RESEARCH ARTICLE

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# TP53 (rs1042522, rs28934571) and TP21 (rs1801270, rs1059234) Polymorphisms and Risk of Breast Cancer among Rural Women of Maharashtra: Findings from a Hospital Based Case- Control Study

**Kailas D Datkhile[1]*, Suresh J Bhosale[2], Pratik P Durgawale[1], Nilam J Jagdale[1], Ashwini L More[1], Rashmi A Gudur[2], Anand K Gudur[2], Satish R Patil[1]**

## Abstract

**Background:** Various studies all around the world depicted the relationship of polymorphisms in tumor suppressor genes with risk of various cancers, but there are unambiguous conclusions on this association. A hospital based case-control study was designed to review the association of polymorphism of tumor suppressor genes p21 and p53 with breast cancer risk in women residing in rural Maharashtra. **Methods:** Two single nucleotide polymorphisms (SNPs) a C>A transversion (Ser>Arg) at codon 31 of exon 2 (rs1801270), C>T transition occurring 20bp upstream from stop codon of exon 3 (rs1059234) in p21 gene and G>C (Arg>Pro) transition at codon 72 of exon 4 (rs1042522), G>T (Arg>Ser) transition at codon 249 in exon 7 (rs28934571) in p53 gene were studied. To precise the quantitative assessment, we enrolled 800 subjects sorted into 400 clinically confirmed breast cancer patients and 400 healthy women from a tertiary care hospital (Krishna Hospital and Medical Research Centre) of south-western Maharashtra. The genetic polymorphisms in p21 and p53 genes was studied by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method using blood genomic DNA isolated from breast cancer patients and controls. The level of association of polymorphisms was assessed using Odds ratio (OR) with 95% confidence interval and p-value identified using logistic regression model. **Results:** After the analysis of SNPs (rs1801270, rs1059234) of p21 and (rs1042522, rs28934571) in p53 gene our analysis suggested that heterozygote Ser/Arg genotype with OR=0.66; 95% CI: 0.47- 0.91; p=0.0003 and homozygote variant Arg/Arg genotype with OR=0.23; 95% CI: 0.13- 0.40; p<0.0001of rs1801270 of p21 was negatively associated with risk of breast cancer in studied population. **Conclusion:** The findings from this study supported that rs1801270 SNP of p21 was inversely associated with breast cancer risk in the studied rural women population.

**Keywords:** Breast cancer- p21- p53- SNP- genetic polymorphism- PCR-RFLP

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## Introduction

Breast cancer (BC) is the most common malignancy among women worldwide estimated with 2261, 419 (24.5%) new cases of all ages females and 684, 994 deaths accounting 15.5% of all cancers in women in year 2020 (GLOBOCAN 2020). Global Cancer Observatory reported BC as the largest cause of cancer causing deaths in India where 178, 361 (26.3%) of new cases and 90, 408 (10.6%) deaths reported in 2020 (Sung et al., 2021). Current trend indicated that the incidence of BC occurring in Indian women at younger age likely to begin in early thirties and peak ages of 50-64 years as compared to western countries (National Cancer Registry Programme 2020). The incidence rate of BC is increasing day by day in rural India because of late stage of disease presentation due to lack of awareness. Etiology of BC is complex and acknowledged to involving the reproductive events that manipulate individual's hormonal status, use of hormonal contraceptive methods, ovulation stimulating drugs, post menopausal hormone therapy in the development of BC. It is also assumed that along with the earlier mentioned risk factors life style (age, diet, obesity, alcohol consumption, smoking, physical activity and other factors including age of first pregnancy, advanced age of menarche and menopause (Meshram 2009, Bhadoria et al., 2013; Momenimovahez and Salehiniva 2019) play a role in breast carcinogenesis. Meanwhile,

*[1]Department of Molecular Biology & Genetics, Krishna Institute of Medical Sciences, "Deemed to be University", Taluka-Karad, Dist- Satara, Pin-415 539, (Maharashtra), India. [2]Department of Oncology, Krishna Institute of Medical Sciences "Deemed to be University", Taluka-Karad, Dist- Satara, Pin-415 539, (Maharashtra), India. *For Correspondence: hodgeneticslab@kimskarad.in*

genetic factors are thought of key determinants in determining host susceptibility towards developing BC along with combination of lifestyle and environmental factors (Collins and Politopoulos 2011; Cobain et al 2016). Studies all around the globe pointed out functional association of polymorphism of several genes with increased risk of developing cancers in different population from different countries. It was previously reported that single nucleotide polymorphisms (SNPs) of the tumor suppressor protein p53 (TP53) and tumor suppressor protein p21 (TP21) might be involved in determining the individual's susceptibility to multiple cancers including BC. The TP53 is a crucial gene involved in critical cellular processes including cell cycle progression, DNA repair and apoptosis. Somatic alterations in the gene lead to an altered expression of various associated genes which are directly or indirectly under the transcriptional control of TP53 (Ali and Kamath 2022; Ghosh et al., 2022; Ali 2023). To date, several literature information on case-control and other epidemiological studies have identified the association of the p53 and p21 SNPs with susceptibility to cervical ( Datkhile et al., 2019; Ratre et al., 2019; Yu et al., 2022), ovarian (Alqumber et al., 2014), lung (Matikodu et al., 2003), head and neck (Bau et al., 2007) bladder (Zang et al., 2018) and gastric (Song et al., 2011) cancer risk, while others pointed out contrast observations where these SNPs did not show any association of either p53 or p21 polymorphism with development of oral (Lin et al., 2018) ovarian (Alqumber et al., 2014), colorectal (Asadi et al., 2017), lung (Nicholas et al., 2019), prostate (Sivonova et al., 2015; Song et al., 2017) and cervical cancer risk (Martinez-Nava et al., 2016). Thus, it is not clear from the earlier evidences that the polymorphisms in tumor suppressor molecules may play a role in genetic susceptibility of variety of cancers and the findings appeared conflicting. Previous studies conferred the association of p53 and p21 polymorphisms with increased risk of BC (Ayobi et al., 2018; Diakite et al., 2021), but others disagree to prove an association with BC risk (Hou et al., 2013, Habyarimana et al., 2018; Zhao et al., 2022). Thus, for determining the role of p53 and p21 SNPs in epidemiology and pathogenesis of BC, most of the studies were conducted in American, European and West and East Asian countries, but limited studies are available from South Asia, especially from India. Findings from Indian case-control and meta-analysis studies declared significant association between p53 polymorphism and increased BC risk in Indian population (Sharma et al., 2014; Akhter et al 2018). Some other Indian studies concluded no association of the SNPs of p53 and p21 with breast cancer risk (Surekha et al., 2011; Vijayaraman et al., 2012). To the best of our knowledge, there have been no such studies from rural population of India on the association between polymorphisms of the above mentioned genes and risk of BC. The present study was intended to identify the risk association of p53 and p21 gene polymorphisms with breast cancer. We conducted a case-control study in order to explore the possible association of (rs1042522, rs28934571) SNPs of p53 and (rs1801270, rs1059234) SNPs of p21 with BC risk in women residing in the rural areas of south-western Maharashtra from India.

