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Molecular Epidemiology of Breast Cancer among Iranian-Azeri Population based on P53 Research

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Abstract

Background: This study was done in order to enhance our understanding about molecular and epidemiological features of breast cancer among the Azeri population with special emphasis on the detection of *TP53* mutations. We also analyzed the role of the *P53*codon72 polymorphism (rs1042522) and its role in susceptibility to breast cancer.

Methods: Tumor and control samples were collected from 248 patients and 189 controls. *TP53* mutations in exons4-9 and adjacent intronic regions were detected by direct sequencing in 130 of these tumor samples. Allele-specific PCR amplification (ARMS-PCR) was used to detect polymorphisms at *P53*codon72 in 248 patients and 189 controls. Data were analyzed using χ^2 test or Fisher's exact and a p value of <0.05 was considered significant.

Results: We identified alterations in 17.69% of the exonic and intronic regions within the *TP53*. We detected 23 mutant and 107 non-mutant samples. These mutations comprised 21 single-base substitutions (15-transitions and 6-transversions), one deletion and one complex. Exon6 was identified as a highly mutable region, with ten out of all 23 (43.47%) observed mutations. We did not observe a significant association between polymorphism and mutation status ($p>0.05$). Also, the results did not show a significant correlation between *P53* mutational status and clinicopathological features. Distribution differences in the *P53*codon72 polymorphism between the cases and controls were not statistically significant ($p>0.05$).

Conclusion: It might be concluded that *P53* mutational status and codon72 polymorphism could not be considered as biomarker for breast cancer risk and its clinical features in the studied population. However, further investigations are needed to support these findings.

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Introduction

Among women, breast cancer is the most prevalent cancer both in the developed and developing countries. Public health schemes in the developed world have led to the stabilization or even a decrease in the incidence rates of breast cancer, whilst in the most developing countries, the incidence of this disease is certainly rising (1, 2). Breast cancer killed 425,000 women in the world in 2010, of whom, 213,700 were from developing countries and 68,000 of them (31.82%) were aged 15–49 years (3). Worldwide, approximately 4.4 million women were living with breast cancer in 2009 and it is estimated that 1.7 million will be diagnosed in 2020, with most of these incidences happening in the developing world, especially in Asia. Although traditionally in Asia the rates have always been low, in the future the rates are going to increase significantly (3, 4). This escalation in incidence may be due to the changes in reproductive factors, and westernization of lifestyle. Compared with developed countries, the cause specific mortality is remarkably higher in developing countries (2). Breast cancer is a complex, multifactorial disease with critical interaction between genetic and environmental factors (5, 6). Currently most of our knowledge on breast cancer has been derived from studies carried out in Western populations. However, the socioeconomic profiles, genetic backgrounds, migration history, lifestyles, and cultures are considerably different in Asia from those in the United States and Europe. Therefore, it is crucial that we improve our understanding of breast cancer among Asian women, both for the subject of genetic susceptibilities and environmental risk factors. As mentioned above, breast cancer has remained a major public health problem in most developing countries in Asia. Most of the molecular aspects of this problem are unidentified, and not a great deal is known about its epidemiology and clinicopathological features. It is not yet clear what proportion of all cases of breast cancer can be attributed to familial reasons, or how much of the variation between Asian countries is attributable to genetic factors. From genetic factors, *BRCA1* and *BRCA2* are two major breast cancer susceptibility genes. These genes are normally expressed in the cells of breast and other tissues to help repair damaged DNA. Harmful mutations in these tumor suppressor genes predispose individuals to hereditary breast and ovarian cancers. Aside from these two genes, mutations in *TP53* greatly increase the risk of developing breast cancer. Since the discovery of this gene in 1979, as a tumor antigen, until 2016 that it has been established as an important tumor suppressor gene, over seventy thousand papers and research reports including this protein name have been published in PubMed and it has been led to creation of one of the richest databanks in molecular biology by the International Agency for Research on Cancer (IARC,http:// P53.iarc.fr). Based upon this accumulated knowledge on *P53*, new opportunities have emerged in translational research for the improvement of cancer prevention and therapy (7-10). P53 is now increasingly evolving as a multidimensional transcription factor that can occasionally exert contrasting influences on biological processes. Therefore, the renowned “guardian of the genome” is changing into the guardian of homeostasis (11). Undoubtedly, *P*53, in addition to its traditional role in cell cycle arrest, senescence, and apoptosis as a new player in various aspects of differentiation, development and cell migration as well as its ability to regulate metabolism and fecundity (8), can span basic molecular biology and molecular epidemiology. This branch of medical science has the power

