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TP53 Codon 72 Genetic Polymorphism, rs1042522, Modifies the Association Between Tobacco Smoking and Breast Cancer Risk

Maryam Moradinasab,[1,*] Afshin Ostovar,[1] Iraj Nabipour,[2] Seyed Sajjad Eghbali,[3] Katayoun Vahdat,[1] Abbas Ghaderi,[4] Mohamad Reza Farzaneh,[3] and Mohammad Reza Ravanbod[5]

[1]The Persian Gulf Tropical Medicine Research Center, The Persian Gulf Biomedical Sciences Research Institute, Bushehr University of Medical Sciences, Bushehr, IR Iran
[2]The Persian Gulf Marine Biotechnology Research Center, the Persian Gulf Biomedical Sciences Research Institute, Bushehr University of Medical Sciences, Bushehr, IR Iran
[3]Department of Pathology, Bushehr University of Medical Sciences, Bushehr, IR Iran
[4]Shiraz Institute for Cancer Research, Shiraz University of Medical Sciences, Shiraz, IR Iran
[5]Department of Oncology and Hematology, Bushehr University of Medical Sciences, Bushehr, IR Iran

[*]*Corresponding author*: Maryam Moradinasab, The Persian Gulf Tropical Medicine Research Center, The Persian Gulf Biomedical Research Institute, Boostan 19 Alley, Postal Code: 7514763448 , Imam Khomeini Street, Bushehr, IR Iran. Tel: +98-7733341828, Fax: +98-7733341828, E-mail: m.moradinasab85@gmail.com

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Abstract

Background: TP53 tumor suppressor gene participates in several pathways involving in carcinogenesis such as cell cycle control, DNA repair, and apoptosis. A common TP53 SNP (guanine/cytosine nucleotide substitution at codon 72), rs1042522, affects the function of p53 protein and may influence tumor behavior in response to environmental carcinogens.
Objectives: This study investigates the association between TP53 codon 72 polymorphisms, tobacco smoking, and breast cancer risk in southern Iranian women from Bushehr.
Methods: A case-control study was conducted on 144 cases with histologically confirmed invasive breast carcinoma and 162 randomly selected healthy controls with no previous cancer history in their family. TP53 codon 72 genotype was determined by using restriction fragment length polymorphism- polymerase chain reaction (RFLP-PCR) technique.
Results: Analysis revealed that smoking frequency was significantly higher in cases compared to controls (OR = 2.31, 95%CI = 1.33-3.99, P = 0.003) and the association between smoking and breast cancer was only significant for individuals with Arg/Pro genotype (OR = 3.23, 95% CI = 1.47 - 7.06, P = 0.003). On the other hand, there was no statistically considerable difference in the allele and genotype distribution between cases and controls.
Conclusions: These results should be confirmed in larger studies, but suggest that TP53 Arg/Pro genotype modifies the risk of breast cancer in tobacco smokers and causes significantly more susceptibility to breast cancer due to smoking.

Keywords: P53, Breast Neoplasms, Polymorphism, Smoking

1. Background

The TP53 gene, as one of the most important tumor suppressors, protects the genome against genotoxic stress, hypoxia, or oncogene activation by stopping cell-cycle progression or promoting apoptosis (1, 2). TP53 somatic mutations are the most common genetic alterations found in up to 50% of all human cancer types that can disrupt the activity of more than 150 genes associated with cell-cycle arrest, apoptosis, and/or DNA repair (3, 4). In addition to mutations, some polymorphisms may also affect p53 function and increase the risk of cancer (4). A common TP53 single-nucleotide polymorphism (SNP) is guanine/cytosine nucleotide substitution at codon 72 on exon 4 (Arg72Pro, Ex4 +119C > G, rs#1042522) (5). The different codon 72 polymorphic variants have been shown to affect p53 function differentially. Investigations indicate that the arginine variant has a stronger capacity to induce mitochondrial mediated apoptosis (6, 7). In contrast, the proline variant has been found to have a higher ability to induce DNA-repair and cell cycle arrest (7, 8); nevertheless, it has been observed that in the context of p53 mutations, cells that express mtp53-codon72-Pro have greater apoptotic potential (9, 10). Concerning the association between TP53 codon72 polymorphism and the risk of breast cancer, studies have revealed conflicting results (11-13).