## Materials and Methods

*Study subjects*

We conducted a case-control study in a consecutive sample of 400 histopathologically confirmed BC cases. All BC cases ranged in age from 23-85 years (Mean ± SD) (52.43 ±12.37) were enrolled immediately after diagnosis during the year 2018-2021. The cases already receiving treatment for malignancy were excluded. Equal numbers (400) of healthy women volunteers without history of any disorders and history of hysterectomy or mastectomy were randomly selected from a group of women visiting to Krishna Hospital & Medical Research Centre (KH&MRC) for blood donation and other purposes. Controls were frequency matched to cases by the age group at enrolment (42.37 ± 13.90). The control group and the study group resided in the same geographical location (Western Maharashtra region). Trained interviewers used a structured questionnaire to collect demographic and clinical data from the participants. The study protocol was approved by Institutional Ethics Committee for the utilization of human subjects in the research.

*SNP Selection and Genotyping*

Genomic DNA was isolated from venous blood of BC cancer patients and normal controls by a modified method where red blood cells are processed with red cell lysis buffer (10mM Tris-HCl pH 7.6, 320 mM sucrose, 5mM MgCl2, 1% Triton X-100, pH 7.6), thereafter treated with nucleic lysis buffer (10mM Tris-HC1, 11.4 mM sodium citrate, 1 mM EDTA, 1 % SDS, pH 8.0). After treatment with 100 µg/mL concentration of proteinase K at 55 degree celsius and subsequently RNase A (100 µg/mL) at 370C, precipitated and purified DNA was checked on 1% agarose gel for its quality as well as quantity. The DNA samples were then genotyped at p21 codon 31 and p53 codon 72, codon 249 loci by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The polymorphisms of both p21 and p53 were chosen for this study based on previously described studies (Ressiniotis et al., 2005; Vijayaraman et al 2012). The primers, PCR and RFLP conditions for the studied polymorphisms were selected from earlier publications by Ressiniotis et al., 2005. The primers and PCR-RFLP conditions optimized for amplification of codon 72 of exon 4 (309 bp) and codon 249 of exon 7 (286 bp) of p53 gene were selected from earlier prescribed by Vijayaraman et al., 2012.

*Statistical analysis*

The chi-square test was used to test the deviations from Hardy-Weinberg equilibrium in the genotype frequencies of the polymorphism in controls along with the differences in demographic variables between cases and controls. The association between the p21 and p53 genotypes and risk of developing BC was determined as an Odds ratio (OR) within 95% confidence interval (CI) based on univariate and multivariate logistic regression analyses. All statistical analyses were performed using SPSS IBM Version 11.0) software. Statistical significance was set at $p < 0.005$.

## Results

*Comparison of selected demographic and clinicopathological characteristics of study subjects*

In this case-control study p21 (rs1801270, rs1059234) and p53 (rs1042522, rs28934571) SNPs were analyzed by PCR-RFLP in 400 cases of BC and equal number of control women from a tertiary care hospital in Karad city. The age range of BC patients were 23-85 years (Mean ± SD: 52.43 ±12.40; Median age, 50yrs) whereas healthy control females ranged from 24-81 yrs (Mean ± SD: 42.37 ±13.90; Median age 40yrs). The demographic variables of BC cases and controls (age at cancer occurrence, age at first pregnancy, diet, tobacco habit, education and economic status, family history) and clinicopathological characteristics of BC patients including hormone status, histological subtypes, histological grade, tumor localization, tumor size were recorded (Table 1). Occurrence of BC frequency was high among the individuals more than 40 years of age (80.50%) than those of patients less than 40 years (19.50%). Significant difference was observed in BC cases when compared for their age of cancer occurrence (OR 4.42; 95%CI, 3.22-6.07.20; p<0.0001). There was no significant difference in diet (OR 1.57; 95%CI, 1.14-2.17; p=0.05) and economic status (OR 0.74; 95%CI, 0.55-1.01; p=0.06) were considered for the relationship with BC development but surprisingly, when we checked for tobacco chewing status we observed significant association with BC (OR 3.07; 95%CI, 2.29-4.12; p<0.0001) in women of rural population.

*Comparative analysis of genotypic polymorphism of p21 and p53 gene in breast cancer cases and controls*