to create the data to open the black box of cancer incidence in different populations. The increasing incidence of breast cancer in the Azeri population, the absence of genetic data, the absence of reliable and accurate epidemiological studies among Iranian-Azeri women similar to most other Asian populations and the complex nature of breast cancer have made this problem tantamount to a Gordian knot. In order to untie this knot, we decided to design a molecular epidemiological study, based on research on *P53*, among Azeri population. This was due to several reasons: firstly, *P53* is the most studied gene in cancer and there is a lot of data from different ethnicities and populations needed for comparative analysis. Secondly, the frequencies and patterns of somatic *P53* mutations may reflect the nature of specific mutagens which affect the Azeri population and may contribute to the aforementioned racial disparity in breast cancer occurrence and survival. Finally, *TP53* is highly polymorphic in coding and noncoding regions and some of these polymorphisms (such as codon72Arg/Pro) have been shown to increase cancer susceptibility and alter cancer phenotypes in *TP53* mutation carriers (9, 10, 12, 13). This study aimed to reveal the mutation spectrum of the *TP53* among Iranian-Azeri population and introduced several unreported mutations in this tumor suppressor gene. We also followed our research with a case-control study in order to identify the association of the *P53*codon72Arg/Pro polymorphism with breast cancer in the studied population.

Materials & methods

Epidemiology

In 2007, we started our studies about breast cancer among Iranian-Azeri population by establishing a research group (Tabriz Breast Cancer Research Group or TBCRG) including the members of molecular biologists, oncologists, surgeons, and epidemiologists from University of Tabriz and Tabriz University of Medical Sciences. In order to access any medical records of each hospital used in this study, we obtained the required permissions based on the privacy rules and ethics of each hospital and the general rules related to the Ministry of Health and Medical Education. Our projects on *P53* and its gene family members (*P63* and *P73*) and breast cancer patients were approved by the 135th Ethics Committee of Tabriz University of Medical Sciences research center (www.tbzmed.ac.ir/Research) with code number: 5.4.3259/13.3.92 (2013). Also, all participants filled a questionnaire, and signed informed consent. All documents were archived in our research center. Tabriz is located in northwest of Iran according to the official map of Iran. To date, we have reviewed more than 2500 breast cancer pathological records collected from five main government and private hospitals of Tabriz for our study concerning the primary epidemiological and clinical information.

Sample collection and clinical data

A total of 248 breast cancer patients, regardless of their family history, were selected for this study. Two hundred thirty six patients had pathologically confirmed invasive ductal carcinoma (IDC), 10 of 248 patients had invasive lobular carcinoma (ILC) and 2 patients represented ductal carcinoma in situ (DCIS). The collected tissues were carried to the laboratory in liquid nitrogen and stored at -80˚C until use. In the selection of a control group, 189 women with no previous history of cancer-related illness were collected. All control subjects were selected from the Iranian Azeri

population, with full consent obtained. We tried to select the cases and controls in the same age range to minimize demographic differences. All participants were women. The patients with benign breast diseases were excluded from the study.

Mutational analysis

DNA extraction, PCR and sequencing

Genomic DNA was isolated from blood and then stored according to standard procedures, using SDS/proteinase-K and based on DNA extraction method as described previously (14), and was subsequently frozen at -20˚C until further analyses. Mutational analysis was carried out using the four primer sets (Table1) for amplification of *P53* exons4-9. Genomic DNA (0.1-0.5µg) was amplified separately using 0.4µM from each set of primers in a 25µl PCR reaction, containing 1X PCR buffer, 0.2mM dNTPs, 1.5mM $MgCl_2$ and 1U Taq DNA polymerase. PCR reactions were carried out by a thermal cycler (Sensoquest, GmbH, Germany) at 35 cycles consisting of the following steps: denaturation at 95˚C for 5 min, annealing at 59˚C for fragment B and 60˚C for others for 30s and extension at 72˚C for 30s in each cycle and also a final extension for 10 min at 72˚C. The results were visualized using a 2% agarose gel and ethidium bromide staining. DNA sequencing was performed for all fragments, including exons4, 9, 5-6 and also exons7-8, as well as adjacent intronic regions by an automated sequencer, ABI 3730 XL [Fazabiotech Co., Tehran, Iran (http://www.fazabiotech.com)] in 130 patients.

