

Brief Report

Analysis of BRCA1 and BRCA2 Mutations in Southern Iranian Breast Cancer Patients

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The germline mutations of breast cancer susceptibility genes 1 (BRCA1) and breast cancer susceptibility genes 2 (BRCA2) have been associated with a significant increase in breast cancer risk and certain other cancers. Among the most known mutations in these tumor suppressor genes are 5382insC and 185delAG in BRCA1 and 6174delT in BRCA2.

The aim of the current study was to investigate the frequency of these BRCA1 and BRCA2 mutations in southern Iranian familial and sporadic cases with breast cancer.

Two hundred fifty women with sporadic breast cancer, 55 women with a familial history of breast cancer in their first degree-relatives and 200 healthy women formed the studied groups. DNA from peripheral blood mononuclear cells was extracted and analyzed by a multiplex polymerase chain reaction method.

The data of this investigation indicated that the aforementioned founder mutations were not detected in the groups studied.

Our results indicate that 5382insC and 185delAG mutations in BRCA1 and 6174delT in BRCA2 have much less frequency in Iranian breast cancer patients.

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Keywords: BRCA1 • BRCA2 • breast cancer • Iran • mutation

Introduction

Breast cancer susceptibility genes 1 (BRCA1) and breast cancer susceptibility genes 2 (BRCA2) encode key tumor suppressor proteins that prevent uncontrolled proliferation of cells by interacting with a number of important regulatory elements for DNA repair, transcription and cell cycle regulation. Germline mutations in these genes usually result in truncation or absence of the protein that confers up to a 90% lifetime risk of breast cancer in carrier females.^{1,2}

Approximately 5 – 10% of all breast cancer cases are familial diseases with earlier age at onset while others are sporadic. A varying degree of

breast cancer patients carry a germline mutation in the BRCA1 or BRCA2 gene depending on the ethnicity of the study population and familial versus sporadic definition.²

BRCA1 gene mutations are reported in ~45% of familial breast cancer patients and 2 – 30% of sporadic breast cancer patients. It has also been suggested that mutations in BRCA2 account for a comparable percentage of familial breast cancer cases.²

The number of germline mutations identified within BRCA1 and BRCA2 genes is growing; most of them are unique to each high risk family. However, BRCA1 and BRCA2 genes have a few dominant mutations, notably the 5382insC and the 185delAG in BRCA1 and the 6174delT in BRCA2. These three founder mutations are frequently observed in Ashkenazi Jewish breast cancer patients. The 185delAG was also observed in non-Ashkenazi Jews, including Iranian-born Jews,¹ and in non-Jewish individuals from several ethnic backgrounds³, and the 5382insC in Russian⁴ and Turkish⁵ populations.

We previously studied BRCA1 185delAG

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mutation in a small number of patients (80) with breast cancer from the Fars province in Iran. None of the patients had this mutation.⁶ However, the low prevalence of 5382insC or 185delAG in BRCA1 has been reported from a province of Iran and a neighboring country.^{5,7} In the present larger study (more than 300 patients), cases from five southern provinces of Iran were analyzed regarding to the 5382insC and 185delAG in BRCA1 and 6174delT in BRCA2.

Patients and Methods

Subjects

Two hundred fifty women with sporadic breast cancer (mean age: 45.1 ± 9.2 years), 55 women with a familial history of breast cancer in their first-degree relatives (mean age: 32.0 ± 7.3 years), and 200 unrelated healthy women with no signs and symptoms of any malignancy or familial history of cancer (mean age: 45.3 ± 8.4 years) formed the studied groups. All patients were recruited during 2005 – 2007 from the Surgery and Gynecology Departments of Namazee Hospital in Shiraz, Iran. Namazee Hospital is a referral hospital that admits patients from the southern provinces of Iran including Fars, Bushehr, Hormozgan, Kohgiluyeh Boyer Ahmad, and a part of Khuzestan. Two hundred healthy women were also included in this study to find the possible presence of this mutation in the general population. The native language of all participants was Persian.

All participating women were informed that their DNA samples would be analyzed for genotyping and the informed consents for the use of their DNA were signed. The protocol was approved by the Ethical Committee of the Shiraz University of Medical Sciences.