The genotypic distribution of p21 and p53 genes determined in BC cases and healthy age and sex matched controls is summarized in Table 2. When we studied frequency distribution of serine and arginine in codon 31 of exon 2, the Ser/Ser genotype of patients (71.75 %) and controls (56.50%) where Arg/Arg genotype in p21 showed negative association for the development of BC (OR=0.23, 95% CI: 0.13-0.40; p<0.0001). Similarly when we studied polymorphisms in C/T and T/T genotypes of exon 3 of p21 we noted, neither of heterozygous C/T genotype (OR=1.50, 95% CI: 1.08-2.08; p=0.01) nor homozygous T/T genotype (OR=1.11, 95% CI: 0.38-3.19; p=0.85) showed relationship with BC development. The distribution of codon 72 and codon 249 genotypes of p53 in patients and control did not deviate from the Hardy-Weinberg equilibrium. The genotype frequencies in cases and controls are presented in Table 2, with no significant statistical association of Pro/Pro (OR 1.36: 95% CI 0.92-.2.05; p< 0.13) and Arg/Pro heterozygous variant (OR 1.19: 95% CI 0.83-1.69; p< 0.32) with BC risk. The genotype frequencies in cases and controls demonstrated no statistically significant association of the 249 Ser genotype (OR 1.38: 95% CI 0.97-1.97; p< 0.07) with BC risk. The frequency of each genotype was 79% for Arg and 21% for Ser in patients with cancer (n=400), and 83.50 for Arg and 16.50 for Ser in normal controls (n=400). When we studied the polymorphism of variant genotypes of p21 and p53 genes with breast cancer risk in a recessive genotype model, we noted negative correlation of p21 (SNP: rs1801270), (OR=0.26; 95% CI: 0.15- 0.45; p<0.0001) whereas rs1059234 SNP (OR=1.00; 95% CI: 0.34- 2.87; p=1) showed no relation with BC risk. Similarly the recessive model for variant genotype of p53 (rs1042522) (OR=0.80; 95% CI: 0.57-1.12); p=0.20) and (rs28934571) (OR=1.38; 95% CI: 0.97-1.97); p=0.07) showed no relationship with BC risk (Table 3). The dominant model showed negative association of p21 (rs1801270) (OR=0.51; 95% CI: 0.38-0.68); p<0.0001) with BC risk and lack of involvement of (rs1059234) (OR=1.47; 95% CI: 1.07-2.02); p=0.01) was reported in BC development. However, lack of significance observed in dominant model of p53 (1042522, rs2893457) (OR=0.82; 95% CI: 0.60-1.14); p=25) and (OR=1.38; 95% CI: 0.97-1.97); p=0.07) (Table 4).

*Correlation between p21 and p53 genotypes and clinicopathologic characteristics among breast cancer cases*

When we analyzed correlation of both p21 and p53 genotypes with clinicopathologic characterstics among 400 breast cancer cases, we observed that there were no significant correlations between the p21 codon 31 Arg or Ser alleles (Table 5) and p53 codon 72 alleles (Pro or Arg) or codon 249 alleles (Ser or Arg) and patients' clinical parameters (Table 6). We observed that all the studied clinicopathological characterstics showed no significant differences in breast cancer patients.

*Correlation of p21 and p53 gene polymorphisms with confounding factors associated with breast cancer risk*

The association of p21 and p53 with the risk of BC was further examined after stratification of confounding factors such as age of cancer occurence, age at first pregnancy and tobacco habit status. The genotype distributions of the selected p21 and p53 gene polymorphisms in cases and controls and their associations with CC risk are summarized in Table 7. The logistic regression analysis showed that none of the polymorphisms except Ser/Arg genotype of p21 codon 31 of exon 2 was negatively associated with BC risk (OR=0.46; 95% CI: 0.32-0.67 p=0.0001) after being adjusted for age >40 years and earlier age at first pregnancy (OR=0.40; 95% CI: 0.26-0.60 p< 0.0001) with development of BC. In Maharashtrian patients, the age of BC occurred at 23 years which was considerably lesser than reported in other reports. Also, the association of BC with first delivery age was reviewed in this study which showed that 15-20 yrs age of first delivery, considerably associated with increased BC risk. When we considered the association of p53 codon 72 and codon 249, there was no evidence of any association of Arg/Pro alleles of codon 72 of exon 4 (OR=1.11; 95% CI: 0.74-1.67 p=0.59) and Arg/Ser of codon 249 of exon 7 (OR=1.28; 95% CI: 0.82-1.99 p=0.72) with subgroups defined by age >40 yrs. Similarly the arginine/proline alleles of codon 72 (OR=1.11; 95% CI: 0.71–1.74, p=0.63) and Arg/Ser allele of codon 249 were (OR=1.09; 95% CI: 0.67 –1.78, p=0.72) not associated with breast cancer development in women with early age of first pregnancy.

Table 1. Distribution Comparisons of Selected Demographic and Clinicopathological Characteristics of Breast Cancer Cases and Healthy Controls from Rural Areas of Maharashtra.

| Variable | Cases (N=400) | | Controls (N=400) | | p-Value based |
|---|---|---|---|---|---|
| | No. | (%) | No. | (%) | on $\chi 2$ |
| Age (Mean ± SD) years | 52.43 ±12.37 | | 42.37 ± 13.90 | | |
| ≤ 40 | 78 | 19.5 | 207 | 51.75 | 0.001 |
| >40 | 322 | 80.5 | 193 | 48.25 | |
| Tobacco smoking Status | | | | | |
| Tobacco users | 219 | 54.75 | 113 | 28.25 | 0.001 |
| Tobacco no users | 181 | 45.2 | 287 | 71.75 | |
| Diet | | | | | |
| Vegetarian | 84 | 21 | 118 | 29.5 | 0.006 |
| Mixed | 316 | 79 | 282 | 70.5 | |
| Education | | | | | |
| High School | 66 | 16.5 | 108 | 27 | 0.001 |
| High School graduate (12 y) | 53 | 13.25 | 50 | 12.5 | |
| College /Graduate | 20 | 5 | 128 | 32 | |
| No School | 261 | 65.25 | 114 | 28.5 | |
| Economic status | | | | | |
| Poor | 295 | 73.75 | 271 | 67.75 | 0.001 |
| Middle | 105 | 26.25 | 129 | 32.25 | |
| Age @ I$^{st}$ delivery (yrs) | | | | | |
| (Mean Age ± SD) | 19.63 ± 2.66 | | 21.06 ± 3.55 | | |
| ≤20 | 218 | 54.5 | 183 | 45.75 | 0.001 |
| >20 | 182 | 45.5 | 217 | 54.25 | |
| Family history of Cancer | | | | | |
| Yes | 100 | 25 | 10 | 2.5 | |
| No | 300 | 75 | 390 | 97.5 | |
| Tumor localization | | | p-value =0.01 | | |
| Left breast | 213 | 53.25 | | | |
| Right breast | 185 | 46.25 | | | |
| Right + Left breast | 2 | 0.5 | | | |
| Hormone Status | | | p-value< 0.001 | | |
| ER+ve | 218 | 54.5 | | | |
| ER-ve | 182 | 45.5 | | | |
| PR+ve | 197 | 49.25 | | | |
| PR-ve | 203 | 50.75 | | | |
| Her2 +ve | 57 | 14.25 | | | |
| Her2 -ve | 343 | 85.75 | | | |
| ER/PR/Her2+ve | 8 | 2 | | | |
| ER/PR/Her2-ve | 134 | 33.5 | | | |
| Histological Subtypes | | | p-value <0.001 | | |
| Invasive ductal Carcinoma | 299 | 74.75 | | | |
| Lobular Carcinoma | 15 | 3.75 | | | |
| Mucinous Carcinoma | 12 | 3 | | | |
| Medulary Carcinoma | 27 | 6.75 | | | |
| Invasive Apocrine Carcinoma | 12 | 3 | | | |
| Others | 35 | 8.75 | | | |
| Histological Grade | | | p-value= 0.147 | | |
| I, II | 195 | 48.75 | | | |
| III, IV | 205 | 51.25 | | | |
| Tumor size in cm | | | p-value=0.001 | | |
| ≤ 2 | 180 | 45 | | | |
| > 2 | 220 | 55 | | | |

