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Three Common *TP53* Polymorphisms and the Risk of Breast Cancer among Groups of Iranian Women

Mahin Ahangar Oskouee*[a], Shohreh Shahmahmoudi[b], Rakhshandeh Nategh[b], Heidar-Ali Ismaili[d], Firozeh Safaeyan[a], Maryam Zareinghalame Moghaddam[c], Mohammad Agazadeh[a], Ayda Savadi Oskouee[e]

[a] *Department of Microbiology &Virology, Faculty of Medicine , Tabriz University of Medical Sciences, Tabriz, Iran*
[b] *Department of Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
[c] *Department of Microbiology, International Branch of Shahid Beheshti Medical University , Tehran, Iran*
[d] *Department of Pathobiology, Tabriz University of Medical Sciences, Tabriz, Iran*
[e] *Faculty of Dentistry, Tabriz University of Medical Sciences, Tabriz, Iran*

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ABSTRACT

Background: The *TP53* gene is the most important tumor suppressor gene in humans. The aim of our study was to determine the genotype frequency of three common TP53 polymorphisms (codon 72 BstUI and intron 6 MspI, as well as the intron 3) in a group of Iranian women with and without breast cancer.

Methods: Paraffin-embedded specimens of 65 malignant breast cancer cases and 65 cases with benign breast lesions were investigated for the presence of three common *TP53* polymorphisms by polymerase chain reaction. Samples were genotyped by polymerase chain reaction followed by variant specific restriction enzyme digestion.

Results: In our study, age grouping as >50 and ≤50 showed that the highest number of cancerous and non-cancerous patients was in the age group under 50; according to statistical tests, the difference was significant and recessive alleles of all three hot spots of *TP53* had the highest frequency in the cancerous group. The majority of the cases with recessive alleles of all three hot spots of *TP53* were in the age group ≤ 50. The difference between cancerous and noncancerous groups was statistically significant.

Conclusions: Our results indicate that recessive alleles in three hot spots of TP53 gene might play a role in the breast cancer development, especially in women younger than 50 years.

Introduction

Many risk factors of the development of breast cancer have been identified. One strong candidate for the genetic susceptibility to familial and or sporadic breast cancer is the *TP53* gene polymorphism.[1,2] Breast cancer is the most common cancer among women around the world. Epidemiological studies in Iran show an annual incidence of 10,000 breast cancer cases in women which is on the rise every year and approximately 14% of cases end in the patient's death.[3] The *TP53* tumor-suppressor protein plays a critical role in the prevention of human cancer. The *TP53* protein has a very important function in many physiological processes, such as inhibition of cell growth, and stimulation of the apoptosis in response to cellular stresses.[4] It is reported that the *TP53* gene is mutated in 20%–30% of the sporadic breast cancers.[5] Polymorphisms at *TP53* are also found in the intronic regions. The role of PIN3 Ins 16 bp and PIN6 G13494A polymorphisms

Address for correspondence:
Mahin Ahangar Oskouee
Address: Department of Microbiology &Virology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.
Tel/fax: +98 4133364661
Email: Ahangarm@tbzmed.ac.ir

in breast cancer is controversial.[6] The status of the three common *TP53* gene polymorphisms in Iranian women with breast cancer is not known. Therefore, we analyzed three common *TP53* gene polymorphisms, codon 72, intron 3, and intron 6, and their haplotypes in Iranian women with breast cancer. It seems that detection of *TP53* gene polymorphism in Iranian women with breast cancer helps to adopt appropriate therapeutic approaches to better manage the patients.

Methods

A total of 65 formalin-fixed paraffin-embedded breast cancer tissue blocks and 65 non-cancerous breast specimens were obtained from the Pathology Department of Tabriz University, East Azerbaijan, Iran. All samples were cut and collected in sterile tubes. Deparaffinization was performed according to protocols mentioned in previous studies.[7] For each sample, PCR amplification with specific primers (Table 1) targeting a 268 bp fragment of the β-globin gene was carried out as an internal control to assess the quality of the extracted DNA.[8] For the intron 6 polymorphism, and exon4, intron3, PCR amplification with specific primers targeting a 404 bp fragment of the intron6, 396bp of exon4 gene, and then the PCR product of the intron6 was digested with MspI, and for exon4 polymorphism, was digested with BstUI. All product of PCR- RFLP (intron6, exon4) were visualized on the 3% agarose gel. For intron 3 polymorphism, the PCR products were visualized on 2.5% agarose gel.[9] (Figures 1, 2, and 3)