Multiplex Polymerase Chain Reaction

Peripheral blood samples were collected and genomic DNA was extracted by the salting-out method from blood mononuclear cells.

For detection of 5382insC and 185delAG in BRCA1 and 6174delT in BRCA2, a multiplex polymerase chain reaction (PCR) was performed with allele-specific oligonucleotide primers as described by Chan et al.⁸ In this method, three primers (one common, one specific for the mutant, and one specific for the wild-type allele) were designed for each mutation (Table 1). The mutant and wild-type primers differed by ~20 bp in size,

so the size of amplified mutant and wild-type segments differed by ~20 bp. For the 185delAG mutation, the mutant and wild-type PCR products were 354 and 335 bp, while those of 5382insC were 295 and 271 bp, and those of 6174delT were 171 and 151 bp (Table 1). In the absence of any mutant allele a minimum of three bands, and in the presence of all three mutations, a maximum of six bands were observed in a single sample of this multiplex PCR.

PCR was performed in a 25 μ L volume containing 1 \times PCR reaction buffer (20mM Tris-HCl, pH=8.3, 50mM KCl), 1.5mM MgCl₂ (Cinnagen, Tehran, Iran), 0.3mM dNTPs (Cinnagen, Iran), 2U Taq DNA polymerase (Cinnagen, Iran) and dimethyl sulfoxide as co-solvent. The concentrations of primers used were 2 μ M for P1 and P3, 0.4 μ M for P2, 0.12 μ M for P4, P5 and P6, 0.31 μ M for P7 and P9, and 0.24 μ M for P8. PCR reaction was performed with an initial denaturation for 5 min at 94°C, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 57°C for 30 sec, with extension at 72°C for 30 sec, and a final extension step of 5min at 72°C.

PCR products were separated by electrophoresis on 3% agarose gel (Invitrogen, UK) and stained with ethidium bromide.

Results

We used a multiplex allele-specific PCR for the simultaneous detection of 5382insC and 185delAG in the BRCA1 gene and 6174delT in the BRCA2 gene.

Data of this investigation indicated that the mutation was not detected either in patients (250 patients with sporadic breast cancer and 55 familial breast cancer patients) nor in 200 healthy individuals. Positive controls of known BRCA mutant genotypes were kindly provided by Prof. Mehdipour from Department of Medical Genetics, Tehran University of Medical Sciences, Tehran, Iran.

Discussion

The 5382insC and the 185delAG mutations in BRCA1 and the 6174delT in BRCA2 occur in the general Ashkenazi Jewish population with a carrier frequency of 1.09% for the 185delAG mutation, 0.13% for the 5382insC mutation, and 1.52% for

Table 1. Nucleotide sequences of the primers used for detection of three founder mutations

Primer	Primer sequence	Size of amplified fragment
BRCA1 185delAG		
Common forward (P1)	5' ggttggcagcaatatgtgaa	
Wild-type reverse (P2)	5' gctgacttaccagatgggactctc	335 bp
Mutant reverse (P3)	5' cccaaattaatacactcttctcgtgacttaccagatgggacagta	354 bp
BRCA1 5382insC		
Common reverse (P4)	5' gacgggaatccaaattacacag	
Wild-type forward (P5)	5' aaagcgagcaagagaatcgca	271 bp
Mutant forward (P6)	5' aatcgaagaaccaccaaagtcttagcgagcaagagaatcacc	295 bp
BRCA2 6174delT		
Common reverse (P7)	5' agctggtctgaatgttcgttact	
Wild-type forward (P8)	5' gtgggatttttagcacagctagt	151 bp
Mutant forward (P9)	5' cagtctcatctgcaaatacttcagggttttagcacagcatgg	171 bp

the 6174delT mutation. Of these, the 185delAG mutation in the BRCA1 gene has the highest prevalence and penetrance in breast cancer.² There are several studies reporting these founder mutations in other populations.^{1,3-5} Here, we investigated the potential role of the three mentioned mutations in the south of Iran by a multiplex PCR method.⁸ The total mean age of our patients was less than 45 years, as the likelihood of finding a mutation is highly age dependent.⁹

We observed that neither the patients (250 patients with sporadic breast cancer and 55 familial breast cancer patients) nor the 200 healthy individuals had any of the three mutations. Our result was consistent with prior studies on Iranian patients with breast cancer. Mehdipour et al. reported a low frequency of the 185delAG founder mutation in the BRCA1 gene in Iranian breast cancer patients but not 5382insC and 6174delT mutations.⁷ However, they did not identify whether the two sisters harboring this mutation were from a Jewish or non-Jewish population. Low frequency of the 185delAG founder mutation in BRCA1 gene has previously been reported in Iranian-born Jewish breast cancer patients.¹ In our study, neither the patients nor the healthy volunteers included Jewish individuals.