Significance p< 0.005

Table 2. The Genotype Frequencies of p21 and p53 Gene Variants and Their Association with Breast Cancer in Untreated BC Patients and Healthy Controls

| Gene | Genotype | Cases (n= 400) (%) | Control (n = 400) (%) | Odds Ratio (95% CI) | P value | Adjusted Odds Ratio (95% CI) | P value |
|---|---|---|---|---|---|---|---|
| *p21* | Serine/Serine | 287 (71.75) | 226 (56.50) | 1 (Reference) | | 1 (Reference) | |
| *codon 31* | Serine/Arginine | 95 (23.75) | 113 (28.25) | 0.66 (0.47-0.91) | <0.0003 | 0.58 (0.41-0.82) | 0.002 |
| *exon-2* | Arginine/Arginine | 18 (4.50) | 61 (15.25) | 0.23 (0.13-0.40) | <0.0001 | 0.24 (0.14-0.42) | <0.001 |
| *rs1801270* | | | | | | | |
| *p21* | CC/CC | 279 (69.75) | 309 (77.25) | 1 (Reference) | | 1 (Reference) | |
| *exon-3* | CC/TT | 114 (28.50) | 84 (21.00) | 1.50 (1.08- 2.08) | 0.01 | 1.64 (1.16-2.32) | 0.005 |
| *rs1059234* | TT/TT | 7 (1.72) | 7 (01.75) | 1.1 (0.38- 3.19) | 0.85 | 1.50 (0.48-4.70) | 0.485 |
| *p53* | Arginine/Arginine | 80 (20.00) | 95 (23.75) | 1 (Reference) | | 1 (Reference) | |
| *Codon72* | Arginine / Proline | 214 (53.50) | 213 (53.25) | 1.19 (0.83-1.69) | 0.33 | 1.15 (0.80-1.65) | 0.45 |
| *Exon-4* | Proline/Proline | 106 (26.50) | 92 (23.00) | 1.36 (0.90-2.05) | 0.13 | 1.32 (0.87-2.00) | 0.19 |
| *rs1042522* | | | | | | | |
| *p53* | Arginine/Arginine | 316 (79.00) | 334 (83.50) | 1 (Reference) | | 1 (Reference) | |
| *Codon249* | Arginine/Serine | 0 | 0 | | | | |
| *Exon-7* | Serine / Serine | 84 (21.00) | 66 (16.50) | 1.38 (0.97-1.97) | 0.07 | 1.32 (0.92-1.9) | 0.137 |
| *rs28934571* | | | | | | | |

Significance p< 0.005; OR, Odds ratio; CI, Confidence Interval

Table 3. Association between Breast Cancer Risk and the Single Nucleotide Polymorphism Variant of p53 and p21 Genes in the Recessive Model

| Genes | Genotype | Cases | | Control | | OR (95% CI) | p value |
|---|---|---|---|---|---|---|---|
| | | (n= 400) | (%) | (n= 400) | (%) | | |
| *p21* | Ser/Ser + Ser/Arg | 382 | 95.50 | 339 | 84.75 | 1 (Reference) | |
| *rs1801270* | Arg/Arg | 18 | 4.50 | 61 | 15.25 | 0.26 (0.15-0.45) | <0.0001 |
| *p21* | CC/CC+CC/CT | 393 | 98.25 | 393 | 98.25 | 1 (Reference) | |
| *rs1059234* | TT/TT | 7 | 1.75 | 7 | 1.75 | 1.00 (0.34-2.87) | 1.00 |
| *p53* | Arg/Arg+ Arg /Pro | 294 | 73.50 | 308 | 77.00 | 1 (Reference) | |
| *rs1042522* | Pro/Pro | 106 | 26.50 | 92 | 23.00 | 1.20 (0.87-1.66) | 0.25 |
| *p53* | Arg/Arg+Arg/Ser | 316 | 79.00 | 334 | 83.50 | 1 (Reference) | |
| *rs28934571* | Ser/Ser | 84 | 21.00 | 66 | 16.50 | 1.38 (0.97-1.97) | 0.07 |

Significance p< 0.005; OR, Odds ratio; CI, Confidence Interval

Neither of p21 nor p53 gene SNPs showed positive or negative association with breast cancer development when confounding factor tobacco habit was considered.

## Discussion

The present study was intended to investigate whether

Table 4. Association between Breast Cancer Risk and the Single Nucleotide Polymorphism Variant of p53 and p21 Genes in the Dominant Model

| Genes | Genotype | Cases | | Caontrols | | OR (95% CI) | P value |
|---|---|---|---|---|---|---|---|
| | | (n= 400) | (%) | (n= 400) | (%) | | |
| *p21* | Ser/Ser | 287 | 71.75 | 226 | 56.50 | 1 (Reference | |
| *rs1801270* | Ser/Arg + Arg/Arg | 113 | 28.25 | 174 | 43.50 | 0.51 (0.38-0.68) | <0.0001 |
| *p21* | CC/CC | 279 | 69.75 | 309 | 77.25 | 1 (Reference) | |
| *rs1059234* | CC/CT + TT/TT | 121 | 30.25 | 91 | 22.75 | 1.47 (1.07-2.02) | 0.01 |
| *p53* | Arg/Arg | 80 | 20.00 | 95 | 23.75 | 1 (Reference) | |
| *rs1042522* | Arg/Pro +Pro/Pro | 320 | 80.00 | 305 | 76.25 | 0.80 (0.57-1.12) | 0.2 |
| *p53* | Arg/Arg | 316 | 79.00 | 334 | 83.50 | 1 (Reference) | |
| *rs28934571* | Arg/Ser + Ser/Ser | 84 | 21.00 | 66 | 16.50 | 1.38 (0.97-1.97) | 0.07 |

