Polymorphism of TP53 codon 72 showed no association with breast cancer in Iranian women

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Abstract

Breast cancer is the most common female malignancy worldwide. Despite the high incidence of sporadic cases, the rate of familial breast cancer is low. The tumor suppressor gene TP53 (alias p53), located on chromosome 17, has been involved in various malignancies. Mutations in codon 72 of TP53 have been studied in breast cancer and most solid tumors. For study of polymorphisms and allele frequency, 221 female patients with sporadic breast cancer and 205 healthy blood donors as control group were recruited. DNA from peripheral blood mononuclear cells was extracted and amplified using allele-specific polymerase chain reaction. Frequency of homozygotic arginine at codon 72 was 37.6% in patients and 36.6% in controls, for homozygotic proline it was 13.1 and 19.5%, and for heterozygotic Arg/Pro it was 49.3 and 43.9%, respectively. No significant difference was found between patients and controls regarding allele frequencies. Mutation in codon 72 of TP53 gene was not associated with breast cancer in Iranian patients.

1. Introduction

Breast cancer is a malignant proliferation of epithelial cells lining the ducts or lobules of the breast and is the most common malignancy in women [1], accounting for about one third of all cancers in women worldwide [2]. Despite the high incidence of sporadic cases, the rate of familial breast cancer is low [1]. The tumor suppressor gene TP53 (alias p53), located on chromosome band 17p13.1, is one of the most mutated genes; it is found in ~50% of human cancers, including cervical, prostate, gastric, breast, endometrial, hepatocellular, and ovarian carcinomas [3–12].

TP53 controls numerous downstream targets, resulting in gene transcription, DNA repair, cell cycle arrest, and apoptosis [13]. The gene comprises 11 exons. More than 90% of mutations in breast cancer occur in exons 5–9, which encode the DNA binding domain of p53 protein [14]. At least 10 polymorphisms are described in this gene; the most commonly studied one is at codon 72, which encodes either arginine (CGC) or proline (CCC) [15].

Hui et al. [10] investigated the association of TP53 codon 72 polymorphisms with susceptibility to hepatocellular carcinoma in a Chinese population and reported an increased risk of hepatocellular carcinoma in hepatitis B surface antigen-negative (HBs-negative) subjects with the proline allele. Zehbe et al. [16] reported a higher risk of cervical carcinoma in patients harboring the TP53 arginine 72 variant.

In India, the Arg/Pro genotype in patients with lung cancer was associated with early progression of the disease, compared with Arg/Arg carriers [17]. According to a meta-analysis conducted by Matakidou et al. [18], however, there was no relationship between TP53 codon 72 polymorphisms and risk of lung cancer. Dumont et al. [19] suggested that the TP53 proline 72 variant is associated with increased risk of cancer due to a decreased ability to induce apoptosis.

A strong association between the Arg/Arg genotype and breast cancer was reported in Turkish patients [20]. Similarly, Langerød et al. [21] reported a growth advantage of breast carcinoma cells with the arginine 72 allele in...
a Norwegian population, and Papadakis et al. [22] reported
the Arg/Arg genotype as a risk factor for breast cancer in
a Greek population. In contrast, Tommiska et al. [23] did
not observe any association between \( TP53 \) codon 72 vari-
ants and breast cancer risk.

The present study was designed to investigate \( TP53 \) gene
codon 72 polymorphism and allele frequency in patients
with sporadic breast cancer and healthy individuals in
southern Iran.

2. Materials and methods

2.1. Patients

A total of 221 female patients with sporadic breast
cancer (age range, 25–80; mean, 47 years) were included,
referred from the Surgery and Gynecology Departments of
Shiraz Medical School. Control cases were 205 healthy
blood donors with no signs and symptoms of autoimmune
disease or malignancy (age range, 23–72; mean, 45.3
years).

2.2. DNA preparation and polymerase
chain reaction method

Blood samples were collected and genomic DNA was
extracted by means of the salting method from blood lym-
phocytes. Amplification of genomic DNA was performed
by allele-specific polymerase chain reaction (PCR). Two
sets of primers (forward, \( F \); reverse, \( R \)) were used to
amplify the arginine and proline alleles, as follows:

\[
\begin{align*}
\text{Arg F:} & \quad \text{TCC CCC TTG CCG TCC CAA} \\
\text{Arg R:} & \quad \text{CTG GTG CAG GGG CCA CGC} \\
\text{Pro F:} & \quad \text{GCC AGA GGT TGC TCC CCC} \\
\text{Pro R:} & \quad \text{CGT GCA AGT CAC AGA CTT}
\end{align*}
\]

Each set of primers was added to a separate tube with
a total volume of 25 \( \mu \)L, containing 0.3 mmol/L dNTPs,
1.5 mmol/L \( \text{MgCl}_2 \), 2 \( \mu \text{Taq} \) polymerase, and 1× buffer
(20 mmol/L Tris-HCl, pH 8.4, and 50 mmol/L KCl). Am-
plification was performed at 35 cycles with a touch-down
program: denaturation at 94°C for 30 seconds, annealing
at 68–62°C for 10 cycles and 62–58°C for the remaining
25 cycles, with extension at 72°C for 30 seconds in each
cycle. The PCR product was 177 bp for the Pro allele and
141 bp for the Arg allele (Fig. 1).

2.3. Statistical analysis

Data regarding age, tumor type, invasion, metastasis,
menopause status, age of diagnosis, presence of estrogen
(ER) and progesterone (PR) receptors, and lymph node in-
volvement were recorded. Results were analyzed with
SPSS software (version 11.5 for Windows software); χ² test was used to compare the results.