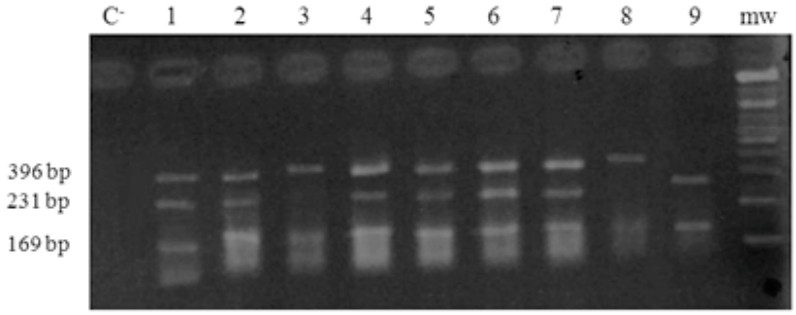

Figure 1. Genotype analysis by digestion of the amplified product and RFLP of exon 4 *TP53*; lanes 1, 4, 5, and 6 are heterozygote (WM) samples for Arg/Proallele; lanes 3 and 8 are mutant homozygote (MM) Samples for Pro/Pro, and lane 9 is homozygote (WW) Arg/Arg samples. The lane MW is the 100 bp DNA ladder.

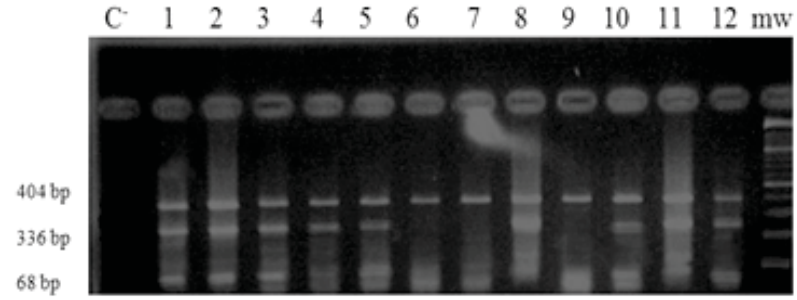

Figure 2. Genotype analysis by digestion of the amplified product and RFLP of Intron 6 *TP53*; lanes 1-5, 8, 11, 12 are WM heterozygote samples, lanes 3 and 4 are MM homozygote samples. The lane mw is the 100 bp DNA ladder .

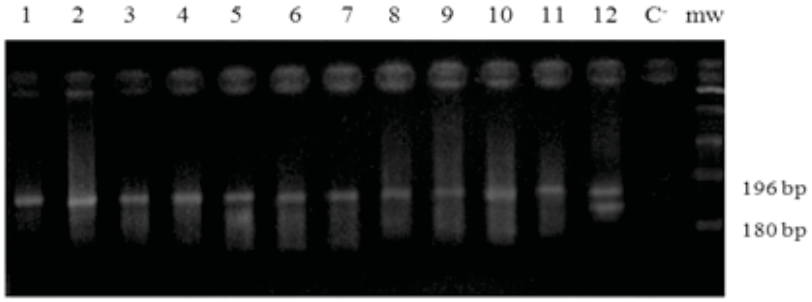

Figure 3. Genotype analysis by PCR amplified product of Intron 3 *TP53*; lanes 5 and 12 are WM heterozygotes, lanes 1-4 and 6-11 are MM homozygotes and the lane MW is the 100 bp DNA ladder

Table 1. Primers and location in the *TP53* gene and β-Globin internal control

Genes and their regions	Primers	Product Length	reference
B-Globin			(7)
PCO4	5'-CAACTTCATCCACGTTCACC-3'	268 bp	
Gh20	5'-GAAGAGCCAAGGACAGGTAC-3'		
TP53			(8)
Exon 4	5'-TGGTAAGGACAAGGGTTGG-3'	396 bp	
Codon 72	5'-ACTGACCGTGCAAGTCACAG-3		
	WW homozygotes	165 bp, 231 bp	
	WM heterozygotes	165 bp, 231 bp, 396 bp	
	MM homozygotes	396 bp	
Intron 3	5'-TGGGACTGACTTTCTGCTCTT-3'	180 bp or 196 bp	
	5'- TCAAATCATCCATTGCTTGG-3'		
	WW homozygotes	180 bp	
	WM heterozygotes	180 bp,196 bp	
	MM homozygotes	196bp	
Interon 6	5'- TGGCCATATACAAGCAGTCA-3'	404 bp	
	5'- TTGCACATCTCATGGGGTTA-3'		
	WW homozygotes	68 bp, 336 bp	
	WM heterozygotes	68 bp, 336 bp, 404 bp	
	MM hemozygotes	404 bp	

Statistical analysis

For all samples (130), the allelic frequency of three hot spots of the *TP53* gene polymorphism was calculated and the genotype frequencies of Intron 3, Intron 6, and Exon 4 of the *TP53* gene polymorphism were assessed using the $\chi 2$ test. The strength of the association between the three hot spots of *TP53* gene polymorphism and the breast cancer risk was measured by odds ratio (OR) with 95% confidence intervals (CIs).