Significance p< 0.005; OR, Odds ratio; CI, Confidence Interval

Table 5. Association between p21 codon 31 of exon 2, exon3 Genotypes and Clinicopathologic Characteristics among Breast Cancer Cases

| Variables | Total (n) | p21 codon -31 (Genotypes) | | | | p21 exon-3 (Genotypes) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Ser/Ser (287) | Ser/Arg (95) | Arg/Arg (18) | $X^2$ (p value) | CC (279) | C/A (114) | AA (7) | $X^2$ (p value) |
| Age group (yrs) | | | | | | | | | |
| ≤40 | 78 | 54 (18.82) | 22 (23.16) | 2 (11.11) | 1.238 | 53 (19.00) | 25 (21.93) | 0 | 2.16 |
| >40 | 322 | 233 (81.18) | 73 (76.84) | 16 (88.89) | 0.53 | 226 (81.00) | 89 (78.07) | 7 (100.0) | 0.33 |
| ER status | | | | | | | | | |
| Positive | 218 | 158 (55.05) | 52 (54.74) | 8 (44.44) | 0.154 | 154 (55.20) | 61 (53.51) | 3 (42.86) | 0.66 |
| Negative | 182 | 129 (44.95) | 43 (45.26) | 10 (55.56) | 0.92 | 125 (44.80) | 53 (46.49) | 4 (57.14) | 0.71 |
| PR status | | | | | | | | | |
| Positive | 197 | 141 (49.13) | 47 (49.47) | 9 (50.00) | 0.002 | 141 (50.54) | 53 (46.49) | 3 (42.86) | 0.75 |
| Negative | 203 | 146 (50.87) | 48 (50.53) | 9 (50.00) | 0.99 | 138 (49.46) | 61 (53.51) | 4 (57.14) | 0.68 |
| Her2-neu status | | | | | | | | | |
| Positive | 57 | 41 (14.29) | 14 (14.74) | 2 (11.11) | 0.115 | 204 (73.11) | 13 (11.40) | 1 (14.29) | 0.74 |
| Negative | 343 | 246 (85.71) | 81 (85.26) | 16 (88.89) | 0.94 | 75 (26.89) | 101 (88.60) | 6 (85.71) | 0.69 |
| Clinical stage | | | | | | | | | |
| I | 48 | 39 (13.59) | 9 (9.47) | 0 | 14.24 | 37 (13.26) | 11 (9.65) | 0 | 11.53 |
| II | 147 | 104 (36.23) | 41(43.16) | 2 (11.11) | 0.025 | 103 (36.92) | 43 (37.72) | 1 (14.28) | 0.08 |
| III | 120 | 87 (30.31) | 26 (27.37) | 7 (38.89) | | 89 (31.90) | 28 (24.56) | 3 (42.86) | |
| IV | 85 | 57 (19.87) | 19 (20.00) | 9 (50.00) | | 50 (17.92) | 32 ( 28.07) | 3 (42.86) | |
| Tumor localization | | | | | | | | | |
| Right breast | 185 | 134 (46.69) | 43 (45.26) | 8 (44.44) | 0.841 | 131 (46.95) | 51 (44.74) | 3 (42.86) | 28.54 |
| Left breast | 213 | 152 (52.96) | 51 (53.68) | 10 (55.56) | 0.93 | 148 (53.05) | 62 (54.39) | 3 (42.86) | <0.001 |
| Right + Left breast | 2 | 1 (0.35) | 1 (1.06) | 0 | | 0 | 1 (0.87) | 1 (14.28) | |

ER, Estrogen Receptor; PR, Progesterone Receptor; Significance p< 0.005

Table 6. Association between p53 codon 72 of exon 4 and codon 249 of exon 7 Genotypes and Clinicopathologic Characteristics among Breast Cancer Cases

| Variables | Total (n) | P53 codon -72 (Genotypes) | | | | P53 codon -249 (Genotypes) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Arg/Arg (106) | Arg/Pro (214) | Pro/Pro (80) | $X^2$ (p value) | Arg/Arg (316) | Arg/Ser 0 | Ser/Ser (84) | $X^2$ (p value) |
| Age group (yrs) | | | | | | | | | |
| ≤40 | 78 | 26 (24.53) | 38 (17.76) | 14 (17.50) | 1.55 | 69 (21.84) | 0 | 9 (10.71) | 5.22 |
| >40 | 322 | 80 (75.47) | 176 (82.24) | 66 (82.50) | 0.46 | 247 (78.16) | 0 | 75 (89.29) | 0.02 |
| ER status | | | | | | | | | |
| Positive | 218 | 64 (60.38) | 111 (51.87) | 43 (53.75) | 2.09 | 179 (56.65) | 0 | 39 (46.43) | 2.79 |
| Negative | 182 | 42 (39.62) | 103 (48.13) | 37 (46.25) | 0.35 | 137 (43.35) | 0 | 45 (53.57) | 0.09 |
| PR status | | | | | | | | | |
| Positive | 197 | 60 (56.60) | 128 (59.81) | 39 (48.75) | 3.05 | 160 (50.63) | 0 | 37 (44.05) | 1.26 |
| Negative | 203 | 46 (43.40) | 86 (40.19) | 41 (51.25) | 0.27 | 156 (49.37) | 0 | 47 (55.95) | 0.65 |
| Her2-neu status | | | | | | | | | |
| Positive | 57 | 20 (18.87) | 21 (9.81) | 16 (20.00) | 7.69 | 41 (12.97) | 0 | 16 (19.05) | 2.51 |
| Negative | 343 | 86 (81.13) | 193 (90.19) | 64 (80.00) | 0.02 | 275 (87.03) | 0 | 68 (80.95) | 0.11 |
| Clinical stage | | | | | | | | | |
| I | 48 | 15 (14.15) | 25 (11.68) | 8 (10.00) | 3.3 | 37 (11.71) | 0 | 11 (13.10) | 2.71 |
| II | 147 | 43 (40.57) | 73 (34.11) | 31 (38.75) | 0.76 | 120 (37.97) | 0 | 27 (32.14) | 0.43 |
| III | 120 | 25 (23.58) | 69 (32.24) | 26 (32.50) | | 97 (30.70) | 0 | 23 (27.38) | |
| IV | 85 | 23 (21.70) | 47 (21.97) | 15 (18.75) | | 62 (19.62) | 0 | 23 (27.38) | |
| Tumor localization | | | | | | | | | |
| Right breast | 185 | 49 (46.23) | 96 (44.86) | 40 (50.00) | 6.7 | 148 (46.84) | 0 | 37 (44.05) | 1.1 |
| Left breast | 213 | 56 (52.83) | 117 (54.67) | 40 (50.00) | 0.15 | 167 (52.85) | 0 | 46 (54.76) | 0.57 |
| Right + Left breast | 2 | 1 (0.94) | 1 (0.47) | 0 | | 1 (0.31) | 0 | 1 (1.19) | |

