. Genetic Testing, 4:23-29.

Langerod A, Bukholm IRK, Bregard A, Lonning PE, Andersen TI, Rognum TO, Meling GI , Lothe RA, Borresen-Dale AL, 2002. The Tp53 codon 72 polymorphism may affect the function of Tp53 mutations in breast carcinomas but not in colorectal carcinomas. Cancer Epidemiology Biomarkers and Prevention, 11:1684-1688.

Ma H, Hu Z, Zhai X, Wang S, Wang X, Qin J, Chen W, Jin G, Liu J, Gao J, Wang X, Wei Q, Shen H, 2006. Joint effects of a single nucleotide in p53BP1 and p53 on breast cancer risk in Chinese population. Carcinogenesis, 27:766-771.

Mabrouk I, Baccouche S, El-Abed R, Mokdad-Gargouri R, Mosbah A, Said S, Daoud J, Frikha M, Jlidi R, Gargouri A, 2003. No evidence of correlation between p53 codon 72 polymorphism and risk of bladder or breast carcinoma in Tunisian patients. Annals of the New York Academy of Sciences, 1010:764-770.

Martin Bland J, Douglas G, 2000. Altman Statistics Notes: The odds ratio. British Medical Journal, 320:1468.

Meshram II, Hiwarkar PA, Kulkarni PN, 2009. Reproductive risk factors for breast cancer: a case control study. Online Journal of Health and Allied Sciences, 8:3.

Noma C, Miyoshi Y, Taguchi T, Tamaki Y, Noguchi S, 2004. Association of Tp53 genetic polymorphism (Arg72Pro) with estrogen receptor positive breast cancer risk in Japanese women. Cancer Letters, 210:197-203.

Ohayon T, Gershoni-Baruch R, Papa MZ, Distelman MT, Eisenberg BS, Friedman E, 2005. The R72P P53 mutation is associated with familial breast cancer in Jewish women. British Journal of Cancer, 92:1144–1148.

Oliver M, Eeles R, Hollstein M, Khan MA, Harris CC, Hainaut P, 2002. The IARC TP53 Database: new online mutation analysis and recommendations to users. Human Mutation, 19:607–14.

Papadakis EN, Dokianakis DN, Spandidos DA, 2000. p53 codon 72 polymorphism as a risk factor in the development of breast cancer. Molecular Cell Biology Research Communications, 3:389-392.

Parkin DM, Bray F, Ferlay J, Pisani P, 2001. Estimating the world cancer burden: Globocon 2000. International Journal of Cancer, 94:153-156.

Pim D, Banks L, 2004. p53 polymorphic variants at codon 72 exert different effects on cell cycle progression. International Journal of Cancer, 108:196-199.

Rajeswari N, Ahuja YR, Malini U, Chandrashekar S, Balakrishna N, Rao KV, Khar A, 2000. Risk assessment in first degree female relatives of breast cancer patients using the alkaline comet assay. Carcinogenesis, 21:557-561.

Rao NM, Pai SA, Shinde SR, Gosh SN, 1998. Reduced DNA repair capacity in breast cancer patients and unaffected individuals from breast cancer families. Cancer Genetics and Cytogenetics, 102:65-73.

Sengupta S, Harris CC, 2005. p53: traffic cop at the crossroads of DNA repair and recombination. Nature Reviews Molecular Cell Biology, 6:44-55.

Siddiqi M, Sen U, Mondal SS, Patel DD, Yeole BB, Jussawala DJ, Pratinidhi A, Sunny L, Yeole BB, Jussawala DJ, Rao BN, Sunny L, Yeole BB, Jussawala DJ, Varsha S, Sunny L, Nair MK, Varghese C, Nair MK, Gangadharan P, Jayalekshmi P, 2001. Cancer statistics from non-ICMR registries: Population based registries, CRAB (Cancer Registry Abstract). Newsletter of the National Cancer Registry Project of India, 8(1):47-59.

Siddique MM, Balram C, Fiszer-Maliszewska L, Aggarwal A, Tan A, Tan P, Soo KC, Sabapathy K, 2005. Evidence for selective expression of the p53 codon 72 polymorphs: implications in cancer

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

Sjalander A, Birgander R, Hallmans G, Cajander S, Lenner P, Athlin L, Beckman G, 1996. p53 polymorphisms and haplotypes in breast cancer. Carcinogenesis, 17:1313-1316.

Smith TR, Miller, MS, Lohman KK, Case LD, Hu JJ, 2003. DNA damage and breast cancer risk. Carcinogenesis, 24(5):883-889.

Steiner E, Klubert D, 2008. Assessing breast cancer risk in women. American Family Physician, 78(12):1361-1366.

Suspitsin EN, Buslov KG, Grigoriev MY, Ishutkina JG, Ulibina JM, Gorodinskaya VM, Pozharisski KM, Berstein LM, Hanson KP, Togo AV, Imyanitov EN, 2003. Evidence against involvement of p53 polymorphism in breast cancer predisposition. International Journal of Cancer, 103:431-433.

Thomas M, Kalita A, Labrecque S, Pim D, Banks L, Matlashewski G, 1999. Two polymorphic variants of wild-type p53 differ biochemically and biologically. Molecular Cell Biology, 19:1092–1100.

Tommiska J, Eerola H, Heinonen M, Salonen L, Kaare M, Tallila J, Ristimaki A, von SK, Aittomaki K, Heikkila P, Blomqvist C, Nevanlinna H, 2005. Breast cancer patients with p53 Pro72 homozygous genotype have a poorer survival. Clinical Cancer Research, 11:5098-5103.

Wang-Gohrke S, Becher H, Kreienberg R, Runnebaum IB, Chang Claude J, 2002. Intron 3 16 bp duplication polymorphism of p53 is associated with an increased risk for breast cancer by the age of 50 years. Pharmacogenetics, 12:269-272.

Wang-Gohrke S, Rebbeck TR, Besenfelder W, Kreienberg R, Runnebaum IB, 1998. p53 germ line polymorphisms are associated with an increased risk for breast cancer in German women. Anticancer Research, 18:2095-2099.

Weston A, Godbold, JH, 1997. Polymorphisms of H-ras-1 and p53 in breast cancer and lung cancer: a meta-analysis. Environmental Health Perspectives, 105(4):919-926.

Weston A, Pan C, Ksieski HB, Wallenstein S, Berkowitz GS, Tartter PI, Bleiweiss IJ, Brower ST, Senie RT, Wolff MS, 1997. Tp53 haplotype determination in breast cancer. Cancer Epidemiology Biomarkers and Prevention, 6:105-112.