Animal experiments and in vitro studies have shown that several carcinogenic compounds found in tobacco smoke such as polycyclic aromatic hydrocarbons (PAH), aromatic amines, and N-nitrosamines could play an important role in the etiology of breast cancer (14, 15) and may increase the risk of cancer especially in woman smoking for a long period or starting to smoke before the first pregnancy (16, 17). The available data show that among breast cancer patients TP53 mutation is more prevalent with vast spectrum in smokers than in non-smokers (18). On the other

hand, several studies examined the association between cigarette smoking and the risk of different types of cancer according to TP53 codon 72 genotype (19-21). Caceres et al. reported that combination of p53codon72 Pro allele and smoking habit significantly increases the risk of lung cancer (22) and Malakar et al. reported that smokers with Pro/Pro and Arg/Pro genotypes are at higher risk of stomach cancer (23). Therefore, due to the effect of codon 72 polymorphic variants on the function of p53 protein, they may also influence tumor behavior in response to environmental carcinogens.

2. Objectives

The aim of the present study was to evaluate the association between TP53 codon 72 polymorphisms, tobacco smoking, and breast cancer risk in southern Iranian women from Bushehr.

2. Methods

2.1. Study Population

This is a case-control study recruiting 306 women including 144 cases with histologically confirmed invasive breast carcinoma prior to receiving chemo- or radiotherapy and 162 randomly selected healthy controls with no previous cancer history in their family, between December 2015 and October 2016. Of note, all of the participants were southern Iranian women from Bushehr province and the study was conducted in the Persian Gulf Tropical Medicine Research Center. The cases included in the study were all the cases who were registered in provincial breast cancer registry. Post-hoc analyses using GPower 3.0.10 sample size software showed that this sample size would have provided statistical power between 60% - 80%.

A questionnaire containing information on demographic characteristics and smoking history was completed for all cases and controls. Smoking was defined as current consumption of any kind of cigarette or hookah (shisha) or smoking cessation during last 12 months prior to the study. A written informed consent was signed by all participants and then 2cc peripheral venous blood sample was obtained. The present research was approved by the ethics committee of Bushehr University of Medical Sciences and Health Services (IR.BPUMS.REC.1395.158).

2.2. DNA Extraction

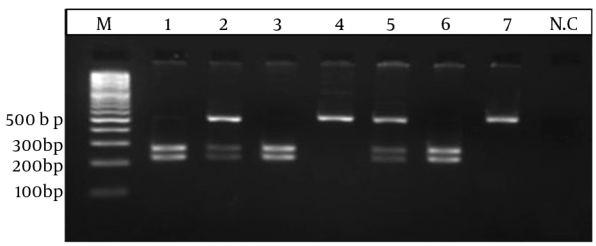
Genomic DNA was extracted from the peripheral blood lymphocytes using High Pure PCR Template Preparation Kit (Roche; Germany). Extracted DNA quality and quantity were determined by agarose gel electrophoresis and NanoDrop 1000 (Thermo Fisher Scientific; USA).

2.3. Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP)

Initially, SNP-containing fragment was amplified by using site-specific primers as follows: forward, 5'-CAA CGT TCT GGT AAG GAC AA-3' and reverse, 5'-AAG CCA AAG GGT GAA GAG GAA-3'. PCR reaction was performed in a total volume of 25 μL including 1 μL template DNA (50 - 100 ng), 2.5 μL PCR buffer (10x), 0.5 μL dNTPs (10 mM), 1 μL of each primers (10 pmol), 1 μL $MgCl_2$ (25 mM), 18 μL sterile distilled H_2O, and 0.2 μL Taq DNA polymerase (5 unit/μL), (Roche; Germany). PCR was conducted in a TC-512 thermal cycler (Techne, Barloworld Scientific Ltd., UK) with an initial denaturation step for 5 minutes at 94°, followed by 35 cycles of 30 seconds at 94°, 20 seconds at 57°, 30 seconds at 72°, and a final extension step for 10 minutes at 72°C. During PCR process, a 504 bp fragment containing polymorphic region of TP53 was amplified.

At the second step, 15 mL PCR products were digested by BstUI restriction enzyme (New England BioLabs; UK) at 60°C for 2 hours. Afterwards, 10 μL of enzyme digested solution were separated on a 2% agarose gel. The product size of Pro72 allele was 504 bp long, and Arg72 allele-specific product, because of the presence of BstUI recognition site, was digested into 238 and 266 bp fragments (Figure 1). It is noteworthy to mention that all of the applied equipment was calibrated.

Figure 1. Agarose Gel Stained with Ethidium Bromide Showing p53 Codon 72 Polymorphism



Lane M = 100 bp DNA ladder; Lane 1, 3, 6 = homozygote wild type Arg/Arg genotype; lane 2, 5 = heterozygote Arg/Pro genotype; Lane 4, 7 = homozygote Pro/Pro genotype.