ER, Estrogen Receptor; PR, Progesterone Receptor; Significance p< 0.005

Table 7. Association of p53 and p21 Gene Variants with Demographic Variables Including Age of Cancer Occurrence, Age at First Pregnancy and Tobacco Smoking in Breast Cancer Cases and Control Group from Population of Maharashtra

| Gene | Genotype | Age (yrs) | Age (yrs) @ 1st pregnancy | | | Tobacco status | |
|---|---|---|---|---|---|---|---|
| | | (Cases/Control) | (Cases/Control) | | | (Cases/Control) | |
| | | ≤ 40 | > 40 | ≤ 20 | > 20 | Users | Non-Users |
| | | N=78/207 | N=322/193 | N=218/183 | N=182/217 | N=219/113 | N=181/287 |
| *p21* | C/C | 54/120 | 233/106 | 157/93 | 130/133 | 156/68 | 131/158 |
| *codon 31* | C/A + A/A | 24/87 | 89/87 | 61/90 | 52/84 | 63/45 | 50/129 |
| *exon-2* | OR (95% CI) | 0.61 (0.35-1.06) | 0.46 (0.32-0.67) | 0.40 (0.26-0.60) | 0.63 (0.41-0.96) | 0.61 (0.37-0.98) | 0.46 (0.31-0.69) |
| *C>A* | p value | 0.08 | 0.0001 | <0.0001 | 0.03 | 0.04 | 0.002 |
| *rs1801270* | | | | | | | |
| *p21* | C/C | 53/165 | 226/144 | 152/139 | 127/170 | 149/79 | 130/230 |
| *exon-3* | C/T + T/T | 25/42 | 96/49 | 66/44 | 55/47 | 70/34 | 51/57 |
| *C>T* | OR (95% CI) | 1.85 (1.03-3.32) | 0.98 (0.65-1.49) | 1.37 (0.87-2.14) | 1.56 (0.99-2.46) | 1.09 (0.66-1.78) | 1.58 (1.02-2.44) |
| *rs1059234* | p value | 0.01 | 0.95 | 0.16 | 0.05 | 0.72 | 0.03 |
| *Codon72* | G/G | 26/40 | 80/52 | 55/50 | 51/42 | 57/27 | 49/65 |
| *Exon-4* | G/C+C/C | 52/167 | 242/141 | 163/133 | 131/175 | 162/86 | 132/222 |
| *G>C* | OR(95% CI) | 0.47 (0.26-0.85) | 1.11 (0.74-1.67) | 1.11 (0.71-1.74) | 0.61 (0.38-0.98) | 0.89 (0.52-1.51) | 0.78 (0.51-1.21) |
| *rs1042522* | p value | 0.01 | 0.59 | 0.63 | 0.04 | 0.67 | 0.27 |
| *p53* | G/G | 69/178 | 247/156 | 172/147 | 144/187 | 177/93 | 139/241 |
| *Codon249* | G/T + T/T | 29-Sep | 75/37 | 46/36 | 38/30 | 42/20 | 42/46 |
| *Exon-7* | OR (95% CI) | 0.80 (0.36-1.77) | 1.28 (0.82-1.99) | 1.09 (0.67-1.78) | 1.64 (0.97-2.78) | 1.10 (0.61-1.98) | 1.58 (0.99-2.52) |
| *G>T* | pvalue | 0.58 | 0.27 | 0.72 | 0.06 | 0.74 | 0.05 |
| *rs28934571* | | | | | | | |

Significance p= 0.005; OR, Odds ratio; CI, Confidence Interval.

the SNPs of tumor suppressor genes p21 and p53 are associated with breast cancer risk in Maharashtra women. In this hospital based case-control study, most studied polymorphisms of p21 (rs1801270 C/A, rs1059234 C/T) and p53 (rs1042522 G/C, rs28934571 G/T) and the risk of breast cancer in women residing in the rural areas of Maharashtra was studied. To the best of our knowledge, we have demonstrated these SNPs in relation with BC in rural population of Maharashtra for the first time, as these SNPs are not studied earlier in relation with BC risk from the rural areas of Maharashtra. The association of the SNPs in p21 and p53 with risk of BC was evaluated by Crude and Adjusted Odds ratio with 95% CI estimated for homozygous and heterozygous genotypes. The rs1801270, rs1059234 SNPs of p21 are not much studied however; p53 is studied for its SNPs (rs1042522, rs28934571) of codon 72 and codon 249 and their association with variety of cancers, but deficit in the literature for their role in breast carcinogenesis. Our results revealed that the C>A polymorphism of p21 gene at codon 31 showed significant association with BC development in women of studied population. The minor variant A allele was significantly (OR=0.23; 95% CI: 0.13-0.40 p<0.0001) associated with decreased risk of BC development. It is evidenced from earlier studies that the polymorphisms in tumor suppressor genes may play an imperative role in genetic susceptibility towards carcinogenesis. Few studies on the polymorphisms in p21 and p53 have been studied and some of them were evidenced to be associated with cancer risk with significance. A polymorphism of p21 i.e., Serine to Arginine transversion at codon 31 of exon 2 and C>T transition 20bp upstream from stop codon of exon 3 and p53 at codon 72 of exon 4 i.e. substitution of arginine to proline, and codon 249 of exon 7 i.e. transition of arginine to serine have been distinctively explored in different populations in relation with carcinogenesis (Zang et al 2018, Ratre et al 2019, Yu et al 2022). The rs1042522, SNP of p53 at codon 72 with a substitution of arginine to proline and rs1801270 SNP of p21 at codon 31 with serine to arginine substitute have been widely studied in diverse group of population for their association with carcinogenesis (Huang et al., 2019; Yu et al 2022), but others reported conflicting results with no association with cancer development (Song et al., 2017; Lin et al., 2018; Nicholas et al., 2019). Similar type of epidemiological studies reported from India for those SNPs and their association with cancer development (Lakshmi et al., 2012; Saikia et al., 2014; Khan et al., 2020) whereas other objected for their association with carcinogenesis in different ethnic population (Pillai and Nasir 2016; Bansal et al., 2016). Various investigations reported some associations of the studied genes with breast cancer development (Ayobi et al., 2018; Diakite et al., 2021) whereas others suggested no association between the polymorphisms either of p21 or p53 genes and the breast carcinogenesis (Habyarimana et al., 2018; Aceto et al., 2019; Zhao et al., 2022). The major attention of this study was to identify the polymorphisms in p53 gene in rural women of Maharashtra and demonstration of their association with risk of BC in studied population. Interestingly, we observed no significant differences in either codon 72 or codon 249 of the p53 gene of controls