Table 1. Primer sequences, product size, annealing temperatures for the different PCR reactions

Primer name Mutation	5'→3'	PCR product(bp)	Annealing Temperature(°C)
E4F E4R	TCCCCCTTGCCGTCCCAA* CGTGCAAGTCACAGACTT	279	59
E5F E6R	TTATCTGTTCACTTGTGCCC* TTAACCCCTCCTCCCAGAGA	472	60
Int6F E8R	GCCCTCCCCTGCTTGCC* TCCACCGCTTCTTGTCCTGC	682	60
E9F E9R Polymorphism	GGAGACCAAGGGTGCAGTTAT* GCCCCAATTGCAGGTAAAAC	233	60
ArgF ArgR	TCCCCCTTGCCGTCCCAA CTGGTGCAGGGGCCACGC	142	61
ProF ProR	GCCAGAGGCTGCTCCCCC CGTGCAAGTCACAGACTT	178	62
BetaF BetaR	CAATGTATCATGCCTCTTTGCACC GAGTCAAGGCTGAGAGATGCAGGA	861	60-62

*Oligonucleotide primers used for sequence analysis

P53 polymorphism analysis

Genotyping of *TP53*codon72 polymorphism was carried out with an allele specific PCR amplification procedure for all samples. Two pairs of primers with different 3´terminal bases were used to amplify *P53* sequences separately: ArgF and ArgR for arginine allele (which yield a 142bp product) and ProF and ProR for proline allele (which yield a 178bp product). Detection of the two codon72 alleles of *P53* was separately carried out from each sample with primers as described by Storey et al. (15). The control primers used were primers BetaF and BetaR (which yield an 861bp product, table1). The PCR reaction was performed in separate tubes for Arg and Pro alleles in a total volume of 25µL. The PCR mixture contained 50mM KCl; 1.5mM $MgCl_2$; 10mM Tris-Cl, pH 8.3; 0.01% gelatin; 0.5U Taq DNA polymerase; 200pmol of each primer; and 100ng DNA. Amplification was performed with denaturation at 95°C for 5 minutes, followed by 30 cycles of 1 minute at 95°C, annealing for 30s (Table 1), 1.5 minutes at 72°C, and a final extension for 15 minutes at 72°C. The resulting PCR products of each sample were then mixed with loading dye and electrophoresed on 2% agarose gels and visualized under UV with ethidium bromide staining.

Statistical analysis

Statistical analysis was performed using SPSS software (v. 16; SPSS Inc., Chicago, IL, USA). The expected allele frequencies were computed by Hardy Weinberg Equilibrium (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). The Pearson's chi-square ($\chi 2$) and Fisher's exact were calculated for the evaluation of genotype and allele frequencies using javastat online statistics package software. The odds ratio (OR) and 95% confidence interval (CI) were calculated to measure the association of *P53* genotypes with breast cancer risk and *P53* mutation status. *P* values less than 0.05 were considered statistically significant.

Results

Epidemiology

In this group of Azeri women with breast cancer, the age at diagnosis ranged between 12 and 91 years. Approximately, 63.70 percent of the patients were diagnosed before the age of 50 years (median, 45±1.045 years). A total of 26.61 percent of patients were aged younger than 40 years at presentation. Only 0.8 percent of the patients presented with ductal carcinoma in situ (DCIS), whilst 55.85 percent of patients had lymph node positive metastasis disease. A great majority of the tumors exhibited invasive ductal histology (95.16%), whilst invasive lobular cancers made up 4.03%. The full data will be released with the surveillance and end results in the future.

Mutations

In this paper, we report the results of our ongoing study on *TP53* mutations in our laboratory. We describe partially important details of two previous papers (16, 17). In our study, we have reported *TP53* mutations in 130 breast tumor tissues (23 mutant cases and 107 non-mutant cases) and have revealed a status of mutations and polymorphisms in exons4-9 and adjacent intronic regions. Sequencing of exon4 revealed multiple mutations in a case (Fig.1a). Exon9 was normal according to sequencing in all samples. The results of direct sequencing are summarized in Table 2, whilst representative results are shown in Fig.1. Exon6 was proved as a highly mutable region in this study, in which we have detected 10 out of the 23 observed mutations in codons193 (1case), 195 (two cases), 198 (1case) and two different mutations of codon213 in two cases (CGA→CTA (missense) (Fig.1b) and

