Research Paper

Low Frequency of 185delAG Founder Mutation of *BRCA1* Gene in Iranian Breast Cancer Patients

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AIM: **The mutations in two breast cancer susceptibility genes, *BRCA1* and *BRCA2*, are frequently associated with familial breast cancer. In this study, we aimed to investigate the probable founder mutations of *BRCA1* and *BRCA2* genes in Iranian breast cancer patients.**

METHODS: **The total 400 patients affected with primary breast cancer were included in this study. Mutation detection was carried out on the basis of a PCR-based amplification, and two founder mutations for *BRCA1* (185delAG and 5382insC) and one for *BRCA2* (6174delT) were screened and considered by pedigree analysis.**

RESULTS: **The positive family histories of breast cancer and other malignancies were recorded in 27.5% and 52% of patient pedigrees, respectively. The most frequent occurrence of breast cancer across four generations revealed to be 50% in the 1st degree in the 3rd generation, 68.8% in the 2nd degree in the 2nd generation, and 59.5% in the 3rd degree in the 3rd generation. Only 185delAG mutation in the *BRCA1* gene was found in 2/400 (0.5%) of investigated pedigrees. There were two sisters of the same family. To our interest both sisters carried 185delAG mutation in the *BRCA1* gene, which had a complete penetrance. However, the mutation was observed with two different organ targeting, at almost an early age of onset (proband: 45 yr, her sister: 30 yr).**

CONCLUSION: **Considering the importance of genetic counseling and recording, the adequate information for the pedigrees of cancer patients put forward the principle approaches in cancer clinics to facilitate early detection for preventing challenges.**

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Keywords:

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Introduction

Cancer genetics research would provide the complementary information to the genetics alteration, which finally could lead to create a database including the adequate data for the patients affected with breast cancer. The carriers of *BRCA1* and *BRCA2* gene mutations have an increased risk for developing both breast and ovarian cancers during their lifetime. Especially, the *BRCA1* defect predisposes to early onset of the hereditary breast and ovarian cancer. The numbers of mutations in *BRCA1* and *BRCA2* were estimated to be 718 and 458, respectively [1]. A large-scale mutation analysis of these two genes indicated that in many populations only 30–60% of familial breast cancer are attributable to *BRCA1* and *BRCA2* gene mutations [2].

Many examples of founder mutations of *BRCA1* and *BRCA2* genes have been reported from different populations such as the Iceland, Finnish, Dutch, and French families [3-5]. In the east European Ashkenazi population, approximately 40 and 29% of patients with breast and ovarian cancer, respectively, were diagnosed before the age of 40 yr [6-8]. Three predominant mutations, including 185delAG and 5382insC of *BRCA1* gene and 6174delT of *BRCA2* gene, dominantly appeared in the substantial proportion of high-risk Ashkenazi families [2,9]. These mutations are observed up to 2.5% of the general Jewish-Ashkenazi population [6]. The 185delAG and Tyr978X alterations were also detected in non-Ashkenazi Jews [9-11], and rarely in non-Jewish individuals [12,13]. In addition, the mutation of 8765delAG in *BRCA2* gene was reported from unrelated Yemenite as well as French-Canadian families, who have exhibited different *BRCA2* haplotypes [14,15].

The present study was planned to investigate the probable occurrence of 185delAG and 5382insC (*BRCA1*) and 6174delT (*BRCA2*) alterations in 400 Iranian breast cancer patients.

Materials and Methods

Patients

The total 400 primary breast cancer patients, who received operation at the Day Hospital from 1994 to 2003, were included in this study. The patients included 396 (99%) women and 4 (1%) men with the mean age of 48.8 ± 11.3 yr, ranging from 15 to 95 yr. The tumors were unilateral

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(388/400, 97%), including 208/388 (53.6%) left-sided tumors. Regarding the histopathological classifications, 332/400 (83%) of tumors were invasive ductal carcinoma, and the grade of tumors were 26/400 (6.5%), 130/400 (32.5%), and 244/400 (61%) for grades I, II and III, respectively. The mean tumor size was 2.97 ± 2.19 cm, ranging from 0.5 to 25 cm. The axillary lymph node metastasis was detected in 222/400 (55.5%) of the patients. The tumors exhibited positive staining for progesterone receptor and estrogen receptor in 228/400 (57%) and 222/400 (55.5%) of patients, respectively.