To control the findings, 10% of samples were repeated randomly, showing that the replicates were 100% concordant. For verifying the authenticity of RFLP-PCR results, 5 randomly selected PCR products for each genotype were sequenced via sanger method using the forward primer (Applied biosystems 3730/3730xl DNA analyzers, Bioneer, Korea).

2.4. Statistical Analysis

All statistical analyses were performed with SPSS software (version 22; SPSS Inc., Chicago, IL). Data were de-

scribed as mean and standard deviation for continuous variables and frequency and percentages for categorical variables. Contingency tables were provided for dichotomous variables and odds ratios and their 95% confidence intervals were used for determining the association of breast cancer as dependent variable with existence of different genotypes and alleles.

3. Results

Participants' general characteristics, genotype, and allelic frequencies for cases and controls are presented in Table 1. Smoking frequency was significantly higher in cases compared to controls (OR = 2.31, 95%CI = 1.33-3.99, P = 0.003) (see Table 1). Distribution of genotype frequency for Arg/Arg, Arg/Pro, and Pro/Pro was similar among cases and controls and there was no statistically significant difference in the frequency of the two variant alleles between cases and controls (Table 1). All genotypes were in Hardy–Weinberg equilibrium.

Further analyses showed that the association between smoking and breast cancer was only statistically significant for individuals with Arg/Pro genotype (OR = 3.23, 95% CI = 1.47 - 7.06, P = 0.003) (Table 2).

Additionally, in case-only analysis, no significant association was found between the mean age at the onset of disease and p53 codon 72 genotypes, suggesting no evidence for age-related alteration. Further analyses showed no significant association between Arg72Pro genotypes and clinicopathological variables, like tumor stage and grade or lymph node metastases, demonstrating that this polymorphism is not a prognostic molecular marker for breast cancer in the study population (data not shown).

4. Discussion

Gene polymorphisms that could influence tumor suppressor genes function, like TP53 codon72 polymorphism, may explain different individuals' susceptibilities to cancer with shared environment exposure. In the present study, we investigated the relationship between TP53 codon 72 polymorphism and tobacco smoking, as the risk modification factors, and the risk of breast cancer. The results showed that there is no significant association between TP53 codon 72 polymorphism and breast cancer; nevertheless, tobacco smoking as a risk factor significantly increased the risk of breast cancer (OR = 2.31,CI = 1.33-3.99) and genetic variation in the TP53 codon 72 modified this association.

Concerning the role of TP53 codon 72 polymorphism on the risk of breast cancer, Hou et al. in a meta-analysis of sixty-one case-control studies suggested that TP53 codon 72 polymorphism is not associated with breast cancer risk (12). On the other hand, another meta-analysis based on thirty-nine case-control studies found no association between TP53 codon 72 polymorphism and breast cancer susceptibility among Asian populations, but significant associations among Europeans and Africans (13). Consistent with these studies, our statistical evidence revealed that none of the codon72 genotypes is associated with breast cancer in southern Iranian women, suggesting that probably this polymorphism does not impress the risk of breast cancer directly. Nevertheless, Goncalves et al. in a meta-analysis of forty-one case-control studies suggested an increased risk of breast cancer due to PP genotype (R vs. P; OR = 1.02; 95% CI 1.00 - 1.05), although in Asians they demonstrated that the risk was associated with the R allele (R vs. P; OR = 1.09; 95% CI 1.01 - 1.17) (11).

Evaluation of association between p53 codon72 genotypes, smoking, and breast cancer risk revealed that for individual with Arg/Pro genotype tobacco, smoking increased the risk of cancer three-fold and caused significantly a more susceptibility to breast cancer (OR = 3.23,95% CI = 1.47 - 7.06, P = 0.003). Although there was a similar trend for Arg/Arg genotype, but the risk was moderately low and non-significant (OR = 2.27, 95% CI = 0.85-6.07, P = 0.079).

In studies conducted in other cancers, Yang et al. reported that p53 Arg/Arg or Arg/Pro genotype in association with HPV16-seropositivity increases the risk of esophageal squamous cell carcinoma especially in smokers ($P < 0.001$, OR 27.05, 95% CI 11.06 - 66.16) (24). In southern India, Devi et al. reported that smokers with Arg/Pro genotype are more prone to lung cancer (25). On the other hand, it has been reported that smokers with Pro/Pro and Arg/Pro genotypes are at higher risk of stomach cancer (23). Cai et al. have reported that smokers with Pro/Pro genotype had a 2.00-fold increased risk of hepatocellular carcinoma in a Chinese population when compared with non-smoking individuals with Arg/Arg genotype (26). In a population in Bangladesh, it has been reported that TP53Pro allele as a risk factor for lung cancer overrepresented among smokers compared to controls (OR = 1.8 - 10.0, $P \leq 0.01 - 0.03$) (27). On the other hand, a significant interaction has been reported between TP53 Pro/Pro genetic polymorphism and smoking in esophageal squamous cell carcinoma (OR = 5.29, 95% CI = 2.91 - 9.61) (28). In pancreatic cancer, it was reported that TP53 Pro/Pro genotype significantly increases the risk of cancer among males, particularly among heavy smokers and excessive alcohol drinkers (29). Liu et al. also reported that TP53Pro allele increases the risk of squamous cell carcinoma of the lung mainly in heavy smokers (OR = 3.84, 95% CI = 1.46 - 10.1) (30).