CGA→CGG (synonymous)), 214 (2cases) and 220 (2cases) of this exon. Codon248, a common mutational hotspot located in exon7, was seen twice in the studied patients, leading to an amino acid change Arg>Glu. A case showed a point mutation in codon257 of exon7. The G to T base change at the first nucleotide of intron7 produced a splicing mutation in one of the tumor samples. Seven point mutations were detected in exons5 and 8, including codons160 (1case), 163 (1case) and 143 (1case) in exon5 and as well as codons272 (1case), 278 (1case) and 273 (as a hotspot residue in 2cases) in exon8 (Fig.1c). Also, we detected a 3bp deletion in exon8 in a 46-year-old patient with invasive lobular carcinoma (Fig.1d). In this study, the frequency of mutations was 17.69%. These mutations comprised 21 single-base substitutions (15 transitions and 6 transversions), one deletion and one complex. Twenty of the substitutions were exonic including 17 missense mutations, two were nonsense (Fig.1e) and one was synonymous mutation. Whilst one of the substitutions was intronic, in which IVS7-1G>T has been previously reported by Takahashi et al (18) as a splicing mutation. No significant associations between age, lymph-node metastasis, side involved, tumor size, stage, pathology and *P53* mutational status were observed (Table3).

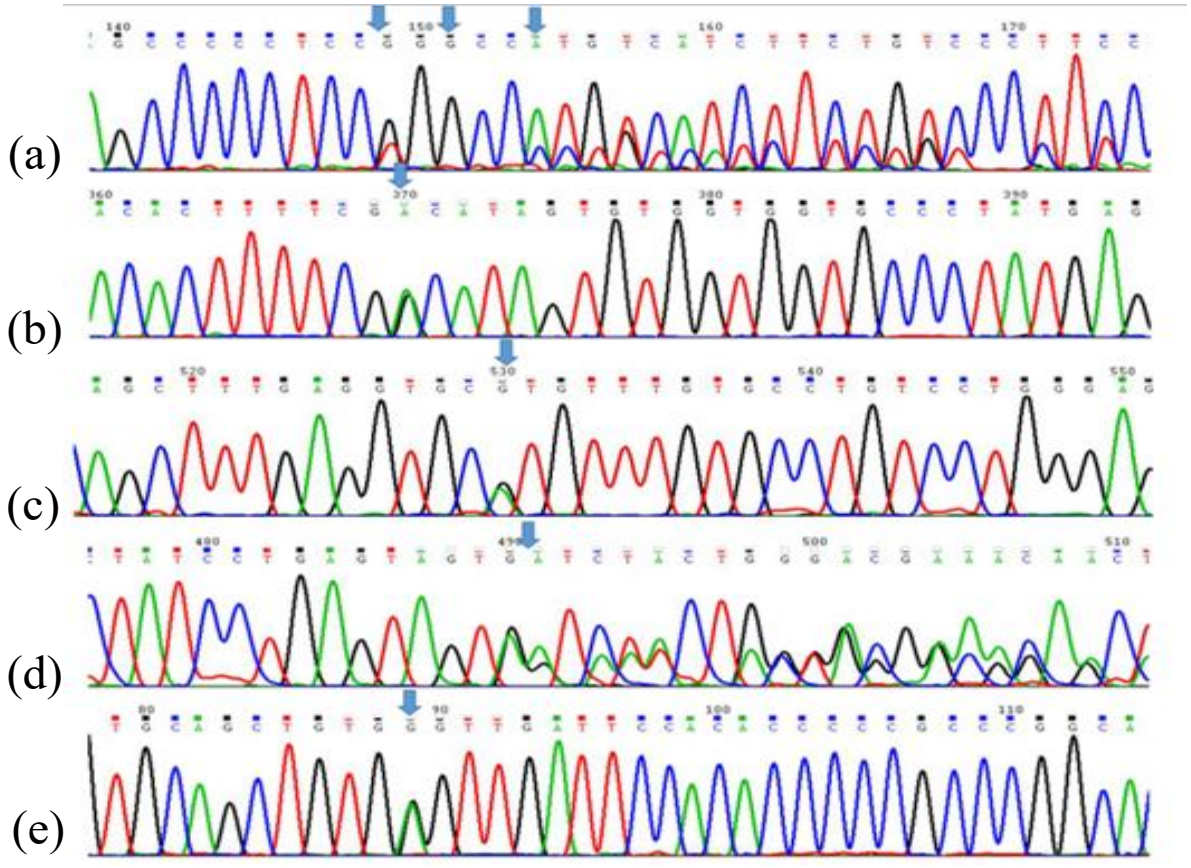

Figure 1. Sequencing results, a: multiple mutations in sample 20, b: a point mutation (missense) in codon213 of exon6 (sample449), c: a point mutation (missense) in codon273 of exon8 (sample405), d: a 3bp deletion in exon8 (sample128), e: a point mutation (nonsense) in codon146 of exon5 (sample344)