Results

In our study, 63.1% and 74.6% of the specimens in case and control groups were below 50 years of age, respectively. The most frequent histological type of breast cancer (55.4%) was invasive ductal carcinoma II (grade II) and fibrocystic (81.5%) in the control group (Table 2). The DNA extracted from tissue samples was positive for the β-globin gene in all specimens, indicating that the quality and quantity of DNA was satisfactory. In this study, heterozygote (recessive alleles) haplotypes of all three hot spots of *TP53* in the cancerous groups had the highest frequency (Table 3). The majority of the cases with recessive alleles of all three hot spots of *TP53* in cancerous groups were in the age group ≤50 (Table 4). The difference between cancerous and non- cancerous groups was statistically significant.

Table 2. General characteristics of the study population according to disease stage and age

Histopathological features	Age		Total	P-value
	≤ 50	> 50		
Stage and histologic type of cancerous lesions				0.3
IDC (I-III)	70.4%	87.5%	76.9%	
ILC (I-II)	22%	8.3%	16.9%	
Others*	7.3%	4.2%	6.2%	
Histologic type of non-cancerous lesions				
Fibrocystic	79.3%	53%	81.5%	
Fibro adenoma	10.3%	0 %	9.2%	
Lipoma	1.7%	0 %	1.5%	
Fat necrosis	1.7%	0 %	1.5%	
Epidermal cystic	3.5%	0 %	3.1%	
Lymph node	1.7%	0 %	1.5%	
Ruptured epidermal cystic	1.7%	0 %	1.5%	

** Mucinous carcinoma, Phyllodes tumor, Metastatic tumor*
Abbreviation: IDC; invasive ductal carcinoma, ILC; invasive lobular carcinoma

Discussion

Studies have shown that various factors increase the risk of breast cancer. Some breast cancers are related with hereditary mutations in genes tumor suppressor genes such as *TP53* and BRCA 1 and 2.[10] The *TP53* gene encodes *TP53* protein in humans; this protein causes tumor suppression in humans. So, any mutation and polymorphism in this gene increase the risk of cancer in humans.[11] Studies show that in addition to mutations, polymorphisms in the *TP53* gene can increase the risk of cancer in humans. Investigation of the pattern of *TP53* mutations in breast cancer has led to the discovery of substantial diversity of the mutational patterns in cohorts from

Table 3. The relative frequency of *TP53* gene polymorphism in both groups with cancer and non- cancerous samples

TP53 polymorphisms and genotypes	Frequency		OR (95% CI)	P-value
	cancer	Non-cancerous		
Exon 4 (R72P)				
WW	32.3%	73.8%	Ref	Ref
WM	61.5%	20.0%	7.03(3.13-15.8)	P<0.001
MM	6.2%	6.2%	2.3(0.52-10)	0.23
WM+MM	67.7%	26.1%	5.9(2.8-20.64)	P<0.001
PIN3Ins/Del 16bp				
WW	21.5%	60.0%	Ref	Ref
WM	60.0%	35.4%	4.7(2.12-10.5)	P<0.001
MM	18.5%	4.6%	11.1(2.73-5.4)	P<0.001
WM+MM	24.5%	40.0%	5.5(2.52-11.8)	P<0.001
PIN6 G13494 A				
WW	33.8%	61.5%	Ref	Ref
WM	53.8%	36.9%	2.65(1.27-5.53)	P<0.001
MM	12.3%	1.5%	14.5(1.7-124)	P<0.001
WM+MM	66.1%	38.4%	3.12(1.52-6.4)	P<0.001

Abbreviation: WW; Dominant homozygous, WM; Heterozygote, MM; Recessive homozygous

Table 4. The relative frequency of *TP53* gene polymorphism among study participants based on their age

	Histologic type		OR (95% CI)	P-value
	Cancerous	Non-cancerous		
Intron 6				
≤ 50				
WW	24.4%	60.3%	Ref	Ref
WM	63.4%	38.0%	4.13 (1.67-20.0)	<0.001
MM	12.2%	1.7%	17.5 (1.82-167.0)	<0.001
WM+MM	75.6%	5.5%	4.7 (1.94-11.4)	<0.001
> 50				
WW	50.0%	71.4%	Ref	Ref
WM	37.5%	28.6%	1.87 (0.29-12.0)	0.41
MM	12.5%	0%	-	-
WM+MM	50.0%	28.6%	2.5 (0.40-15.5)	0.28
Intron 3				
≤ 50				
WW	17.1%	62.1%	Ref	Ref
WM	65.8%	32.7%	7.30 (2.7-19.7)	<0.001
MM	17.1%	5.2%	12.0 (2.5-58.0)	<0.001
WM+MM	83.0%	38.0%	7.94 (3.01-21.0)	<0.001
> 50				
WW	29.2%	43.0%	Ref	Ref
WM	50.0%	57.1%	1.3 (0.22-7.5)	0.56
MM	21.0%	0%	-	-
Exon 4				
≤ 50				
WW	29.3%	74.1%	Ref	Ref
WM	63.4%	20.7%	7.8 (3.04-81.0)	<0.001
MM	7.3%	5.2%	3.6 (0.63-20.8)	0.15
> 50				
WW	37.5%	71.4%	Ref	Ref
WM	58.3%	14.3%	7.3 (0.8-7.8)	0.07
WM+MM	70.7%	25.9%	6.93 (2.83-17)	<0.001