Procedures

The peripheral blood samples were collected from patients, and the genomic DNA was extracted according the standard protocol. Detection of gene mutations was carried out on the basis of a PCR-based amplification and gel electrophoresis described previously [16]. PCR amplification was carried out using the oligonucleotide primers listed in Table 1. In each PCR reaction, 25 ng of genomic DNA was added to 20 μl of reaction mixture consisting of 10 × PCR reaction buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 10 μg/ml gelatin), 3.25 mM $MgCl_2$, 0.2 mM dNTPs, and 3 U Taq DNA polymerase (Roche, Penzberg, Germany). The concentrations of primers used were 2.0 μM for P1 and P3, 0.4 μM for P2, 0.12 μM for P4, P5, and P6, 0.31 μM for P7 and P9, and 0.24 μM for P8. Each PCR reaction consisted of an initial 4 min at 94°C, followed by 35 cycles of 94°C for 30 sec, 57°C for 30 sec, and 72°C for 45 sec, and a final extension step of 10 min at 72°C. Then, 10 μl of PCR product was mixed with 5 μl of loading dye and subjected to gel electrophoresis in 10% non-denaturing polyacrylamide (29:1) gel, and finally were visualized with ethidium bromide staining (Figure 1).

The pedigrees were drawn according to the information provided by the probands and/or their first-degree relatives through multiple interviews.

Results

Totally 400 patients affected with primary breast cancer have been investigated for founder mutations of *BRCA1* (185delAG and 5382insC) and *BRCA2* (6174delT) genes. The family histories positive for breast cancer and other malignancies were recorded in 110/400 (27.5%) and 208/400 (52%) of patients' pedigrees, respectively. The most frequent occurrence of breast cancer across four generations revealed to be 20/40 (50%) in the 1st degree in the 3rd generation, 22/32 (68.8%) in the 2nd degree in the 2nd generation, and 22/37 (59.5%) in the 3rd degree in the 3rd generation (Table 2).

The pedigree of family A (Figure 2A) included the proband

Table 1: Oligonucleotide sequences of primers used for detection of three founder mutations of *BRCA1* and *BRCA2* genes

	Oligonucleotide primer sequence[a]	Size of amplified segment
BRCA1 185delAG		
Common forward (P1)	5'-ggttggcagcaatatgtgaa-3'	
Wild-type reverse (P2)	5'-gctgacttaccagatgggactctc-3'	335 bp
Mutant reverse (P3)	5'-cccaaattaatacactcttgtcgtgacttaccagatgggacagta-3'	354 bp
BRCA1 5382insC		
Common reverse (P4)	5'-gacgggaatccaaattacacag-3'	
Wild-type forward (P5)	5'-aaagcgagcaagagaatcgca-3'	271 bp
Mutant forward (P6)	5'-aatcgaagaaaccaccaaagtccttagcgagcaagagaatcacc-3'	295 bp
BRCA2 6174delT		
Common reverse (P7)	5'-agctggtctgaatgttcgttact-3'	
Wild-type forward (P8)	5'-gtgggatttttagcacagctagt-3'	151 bp
Mutant forward (P9)	5'-cagtctcatctgcaaatacttcagggatttttagcacagcatgg-3'	171 bp

[a]The sequences of primers were previously reported by Chan *et al.* [16].

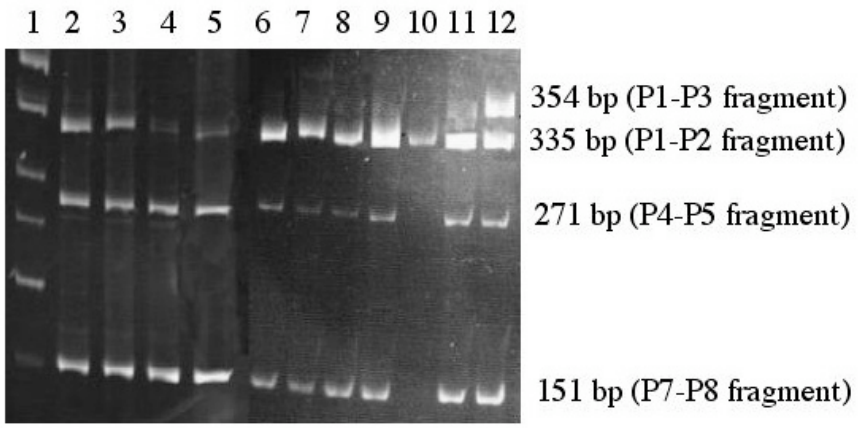

Figure 1: Gel electrophoresis analysis of the PCR products of genomic DNA isolated from primary breast cancer patients. The sequences of the oligonucleotide primers P1~P9 used for PCR reactions are listed in Table 1. The presence of the band with a size of 354 bp indicates the *BRCA1* gene heterozygosity resulting from 185delAG mutation. Lane 1, DNA size marker. Lanes 2-12, examples of the PCR results from 11 breast cancer patients.