Table2. Mutational spectra of *P53* sequence in Iranian-Azeri patients

sample	codon	age	stage	pathology	Exon/ Intron	Nucleotide change	Aminoacid change	Transition/ Transvertion	Codon72
20	91-93	38	II	IDC	Ex4	multiple mutations	multiple	-	AA
99	160	53	III	IDC	Ex5	ATG→AAG	Met→Lys	Tv	AA
100	163	40	II	IDC	Ex5	TAC→TGC	Tyr→Cys	Ts	AA
344	146	42	III	IDC	Ex5	TGG→TGA	Trp→Stop	Ts	PP
40	193	35	III	IDC	Ex6	CAT→AAT	His→Asn	Tv	AP
76	195	61	II	IDC	Ex6	ATC→TTC	Ile→Phe	Tv	AA
125	195	39	I	IDC	Ex6	ATC→ACC	Ile→Tyr	Ts	AP
42	198	45	II	IDC	Ex6	GAA→TAA	Glu→Stop	Tv	PP
120	213	30	II	IDC	Ex6	CGA→CTA	Arg→Leu	Tv	PP
110	214	49	III	IDC	Ex6	CAT→CGT	His→Arg	Ts	AP
39	214	64	III	IDC	Ex6	CAT→CGT	His→Arg	Ts	AA
70	220	49	I	IDC	Ex6	TAT→TGT	Tyr→Cys	Ts	AA
109	220	35	III	IDC	Ex6	TAT→TGT	Tyr→Cys	Ts	AA
449	213	40	III	IDC	Ex6	CGA→CGG	Arg→Arg	Ts	AP
142	248	37	III	IDC	Ex7	CGG→CAG	Arg→Gln	Ts	AA
58	248	55	II	IDC	Ex7	CGG→CAG	Arg→Gln	Ts	AA
173	257	51	III	IDC	Ex7	CTG→CCG	Leu→Pro	Ts	AP
170	Intronic	46	III	IDC	Int7	G→T	-	Tv	PP
166	272	34	III	IDC	Ex8	GTG→ATG	Val→Met	Ts	AA
128	262	46	II	ILC	Ex8	(GGT)3bp Deletion	Gly→-	Deletion	AP
111	278	49	I	IDC	Ex8	CCT→TCT	Pro→Ser	Ts	AA
338	273	36	III	IDC	Ex8	CGT→CAT	Arg→His	Ts	AP
405	273	37	III	IDC	Ex8	CGT→CAT	Arg→His	Ts	AP

AA:Arg/Arg,AP:Arg/Pro,PP:Pro/Pro,EX:Exon,Int:Intron

Table 3. Relationship between P53 mutational status (wild-type/mutant) and clinicopathologic parameters

clinicopathologic parameters	*P53* mutational status			
	Wild-type (107 patients)	Mutant (23 patients)	Total	*p*-value
Tumor-size				
≤3.5cm	40(88.9%)	5(11.1%)	45	0.13
>3.5cm	49(77.8%)	14(22.2%)	63	
Age (years)				
≤43	52(81.2%)	12(18.8%)	64	0.76
>43	50(83.3%)	10(16.7%)	60	
Tumor-stage				
I/II	37(82.2%)	8(17.8%)	45	0.85
III	56(83.6%)	11(16.4%)	67	
Lymph-node metastasis				
Negative	24(82.8%)	5(17.2%)	29	0.93
Positive	64(82.1%)	14(17.9%)	78	
Side-involved				
Left	49(81.7%)	11(18.3%)	60	0.8
Right	52(82.5%)	11(17.5%)	63	
Both	1(100%)	0	1	
Pathology				
IDC	100(82%)	22(18%)	122	0.3
ILC	1(50%)	1(50%)	2	
DCIS	6(100%)	0	6	

Polymorphisms

In general, 248 Azeri breast cancer patients and 189 controls were analyzed for genotyping of *TP53*codon72Arg/Pro polymorphism. The patient's age range at diagnosis of their first episode of breast cancer was 22-82 years [Mean age±standard deviation 47.26±1.044 (years)], with that of controls 17-76 (48.36±1.30). The distribution of codon72 genotype frequencies among the patients was not in agreement with that expected under HWE, while among controls it was in tandem HWE. The distribution of the Arg72Pro polymorphism in cases and controls is presented in Table 4. In the control group, the genotype distribution of *P53* polymorphism showed 34.92%, 46.56% and 18.52% for the Arg/Arg, Arg/Pro, Pro/Pro Genotypes, respectively. In the cancer group, the distribution was 43.14%, 39.52% and 17.34% for the Arg/Arg, Arg/Pro and Pro/Pro genotypes, respectively. Distribution differences which were obtained from javastat online statistics package software in the *P53*codon72 polymorphism between the cases and controls were not statistically significant ($p>0.05$, Table4). The