A large number of distinct mutations in the BRCA1 and BRCA2 genes have been reported worldwide, but little is known regarding the role of these two susceptibility genes on breast cancer in Iran. Ghaderi et al. reported a novel mutation in exon 15 at codon 1534 (G to A) in the BRCA1 gene.⁶ Pietschmann et al. also found several mutations in BRCA2 gene including a novel deletion c.4415_4418delAGAA in Iranian familial breast cancer patients but no mutation in the BRCA1 gene.¹⁰ It was suggested that the pattern of

mutations seen in the BRCA genes among Iranians might be different from other populations.⁷ A complete BRCA1 and BRCA2 gene sequence analysis might be required for identification of specific mutations in Iran, a country with an ethnically diverse population. Furthermore, it seems that penetrance or prevalence of BRCA1 mutations is lower in Iran.¹⁰ Thus, there might be other genes that contribute more significantly to familial breast carcinoma in this population than BRCA.

Results of our investigation and the data previously published from the northern Iran⁷ collectively indicate that the common and known BRCA1 and BRCA2 mutations are less frequent in Iranian breast cancer patients.

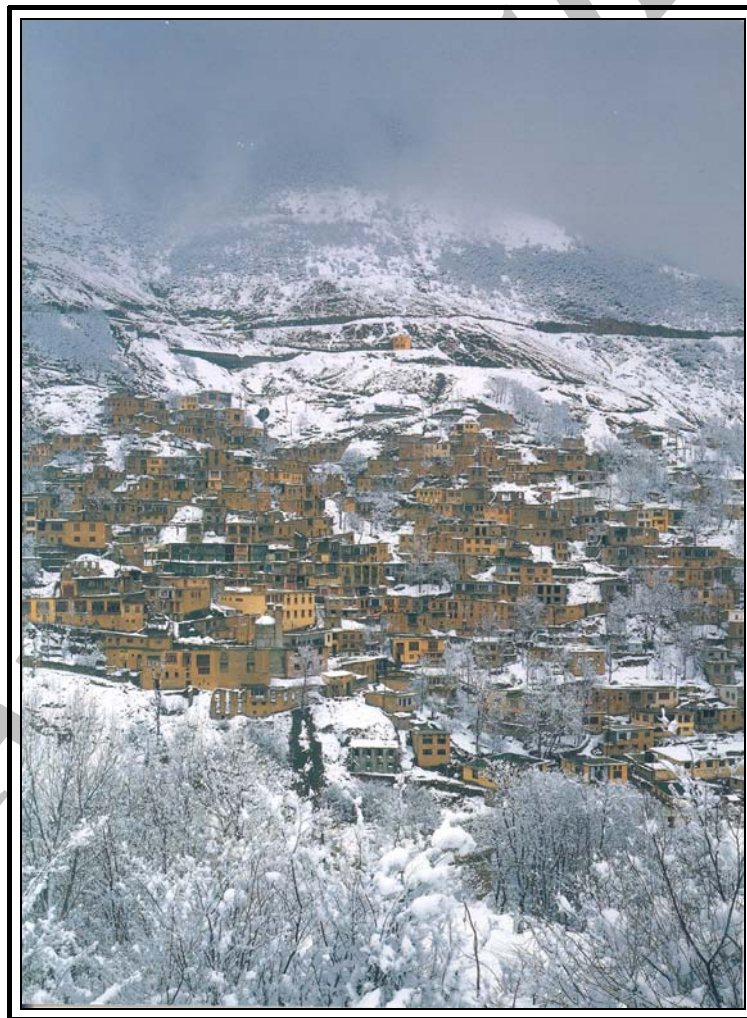
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Masuleh, Gilan Province, North of Iran. Photo by S. M. Aznaveh, Gooya House of Culture and Art