and cases when statistically analyzed for their association with BC development. Our results are in accordance with earlier observations noted by the researchers in South Indian populations (Suresh et al., 2011; Vijayaraman et al., 2012) and North Indian population (Mandal et al., 2014). When we studied the polymorphism of p21 (codon 31 of exon 2 and C>T transition occurring 20bp upstream from stop codon of exon 3, the results indicated that the homozygous variant Arg/Arg genotype of codon 31 of p21 was negatively associated with BC susceptibility. The rs1801270 SNP of p21 gene showed significant negative association when observed for the homozygous variant allele when assessed by recessive model (OR=0.26; 95% CI: 0.15-0.45 p<0.0001) and heterozygous and minor homozygous allele addressed by dominant model (OR=0.51; 95% CI: 0.38-0.68 p<0.0001) which are in agreement with other reported studies (Martinez-Nava et al., 2016).

In conclusion, the results derived from this study stated that the functional SNP rs1801270 of p21 gene showed significant association with decreased risk of BC in studied rural population of south-western Maharashtra which was not reported earlier from this part of the world. The findings obtained from this study also indicated that neither of p53 Arg72Pro nor Arg249Ser was associated with BC risk among studied Maharashtra women

## Author Contribution Statement

Concept: KDD, SJB, AKG, RAG, Design: KDD, SJB, AKG, Experimental Studies: PPD, NJJ, ALM Clinical studies: AKG, RAG, SRP Data analysis: PPD, KDD, Statistical analysis: PPD, KDD, Manuscript preparation: KDD, SJB, AKG, RAG. All authors read and approved the final manuscript.

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*Approval by any scientific Body*

The research protocol (Protocol No: KIMSDU/Protocol-03/2017) was approved by Protocol Committee of Krishna Institute of Medical Sciences, Deemed to be University)

*Ethics Committee Approval*

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*Availability of data*

Not Applicable.

*Abbreviations*

BC: Breast Cancer
p21: Tumor suppressor p21 gene
p53: Tumor Supressor TP53 gene
PCR-RFLP: Polymerase Chain Reaction-Restriction allighnment Length Polymorphism
SNP: Single Nucleotide Polymorphism
OR: Odds Ratio
CI: Confidence Interval
µL: Microliter
µg: Microgram
DNA: Deoxyribose Nucleic Acid
EDTA: Ethylene Diamine Tetra Acetate
SDS: Sodium dodecyl sulphate

*Any conflict of interest*

The authors declare that they have no competing financial or any other interests that could have appeared to influence the work reported in this paper.

## References

Aceto GM, Awadelkarim KD, Di Nicola M, et al (2019). Germline TP53 mutation spectrum in Sudanese premenopausal breast cancer patients: Correlations with reproductive factors. *Breast Cancer Res Treat*, **175**, 479-85.

Akhter N, Dar SA, Chattopadhyay S, et al (2018). Impact of p53 arg72pro SNP on Breast Cancer risk in North Indian Population. *Curr Genom*, **19**, 395-410.

Ali T, Kamath SM (2022). Correlation of P53 expression with clinicopathological parameters, hormone receptors and Her2 status in breast carcinoma. *Asian Pac J Cancer Biol*, **7**, 307-14.

Ali G (2023). Mechanism of action of p53. *Asian Pac J Cancer Biol*, **8**, 63-8.

Alqumber MA, Akhter N, Haque S, et al (2014). Evaluating the association between p53 codon 72 Arg>Pro polymorphism and risk of ovary cancer: a meta-analysis. *PLoS One*, **9**, e94874.

Ayoubi SE, Elkarroumi M, El Khachibi M, et al (2018). The 72Pro Variant of the Tumor Protein 53 is associated with an increased breast cancer risk in the Moroccan population. *Pathobiol*, **85**, 247-53.

Bansal A, Das P, Kannan S, et al (2016). Effect of p53 codon 72 polymorphism on the survival outcome in advanced stage cervical cancer patients in India. *Indian J Med Res*, **144**, 359-65.

Bau DT, Tsai MH, LO YL, et al (2007). Association of p53 and p21(CDKN1A/WAF1/CIP1) polymorphisms with oral cancer in Taiwan patients. *Anticancer Res*, **27**, 1559-64.

Bhadoria A, Kapil U, Sareen N, et al (2013). Reproductive factors and breast cancer: A case-control study in tertiary care hospital of North India. *Indian J Cancer*, **50**, 316–21.

Cobain EF, Milliron KJ, Merajver SD (2016). Updates on breast cancer genetics: Clinical implications of detecting syndromes of inherited increased susceptibility to breast cancer. *Semin Oncol*, **43**, 528–35.

Collins A, Politopoulos I (2011). The genetics of breast cancer: risk factors for disease. *Appl Clin Genet*, **4**, 11-9.