relationship among *P53* mutations and *P53*codon72 genotypes is shown in table 5. Among the breast cancer cases which are revealed in terms of *P53* mutation status (130 patients), 62 were homozygous for the Arg72 allele, of which, 11 (17.74%) also had a *TP53* mutation in their tumors. In contrast, of the 28 cases that were homozygous for the Pro72 allele, only 4 cases (14.28%) had a *TP53* mutation. Also, of the 40 heterozygotes cases, (Arg/Pro) 8 (20%) carried a mutation in the *TP53*. Cases with the Pro/Pro genotype showed a mutation at codons 198, 213 of exon6 and the first nucleotide of intron 7. In addition to the codon72 polymorphism, the results of direct sequencing revealed other polymorphisms: 13399A>G in exon6 and 14181C>T, 14201T>G in intron7. The frequency of polymorphism 13399A>G was 7% and the frequency of two other polymorphisms, 14181C>T and 14201T>G, were 14%.

Table 4. Genotypes and allelic frequency distributions of P53codon72Arg/Pro in patients and controls

Polymorphism	Breast cancer N (%)	Control N (%)	OR[a] (95%CI[b])	*p*-value
Codon72 Arg/Pro				
Arg/Arg	107(43.14%)	66(34.92%)	*Ref*	_
Arg/Pro	98(39.52%)	88(46.56%)	0.687(0.441-1.069)	0.08
Pro/Pro	43(17.34%)	35(18.52%)	0.758(0.425-1.350)	0.315
Arg/Pro+Pro/Pro	141(56.85%)	123(49.2%)	0.707(0.469-1.065)	0.082
Arg/Arg+Arg/Pro	205(82.66%)	154(81.48%)	1.084(0.643-1.825)	0.750
Arg	312(62.90%)	220(58.20%)	*Ref*	_
Pro	184(37.1%)	158(41.8%)	0.821(0.619-1.090)	0.158

[a]odds-ratio; [b]confidence-interval

Table 5. TP53 mutational status (wild-type/mutant) in relation to P53codon72Arg/Pro

***TP53* status (wild-type/mutant)**		***P53*codon72Arg/Pro**			
		Arg/Arg	**Arg/Pro**	**Pro/Pro**	**Total**
Wild-type					
	Count	51	32	24	107
	within wild-types (%)	47.7	29.9	22.4	100
	Total (%)	39.2	24.6	18.5	82.3
Mutant					
	Count	11	8	4	23
	within mutants (%)	47.8	34.8	17.4	100
	Total (%)	8.5	6.2	3.1	17.7
Total					
	Count	62	40	28	130
	Total (%)	47.7	30.8	21.5	100
***p*-value**			0.83		
OR[a](95%CI[b])			0.831(0.966-0.895)		