Abbreviation: WW; Dominant homozygous, WM; Heterozygote, MM; Recessive homozygous

various parts of the world. The differences in these mutation patterns in geographically and/or racially diverse populations reflect an intrinsic (endogenous) pattern of mutation plus exposure to particular environmental carcinogens. A large number of studies have assessed the prognostic and predictive role of *TP53* alterations in breast cancer reporting conflicting results.[12]

Several studies have examined the involvement of *TP53* gene polymorphisms as a risk factor in human breast cancer, and variations due to ethnicity or race have been reported. In our country, a few studies have reported *TP53* polymorphism in human breast cancer but there are no reports on three common *TP53* polymorphisms (codon 72, intron 3, and intron 6) in Iranian women affected with breast cancer.[13-16] Thus, the present report is the first to assess the role of *TP53* polymorphisms at three

genomic sites in Iranian women with breast cancer.

In our study, heterozygote (recessive alleles) haplotypes of all three hot spots of *TP53* had the highest frequency in the cancerous group. The majority of cases with recessive alleles of all three hot spots of *TP53* were in the age group ≤50. The difference between cancerous and noncancerous groups was statistically significant ($P < 0.001$).

The reported frequency of codon 72 of *TP53* was different from other population. In our study, the frequency of the Allele Arg/ Pro was 61.5% in cancerous samples and 20% in non- cancerous sample ($P < 0.0001$). The frequency of the Allele Arg/ Pro has been reported 17% in Sweden, 37% in Turky, and 63% in Nigeria.[17, 18] Its frequency is 43.6% in Arab women, 42% in Iran, and 41.5% in Indians.[13,19]

Some studies have reported that women with allele Pro/Pro have an increased risk of developing breast cancer.[20, 21] However, some other studies have found no correlations between intron 3 genotypes and the risk of breast cancer.[22-24]

The involvement of intron 3 polymorphism and the risk of breast cancer is controversial. In the Slovak population, wild-type intron 3 (A1/A1) genotype is more than six times higher in comparison with the A2/A2 genotype.[25] A Study conducted in Australia failed to showed any significant association between the polymorphism of intron6 *TP53* and breast cancer while another study conducted in Czech Republic showed that G13964C was associated with aggressive breast cancer.[20] Intron 3 16 bp duplication polymorphism of *TP53* has been reported to be associated with breast cancer and other cancers in humans. However, the reported results remain inconclusive. In two meta- analyses (case – control) studies, IVS3 16 bp has been reported to be an important genetic marker contributing to susceptibility to breast cancer.[19,20]

In the Australia population, PIN6 G13494A polymorphisms is not a high-risk mutation in familial breast cancer.[26] while a study from Czech Republic showed that PIN6 G13964C was associated with an aggressive breast cancer phenotype.[27]

Intron 3 16 bp duplication polymorphism of TP53 has been reported to be associated with breast cancer, colorectal cancer, lung cancer, and other cancers; nonetheless, the reported results remain inconclusive.[28]

In 2010, Hu Z *et al.* in a meta- analysis based on case – control studies revealed that the intron 3 16 bp insertion allele was significantly associated with an increased cancer risk in overall analysis and particularly in the breast cancer subgroup.[29]

In another meta-analysis based on case-control studies, IVS3 16 bp was reported to be an important genetic marker contributing to susceptibility to breast cancer.[30]

Hrstka *et al.* suggested that PIN3 Ins 16 bp and PIN6 G13494A polymorphisms might be useful predictive biomarkers in human breast cancer.[27] Two studies demonstrated that PIN3 Ins 16 bp were not associated with an increased risk of breast cancer.[22,25]

In summary, the most probable mechanism of carcinogenesis may involve a combination of genetic alterations, immune system dysfunctions, and viral infections. Based on our findings, it seems that recessive alleles in three hot spots of *TP53* gene can solely increase the risk of the development of breast cancer. However, additional large studies are required to validate this association in different populations. It seems that by extensive assessment of the role of *TP53* gene polymorphism in Iranian women with breast cancer, preventive and treatment strategies can be employed in the future to increase their life expectancy and improve their quality of life.

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Conflict of Interest

The authors declare that they have no conflict of interest in this work.

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