Datkhile KD, Patil MN, Durgawale PP, et al (2019). Role of p53 and p21 polymorphisms in cervical cancer risk among rural population of south-western Maharashtra: A case-control

study. *Indian J Public Health Res Dev*, **10**, 277–81
Diakite B, Kassogue,Y, Dolo G, et al (2020). p.Arg72Pro polymorphism of P53 and breast cancer risk: a meta-analysis of case-control studies. *BMC Med Genet*, **21**, 206.
Ghosh S, Bhattacharjee M, Jana N (2022). Gene regulation by p53 in human cancer system. *Asian Pac J Cancer Biol*, **7**, 97-109.
GLOBOCAN (2020). Glogal Cancer Observatory. (2020). [Cit. March 2021]. Available from https://gco.iarc.fr/today/data/factsheets/populations/356-india-fact-sheets.
Habyarimana T, Attaleb M, Mugenzi P, et al (2018). Association of p53 Codon 72 polymorphism with breast cancer in a Rwandese population. *Pathobiol*, **85**, 186-91.
Hou J, Jiang Y, Tang W, et al (2013). p53 codon 72 polymorphism and breast cancer risk: A meta-analysis. *Exper Ther Med*, **5**, 1397-1402.
Huang X, Wu F, Zhang Z, et al (2019). Association between TP53 rs1042522 gene polymorphism and the risk of malignant bone tumors: a meta-analysis. *Biosci Rep*, **39**, BSR20181832.
Khan MA, Tiwari D, Dongre A (2020). Exploring the p53 connection of cervical cancer pathogenesis involving north-east Indian patients. *PLoS One*, **15**, e0238500.
Lakshmi A, Kumar K, Reddy M, et al (2012). P53 Codon 72 Gene polymorphism and risk of oral squamous cell carcinoma in South Indian population: A case-control study. *J Cancer Sci Ther*, **4**, 188-92.
Lin YM, Shao J, Yin XH, et al (2018). Meta-Analysis results on the association between TP53 codon 72 polymorphism with the susceptibility to oral cancer. *Front Physiol*, **9**, 1014.
Mandal RK, Yadav SS, Panda AK (2014). No evidence of correlation between p53 codon 72 G>C gene polymorphism and cancer risk in Indian population: a meta-analysis. *Tumor Biol*, **35**, 8607-13.
Martinez-Nava GA, Fernandez-Nino JA, Madrid-Marina V, et al (2016). Cervical cancer genetic susceptibility: A systematicr and meta-analyses of recent evidence. *PLoS One*, **11**, e0157344.
Matakidou A, Eisen T, Houlston RS (2003). TP53 polymorphisms and lung cancer risk: a systematic review and meta-analysis. *Mutagenesis*, **18**, 377–85.
Meshram II, Hiwarkar PA, Kulkarni PN (2009). Reproductive risk factors for breast cancer: A case control study. *Online J Health Allied Sci*, **83**, 85.
Momenimovahed Z, Salehiniya H (2019). Epidemiological characteristics of and risk factors for breast cancer in the world. *Breast Cancer Targets Ther*, **11**, 151-64.
National Cancer Registry Programme (2020). Consolidated report of the population based cancer registries 2012-2016. New Delhi: Indian Council of Medical Research; 2020.
Nicholas N, Mitchell K, Zingone A, et al (2019). Relationship between the TP53 SNP rs1800371 and lung cancer in African Americans. *Cancer Health Disparities*, **3**, 1-9.
Pillai RM, Nasir SA (2016). Polymorphism of p53 in cancer prognosis. *Ind J Med Res*, **144**, 314-16.
Ratre YK, Jain V, Amle D, et al (2019). Association of TP53 gene codon 72 polymorphism with incidence of cervical cancer in chhattisgarh. *Indian J Exp Biol*, **57**, 580–5.
Ressiniotis T, Griffiths PG, Keets SK, et al (2005). A polymorphism at codon 31 of gene p21 is not associated with primary open angle glaucoma in Caucasians. *BMC Ophthalmol*, **5**, 1-4.
Saikia BJ, Das M, Sharma SK, et al (2014). Association of a p53 Codon 72 gene polymorphism with environmental factors and risk of lung cancer: a case control study in Mizoram and Manipur, a high incidence region in North East India. *Asian Pac J Cancer Prev*, **15**, 10653-58.
Sharma S, Sambyal V, Guleria K, et al (2014). TP53 polymorphisms in Sporadic North Indian breast cancer patients. *Asian Pac J Cancer Prev*, **15**, 6871-79.
Sivonova MK, Vilckova M, Klimentz J, et al (2015). Association of p53 and p21 polymorphisms with prostate cancer. *Biomed Rep*, **3**, 707-14,
Song F, Zong-Yao H, Meng Z, et al (2017). Association between the rs1042522 polymorphism in TP53 and prostate cancer risk: An updated meta-analysis. *J Chron Dis Transl Med*, **3**, 95-104.
Song HR, Kweon SS, Kim HN, et al (2011). p53 codon 72 polymorphism in patients with gastric and colorectal cancer in a Korean population. *Gastric Cancer*, **14**, 242–7.
Sung H, Ferlay J, Siegel RL, et al (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of incidence and Mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*, **71**, 209-49.
Surekha D, Rao DN, Padma T, et al (2011). Codon 72 and G13964C Intron 6 polymorphisms of TP53 in relation to development and progression of breast cancer in India. *Asian Pacfic J Cancer Prev*, **12**, 1893-98.
Suresh K, Venkatesan R, Chandirasekar R, et al (2011). Association of Trp53 arg72pro polymorphic variants with breast cancer - a case control study in south Indian population. *Biol Med*, **3**, 15-22.
Vijayaraman KP, Veluchamy M, Murugesan P, et al (2012). p53 Exon 4 (codon 72) Polymorphism and Exon 7 (codon 249) mutation in breast cancer patients in southern region (Madurai) of Tamil Nadu. *Asian Pac J Cancer Prev*, **13**, 511-16.
Yu M, Zhang Q, Zhao X (2022). Associations of MDM2 rs2279744 and TP53 rs1042522 polymorphisms with cervical cancer risk: A meta-analysis and systematic review. *Front Oncol*, **12**, 973077.
Zhang L, Wang Y, Qin Z, et al (2018). TP53 codon 72 polymorphism and bladder cancer risk: a meta-analysis and emphasis on the role of tumor or smoking status. *J Cancer*, **9**, 3522-31.
Zhao L, Yin X-X, Qin J, et al (2022). Association between the TP53 polymorphisms and breast cancer risk: An updated meta-analysis. *Front Genet*, **13**, 807466.

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