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Table 2: Distribution of breast cancer among relatives across different generations and relative degrees

	1st Generation	2nd Generation	3rd Generation	4th Generation	Total
1st Degree	-	17	**20**	3	40
2nd Degree	3	**22**	3	4	32
3rd Degree	7	7	**22**	1	37
4th Degree	0	0	1	0	1
Total	10	46	46	46	110

*Data are presented as numbers of involved relatives. The bold outcomes are considered as most frequent involvement.

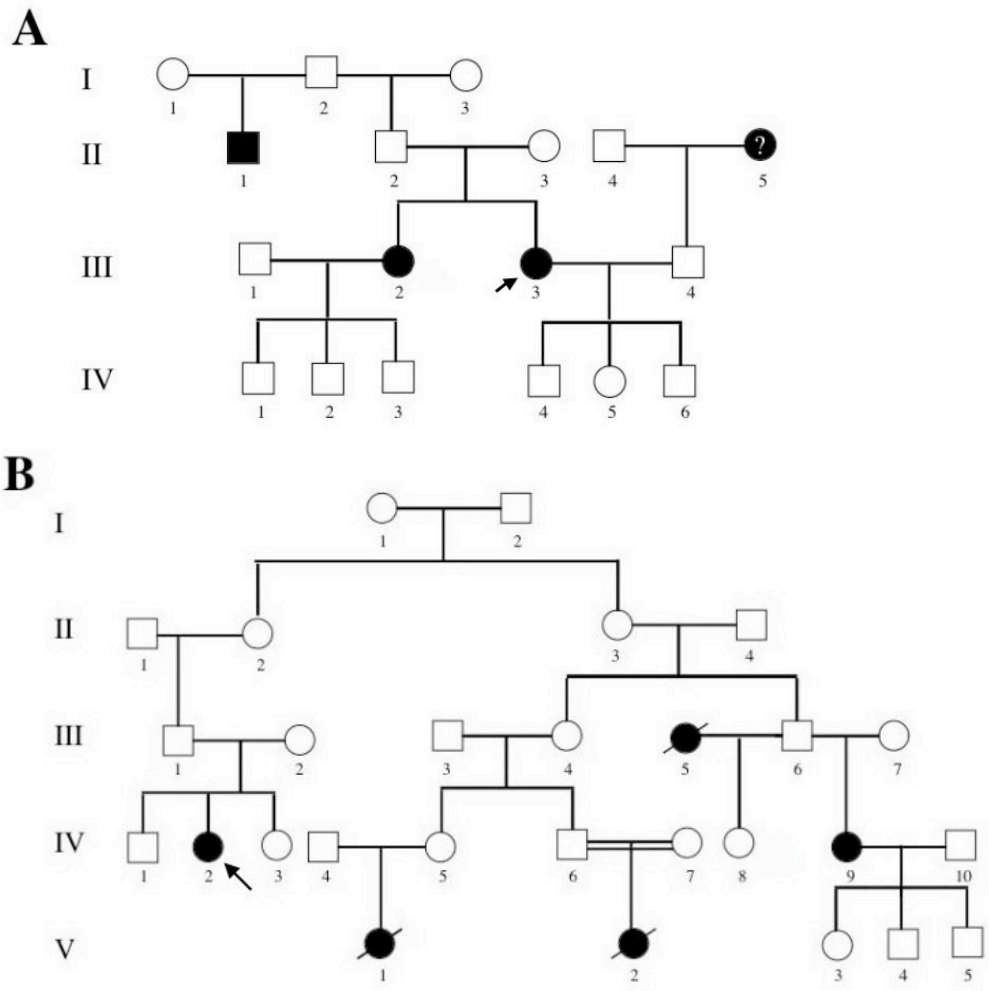

Figure 2: The pedigrees of two breast cancer patients with the 185delAG mutation in *BRCA1* gene. (A) The members of family A included: II/1, affected with gastric cancer at age of 60 yr; II/5, affected with unknown cancer; III/2, affected with brain tumor at age of 35 yr and carried 185delAG mutation but without any mutation in exons 4-9 of *p53* gene; III/3, affected with primary breast cancer at age of 46 yr and carried 185delAG mutation. The proband is indicated by the arrow. (B) The members of family B included: III/5, affected with liver cancer at age of 35 yr and deceased at the same age; IV/2: affected with breast cancer at age of 45 yr; IV/8, having a benign breast tumor at age of 16 yr; IV/9, affected with breast cancer at age of 44 yr and carried 185delAG mutation; V/1, affected with breast cancer at age of 30 yr and deceased at age of 35 yr; V/2: affected with colon cancer at age of 56 yr and deceased at age of 69 yr. Arrow is indicative of proband.

(III/3) afflicted with primary breast cancer at age of 46 yr, and her sister (III/2) manifested the brain tumor diagnosed as meningioma (meningiotheliomatous type) at age of 35 yr. Both of sisters carried the 185delAG mutation in *BRCA1* gene. The proband's sister had no mutation in exons 4-9 of *p53* gene (our unpublished data). An affected uncle (II/1), who manifested gastric cancer at age of 60 yr and had deceased at 65 yr, and the mother-in-law of proband's sister (II/5) with an unknown cancer were also included in this pedigree.