3. Results

A total of 221 female patients with sporadic breast cancer were studied, with a mean age of 48.7 years. Of these patients, 169 (88.5%) had infiltrative ductal carcinoma and 20 (10.5%) had medullary carcinoma; 2 patients (1%) had other types of breast cancer. Of the 221 patients, 58.6% had lymph node involvement, 1.8% had distant metastasis, and 77% had pretumoral invasion (vascular, lymphatic, or preneural).

No genotype differed significantly (P = 0.2) between patients and controls (Table 1). The Arg/Arg genotype appeared in 37.6 and 36.6% of the patients and controls, respectively; the Pro/Pro genotype in 13.1 and 19.5%; and the Pro/Pro genotype in 37.6 and 36.6% of the patients and controls, respectively; the Pro/Pro genotype in 13.1 and 19.5%; and the Arg/Pro genotype in 49.3 and 43.9%.

Likewise, allele frequencies did not differ significantly (P = 0.9) between patients and controls (Table 2). The Arg allele frequency was 62.2 and 58.5% in patients and controls, respectively; the corresponding Pro allele frequency was 37.8 and 41.5%.

In terms of tumor—node—metastasis (TNM) clinical staging, 42 patients (18.2%) were in stage 0 or I, 152 patients (68.7%) were in stage II, and 29 patients (13.1%) were in stage III or IV. Ten (35.7%) patients with stage III or IV disease had Arg/Arg genotype, 1 (3.6%) had Pro/Pro genotype, and 17 (60.7%) had the Arg/Pro genotype (P = 0.308), indicating no statistically significant effect of allele on progression in clinical stage of the disease.

Of the patients with lymph node involvement, 44 patients (34.1%) had the Arg/Arg genotype, 16 (12.2%) had Pro/Pro, and 70 (53.7%) had the heterozygous genotype, revealing no statistically significant relationship between genotype and lymph node involvement (P = 0.15). The same was true considering genotype frequency and distant metastasis.

Neither estrogen receptor (ER) expression (P = 0.94) nor progesterone receptor (PR) expression (P = 0.19) expression was associated with presence of a specific genotype in patients. No correlation was found between histological type of tumor and genotype frequency (P = 0.53).

Patients were divided into two groups based on the age of diagnosis, those under 35 years as early and those over 35 years as late, with still no difference in the prevalence of genotypes between two groups. The same result was achieved considering premenopausal and postmenopausal cases (P = 0.9).

4. Discussion

Several studies have shown an association between polymorphisms at codon 72 and human malignancies. We investigated the allele and genotype frequencies of TP53 codon 72 in 221 female patients with sporadic breast cancer and 205 healthy individuals from southern Iran.

No significant difference in the frequency of genotypes and alleles was found between patients and controls. In addition, no significant association was detected between prevalence of alleles and genotypes and tumor type, clinical stage of tumor, lymph node involvement, metastasis, age of diagnosis, menopause status, and ER and PR expression.

Our results are in agreement with similar studies on bladder cancer [24], but are contrary to some other reports [25–28]. Prevalence of this polymorphism in cervical [29–32], lung [33,34], colon [35], bladder [36,37], and skin [38] carcinoma have previously been investigated.

There are few reports of studies assessing the TP53 codon 72 polymorphisms in breast cancer, with inconsistent results [39–41]. Some investigators reported a difference in the frequency of the Arg allele between breast cancer patients and controls [39,42], and others propose Pro allele as a risk factor for breast cancer [40,43]. Tommiska et al. [23] suggested that codon 72 polymorphism, particularly the Pro/Pro genotype, is an independent prognostic factor in patients with breast cancer and provided evidence that patients harboring this genotype will have a reduced survival.

A report from China indicated an association between HPV oncogenic strains and breast cancer, with evidence that human papilloma virus (HPV) E6 oncoprotein more readily targets the Arg allele than Pro allele for degradation of p53 protein [44]. In light of that finding, Makni et al. [45] reported a correlation between HPV associated cervical cancer and Arg homozygosity of TP53 codon 72. Contrary to these findings, our previous study [46] revealed no association between HPV oncogenic strains in patients with cervical cancer, a result which is explained by the low frequency of HPV oncogenic strains in our population [46].

In conclusion, the data of the present study show that TP53 gene codon 72 polymorphism is not associated with increased risk of breast cancer in southern Iranian patients.

Table 1

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Patients, no. (%)</th>
<th>Controls, no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg/Arg</td>
<td>83 (37.6)</td>
<td>75 (36.6)</td>
</tr>
<tr>
<td>Pro/Pro</td>
<td>29 (13.1)</td>
<td>40 (19.5)</td>
</tr>
<tr>
<td>Arg/Pro</td>
<td>109 (49.3)</td>
<td>90 (43.9)</td>
</tr>
<tr>
<td>Total</td>
<td>221</td>
<td>205</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Allele</th>
<th>Patients, no. (%)</th>
<th>Controls, no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg</td>
<td>275 (62.2)</td>
<td>240 (58.5)</td>
</tr>
<tr>
<td>Pro</td>
<td>167 (37.8)</td>
<td>170 (41.5)</td>
</tr>
<tr>
<td>Total</td>
<td>442</td>
<td>410</td>
</tr>
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This finding may be a reflection the low frequency of oncogenic strains of HPV in our population [46].

Acknowledgments

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References