[a]odds-ratio; [b]confidence-interval

Discussion

In recent years, researchers have encountered several important questions surrounding breast cancer among Asian populations such as "How similar are the characteristics of breast cancer in Asian, compared to the western countries?" (19, 20). To answer such a question, it is first necessary to augment our understanding about multi-aspects of breast cancer in the most populous global continent, accounting for over 60% of the world population. Despite many of the problems such as sociocultural barriers, missed data due to the absence of any treatment and other limitations, some figures and facts were revealed about breast cancer in Asian women by researchers. Compared with western countries, Asian countries have a lower incidence, but significantly higher mortality (2-4). Patients are about one decade younger and the proportion of young patients (<35 years) varies from about 10% in developed to up to 25% in developing Asian countries. In addition, early detection is rare and the cancer is detected at advanced stages (2, 3, 21, 22). These facts are illustrated in our epidemiological results. 22.39% of these patients were aged 40 or younger at presentation. The most important first steps are the attainment of accurate epidemiological data from more heterogeneous communities in the Asian population and the careful monitoring of treatment and survival outcomes among regions and ethnic groups within individual countries. In the next stage, it is necessary to determine the differences in genetic background and environmental risk factors; this helps us determine whether our current knowledge of the basic frameworks and treatment guidelines used in developed countries are applicable in these very different environments and multi-ethnic populations (4). The results of the present study showed the pattern of *TP53* mutations in breast cancer tissues among Iranian-Azeri women, a large Iranian ethnic group living in the north west of Iran. We found 17.69% of alterations at both exonic and intronic regions, which is less than the frequency of *P53* mutation in breast carcinoma in the IARC database (23.56%). In comparison with the data reported in the IARC (23), our *TP53* mutation database points to some differences, including the observed excess of mutations in exon6, the distinct pattern of the *TP53* mutations and the differences in hotspot distribution, which may reflect geographical and ethnic disparity in this study. Despite introducing two unreported mutations, we could not detect mutation in the most frequent hotspot residues in breast cancer -R175H (according to the IARC *TP53* database). Also, we did not find any mutations in codons 245, 249 and 282 that were seen in IARC mutational spectrum (Fig.2). These differences and the observed excess of mutations in exon6, raise the possibility that there may be an influencing factor contributing to mutagenesis which is more prevalent in our population. Also, the marked heterogeneity of breast cancer disease and its risk factor correspond to different results. The high proportion of mutation in exon6 has been previously reported among Kashmir valley (India) by Eachkoti (24). In addition, the extraordinary diversity of mutational patterns among cohorts has been reported in many of the geographical and ethnically diverse populations (25-29). As expected, we also found a distinct pattern of *TP53* mutation spectrum in the studied population. Our findings revealed a hot spot mutation, -R273H in two samples (Fig.2). In the presence of -R273H mutation, the interaction with DNA was lost due to the replacement of the Arg residue 273 that directly contacts with DNA (30). We had also previously analyzed mutation status of exon1 of *P53* and reported a mutation in intron 1 in this region (10). In addition to acquired mutations, inherited mutations in breast cancer susceptibility genes such as *BRCA1*, *BRCA2* and other DNA repair genes significantly increase the person's risk of developing breast malignancy. Germ-line mutations in the *TP53* account for the Li-Fraumeni syndrome, a hereditary cancer predisposition syndrome characterized by a high malignancy penetrance and early

tumor onset (30). *P53*codon72 polymorphism, another genetic variation, in the human population is thus a matter of strong concentration, as this polymorphism in *P53* may affect an individual's possibility of developing malignancy, including breast cancer (31). In the present study, our results did not illustrate a significant association between the codon72 polymorphism and the risk of developing breast cancer in the Azeri population. However, additional well-designed studies on large populations are required to validate this result. In agreement with our results, Kung et al recently demonstrated that R allele alterations have not been related to increased human malignancy incidence (32). Our previous research on thyroid cancer revealed the haplotype status of *P53*codon72Arg/Pro and *P53*intron3 16bp insertion (-16ins-Pro) but it was not associated with the decreased risk of thyroid carcinoma in separate manner (33). Evidence shows that the prevalence of cases with *P53* mutations within the Arg72-containing allele are higher than the Pro72-containing allele (34). Marin et al showed that in the squamous cell tumors with Arg/Pro genotype, the Arg-containing allele was preferentially mutated and retained (35). These findings were followed and confirmed in some other studies (34-36). Also, our results showed that 17.74% of patients carrying the Arg/Arg genotype had tumors harbouring *TP53* mutations, whereas only 14.28% of Pro/Pro genotype carriers had tumors harbouring such mutations, which were not statistically significant. Langerod et al. (2002) reported that the *TP53*codon72 polymorphism may affect the function of *TP53* mutations in breast carcinomas but not in colorectal carcinomas (34). Because conflicting data has been obtained from different cancerous tissues, such as colorectal and lung cancers (34, 38), additional comprehensive studies are needed to illustrate the association between the different codon72 variants and mutant behavior of *P53* in human carcinogenesis. Findings of this study may be regarded as potential information for a meta-analysis conduction in the future. In the battle against cancer, researchers have been through several eras of therapeutical advancement to improve our knowledge on cancer treatment. In the targeted therapy era, we learnt to use small molecules or drugs interfering with specific targeted molecules and genetic changes needed for carcinogenesis and tumor growth (39-41). Since the *P53* mutation is a frequent observation in all of the human cancers, new therapeutic approaches have been introduced to target *P53* alterations. Reactivation of mutant *P53* by a new class of small molecules opens new doors into cancer therapy with less severe side effects (30, 41-43). Phikan059, a carbazole derivative, is one of those that bind to a crevice created by a substitution of tyrosine to cysteine at residue 220 of *P53* and stabilizes and rescues this mutant protein. This cleft is created on the *P53* surface that is opposite to the DNA binding domain. According to the IARC database, the p.Y220C mutation occurs in ≈75,000 new cancer cases per annum (44-46). Our results showed that this residue is one of the hot spots among Azeri population. Currently the genomic era of cancer biology is developing rapidly and bringing about the promise of personalized medicine to improve patient's clinical outcome and quality of life. We are moving beyond the "one size fits all" approach into a new era in which treatments will be targeted according to a patient's individual genetic tumor profile (47). In other words, inefficient and experimental medicine is replaced with the data-driven medicine. Undoubtedly, this form of medicine requires information about a person's genes, proteins, and the environment. Although it provides the excellent opportunities for diagnosis and treatment of disease, access to this platform will remain inequitable, especially in the developing world (48). Unfortunately it means that the gap of accessible treatment facilities between developed and developing countries is widening. While many questions on all aspects of cancer in developing countries remain unanswered, current advances in personalized medicine have yielded new questions. According