Table 1. General Characteristics, Genotype, and Allelic Frequencies in Breast Cancer Cases and Controls[a]

	Case (n = 144)	Control (n = 162)	OR[b] (95% CI)[c]	P Value
Age	47.73 ± 14.44	47.60 ± 11.76		0.932
Smoking status				
Smoker	42 (34.1)	29 (18.4)	2.31 (1.33 - 3.99)	0.003
Non-smoker	81 (65.9)	129 (89.6)		
Genotype				
G/G	46 (31.9)	50 (30.9)	1.05 (0.65 - 1.71)	0.839
C/G	68 (47.2)	90 (55.6)	0.72 (0.46 - 1.12)	0.146
C/C	30 (20.8)	22 (13.6)	1.68 (0.92 - 3.06)	0.094
Allele				
G	161 (55.90)	190 (58.64)	0.89 (0.64 - 1.24)	0.49
C	127(44.09)	134 (41.34)	1.11 (0.80 - 1.56)	0.49

[a]Values are expressed as mean age ± SD.
[b]Odds Ratio.
[c]Confidence Interval.

Table 2. Association Between Smoking and Breast Cancer Risk Stratified by TP53 Genotypes

Genotype	Smoking Status	Case (n = 123)	Control (n = 158)	OR[a] (95 %CI)[b]	P Value
Pro/Pro	+	7 (25.9)	6 (27.3)	0.93 (0.26 - 3.33)	0.584
	-	20 (74.1)	16 (72.7)		
Arg/Pro	+	22 (38.6)	14 (16.3)	3.23 (1.47 - 7.06)	0.003
	-	35 (61.4)	72 (83.7)		
Arg/Arg	+	13 (33.3)	9 (18)	2.27 (0.85 - 6.07)	0.079
	-	26 (66.7)	41 (82)		

[a]Odds Ratio.
[b]Confidence Interval.

Nevertheless, some studies have found no evidence of association between TP53 Arg72Pro polymorphism and smoking (31, 32). In this regard, so far only one study has been conducted in breast cancer indicating that the association between codon 72 polymorphism and breast cancer is not modified by cigarette smoking (33).

Several limitations should be discussed for our study: First, due to the relatively small sample size, the statistical power of our study was low. Second, we did not have access to other breast cancer risk factors information about subjects such as body weight and height, alcohol consumption, premenopausal and postmenopausal status, and hormonal receptors. Third, this was a hospital-based case-control study; therefore, selection bias may not be avoided, and the subjects may be less representative of the general population. Finally, we only genotyped TP53 Arg72Pro and did not analyze other TP53 potentially functional SNPs or TP53 mutations whereas polymorphisms effects that are best represented by their haplotypes and TP53 mutations may affect these results.

In conclusion, the results of the present study, as the first report in Iran and the second report in the world, suggested that TP53 codon 72 polymorphism can modify the risk of breast cancer in tobacco smokers, and smokers with Arg/Pro genotype are significantly more susceptible to breast cancer. Nevertheless, more studies with well-designed and larger sample sizes are required to validate these observations.

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Footnotes

Authors' Contribution: Study concept and design: Maryam Moradinasab and Iraj Nabipour; laboratory experiments: Maryam Moradinasab; analysis and interpretation of data: Afshin Ostovar; drafting of the manuscript: Maryam Moradinasab; Acquisition of data: Seyed Sajjad Eghbali, Abbas Ghaderi and Mohammad Reza Ravanbod; Critical revision of the manuscript for important intellectual content: Afshin Ostovar, Iraj Nabipour, Katayoun Vahdat, and Mohamad Reza Farzaneh.

Conflict of Interest: The authors declare that they have no conflict of interests.

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Implication for Health Policy Makers/Practice/Research/Medical Education: TP53 codon 72 SNP can modify the risk of breast cancer in tobacco smokers, and smokers with Arg/Pro genotype are significantly more susceptible to breast cancer in Southern Iranian women.

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