The pedigree of family B (Figure 2B) involved a breast cancer proband (IV/2) at age of 45 yr who carried the 185delAG mutation in *BRCA1* gene. The relatives affected with cancers included an unrelated individual (III/5) who was diagnosed for liver tumor and had deceased at 35 years old. The proband's cousin (IV/8) was diagnosed to have the benign breast tumor at age of 16 yr. Two more family members included a breast cancer patient (V/1) aged at 30 yr and deceased at 35 yr and one affected relative (V/2) with colon cancer aged at 56 yr and deceased 13 yr later. Both of these

patients were the third cousins from the paternal line of the breast cancer proband of family B (Figure 2B).

In our 400 patients, we did not find the other two mutations, i.e. 5382insC in *BRCA1* gene and 6174delT in *BRCA2* gene.

Discussion

The frequencies of *BRCA1* and *BRCA2* gene mutations varied greatly in the large scale of breast cancer pedigrees of different countries and populations, which included 21% and 9% in Britain, 24% and 18% in France, 40% and 16% in Canada, and 39% and 25% in the USA, respectively, and in either genes 35% represented in the families of Sweden and Hungary [4]. Neither the 1100delAT (*BRCA1*) nor the 8765delAG (*BRCA2*) mutation was detected in the high-risk Jewish, non-Ashkenazi individuals of Yemenite and North African origin [17]. Whereas, the 8765delAG (*BRCA2*) mutation occurred at a high frequency in Sardinian high-risk families [18]. The 5382insC mutation in *BRCA1* was specific for Ashkenazi and Russian populations, and could also serve as a founder mutation in the Turkish population, suggesting a certain level of admixture between Jewish, Russian, and Turkish individuals in spite of religious and cultural barriers separating these diverse populations [19]. Out of 24 familial breast cancer patients studied from two different geographic regions/populations of India, two sisters from a family (12.5%) out of eight families from Goa with Portuguese colonial origin showed the presence of founder Ashkenazi Jewish *BRCA1* mutation (185delAG) [20]. Regarding the mutation data of Iranian population, a novel deletion c.4415_4418delAGAA, an insertion c.6033_6034insGT, one intronic variation g.5075–53C>T, a deletion/insertion g.81_389del9ins29 in the 3'-untranslated region of *BRCA1* and a previously described insertion c.6033_6034insGT have been reported [21-24].

In this study, the 185delAG mutation in *BRCA1* gene was found in 0.5% of investigated pedigrees and in 0.75% of patients. Among them, the family A showed 185delAG mutation in two members, including the proband (III/3) and her sister (III/2). Although they both carried the same genetic alteration and had complete penetrance, but the mutation of *BRCA1* gene was associated with two different organ targeting (i.e. breast *vs.* brain cancer) and neoplastic behaviors (i.e. the malignant *vs.* benign natures) at almost an early age of onset (proband: 45 yr, sister: 30 yr). However, there were important key elements in families A and B, which could lead to a plan to address the challenges of preventive genetics and to consider an appropriate management for the healthy relatives as following. The age of onset for breast cancer, either in the probands or in the proband's relatives of both families A and B, was found to be within the same range (i.e. 44-46 yr) except the 3rd cousin of proband (V/1) in family B who was affected at age of 30 and deceased at age of 35. To draw the attention towards the recorded family history of breast cancer and other malignancies, even the unrelated individuals in the same pedigree, could provide a great impact on the future management for their offspring. In this regard, two individuals (II/1 and II/5) in the family B could be exemplified. If they carried a mutation, the complexity of genetic alterations and the outcome for the offspring in the generation IV and V could be early diagnosed and predicted.

Due to the lack of any report on the founder mutations in *BRCA1* and *BRCA2* from Iran, the result of this study has provided the preliminary information to understand the level of involvement of *BRCA1* and *BRCA2* mutations in the breast cancer occurrence of Iranian population. The screening on other mutations, such as c.4415_4418delAGAA, c.6033_6034insGT, g.5075–53C>T, and g.81_389del9ins29, in *BRCA1* gene is ongoing to confirm the result. In addition, the comprehensive sequencing of *BRCA1* and *BRCA2* and their haplotype analyses will also be performed to determine whether the founder mutations of these genes are unique to Iranian population. In conclusion, our observation of the 185delAG mutation of *BRCA1* gene in three investigated family members could suggest the clinician to provide a preventive genetic test of the *BRCA1* defect in the healthy and affected family members, either affected with breast cancer or other types of cancer, as early as possible.

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