to facts in developing countries, how can we be prepared for the operation of personalized cancer treatment? Overall, there is no doubt that the future of cancer treatment relies on a further understanding about personal medical and population genomics data. In this study, we attempted to enhance our understanding about the molecular and epidemiological features of breast cancer among the Azeri population. For the first time, we could report on the pattern of *P53* mutations and probable correlation of *P53*codon72 polymorphism with breast cancer susceptibility in this population. Two of these mutations were seen in the blood of related tumor tissue samples (data not shown). These patients are probably associated with Li-Fraumeni. These probands draw attention to their families. The proportion of familial breast cancer, in addition to the spectrum of *BRCA1* and *BRCA2* mutations are completely unknown in our population. In future publications, we will talk about the correlation of three polymorphisms in the *TP53* and their haplotype combinations with breast cancer. In addition, we are trying to identify those families that are prone to the *BRCA1*/*BRCA2* mutations. We hope that our team can establish the first molecular database in this geographically and genetically distinct region.

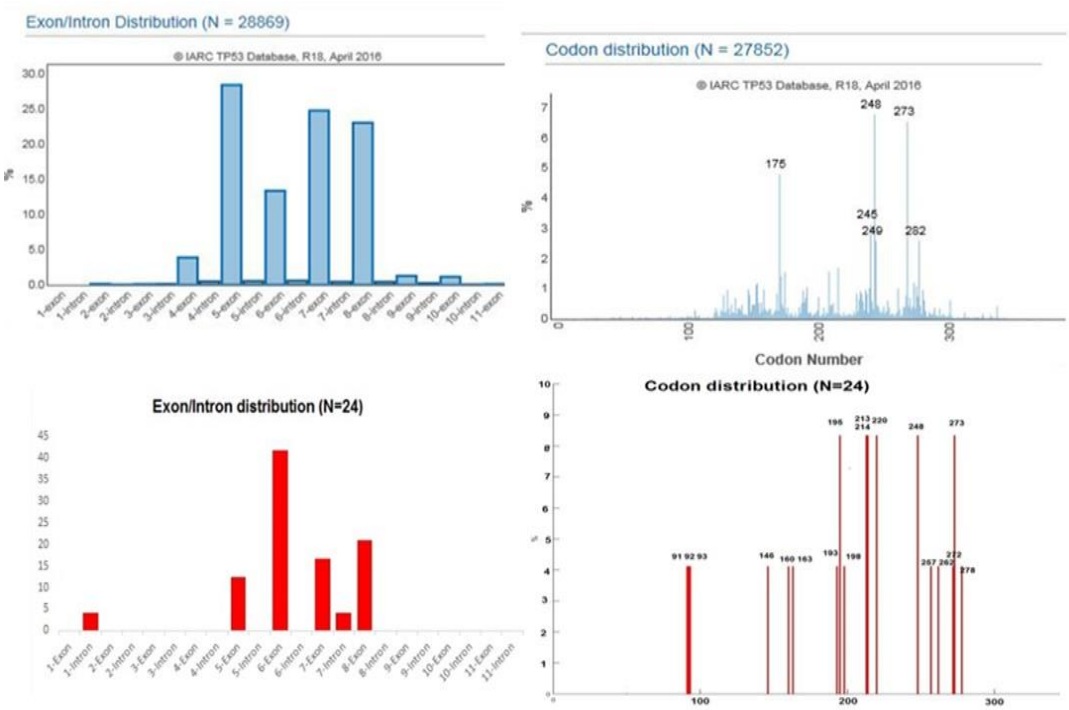

Figure 2. Comparison of *TP53* mutation database in IARC (a, b) and this study (c, d), (a, c): frequency distribution of exons/introns in which mutations have been located, (b, d): Codon frequency distribution of mutations, N: number of samples with mutations, Exons2,3,10,11 are not the focus of this study

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