

Genomic rearrangement screening of the *BRCA1* from seventy Iranian high-risk breast cancer families

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Background: The second leading cause of cancer deaths in women is breast cancer. Germline mutations in susceptibility breast cancer gene *BRCA1* increase the lifetime risk of breast cancer. Eighty-one large genomic rearrangements (LGRs) have been reported up to date in *BRCA1* gene, and evaluation of these rearrangements helps with precise risk assessment in high-risk individuals. In this study, we have investigated LGRs in *BRCA1* among Iranian high-risk breast cancer families. **Materials and Methods:** Seventy patients with breast cancer who were identified negative for point mutations or small deletions/insertions of *BRCA1* gene were selected. Deletions and duplications of *BRCA1* gene were evaluated using multiplex ligation-dependent probe amplification (MLPA). **Results:** Two deletions, deletion of exons 1A/1B-2 and exon 24, were detected in two patients with breast cancer. The former alteration was found in a woman with a strong family history of breast cancer while the latter one was detected in a woman with early onset of breast cancer. **Conclusion:** Although our data confirm that LGRs in *BRCA1* comprise a relatively small proportion of mutations in hereditary breast cancer in the Iranian population, MLPA analysis might be considered for screening of LGRs in high-risk individuals. It is worth to note that our results are consistent with previous studies in various Asian and European countries.

Key words: *BRCA1* gene, breast cancer, large genomic rearrangements, multiplex ligation-dependent probe amplification

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INTRODUCTION

BRCA1 gene, which is located on the long arm of chromosome 17 (17q21) [Figure 1], spans approximately 81 kb and encodes a protein of 1863 amino acids.^[1] *BRCA1* is an essential tumor suppressor, suppressing genome instability.^[2]

Breast cancer is the second leading cause of cancer deaths in women worldwide.^[3] *BRCA*-mutant individuals and families have a notable increased risk of developing breast and ovarian cancers.^[4] Five percent of all breast

cancers are hereditary, and nearly 16% of hereditary breast cancers are due to germline mutations in *BRCA1/2* genes.^[5] The screening of *BRCA1/2* mutations is a common component of risk evaluation and management of familial breast cancer, bilateral breast cancer, and early-onset breast cancer.^[6] Familial breast cancer includes 20–30% of all breast cancer cases. It is also reported that most of the families with <6 breast cancer cases and no ovarian cancer do not carry *BRCA1/2* mutations that can be detected by routine sequencing protocols.^[7] Various mutations including nonsense mutations, small deletions and insertions, and large genomic rearrangements (LGRs) are reported in *BRCA1* gene.^[8] Furthermore, these mutations can result in

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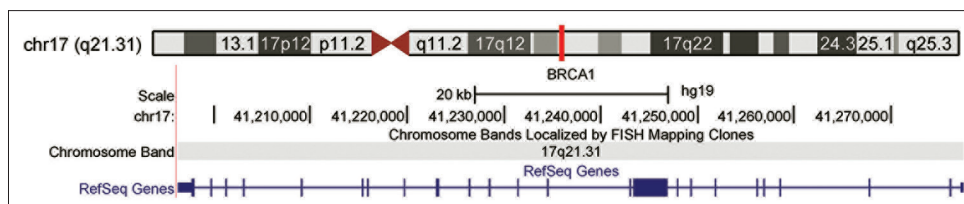


Figure 1: Genomic location of *BRCA1* gene on chromosome 17. Genome assembly used to produce (<http://genome.ucsc.edu>)

recurrent mutations of other specific genes (such as RB1) that may lead to other cancers.^[9] The incidence of LGRs in *BRCA1/2* genes is very low,^[10] and these LGRs occur in a small percentage (<1%) of patients tested for hereditary breast and ovarian cancers.^[11] Furthermore, the prevalence of LGRs varies from 0% (Afrikaner and French-Canadian populations) to 27% (the Dutch population) of all *BRCA1* mutations in different populations.^[12] The characterization of three founder *BRCA1* LGRs, deletion of exon 13, exon 22,^[13] and exons 1A/1B-2,^[14] in the Dutch population explains high frequency of LGRs (27%).

DNA double-strand break (DSB) is one of the most common DNA repair mechanisms within normal cells. The BRCA-mutated breast cancer cells lack the homologous recombination that is required for error-free DSB repair.^[15] Most LGRs detected in *BRCA1* result from unequal homologous recombination events involving Alu repeats, comprising 41.5% of the gene, and *BRCA1* pseudogene (ψ *BRCA1*), which is located 30 kb upstream of the *BRCA1*.^[16]

The description of multiplex ligation-dependent probe amplification (MLPA) test for detection of LGRs in *BRCA1* in 2001 has increased the number of LGRs identified in different populations so that nearly 61 of the 81 reported LGRs are identified by MLPA.^[17] Before the development of MLPA, detection of LGRs was mostly carried out by applying different approaches such as Southern blot, long-range PCR, fluorescence *in situ* hybridization-based methods, and quantitative multiplex PCR of short fragments.

MLPA can be used as the first step for genetic screening of women with family history of breast cancer in populations with high frequency of LGRs. In this study, LGRs in *BRCA1* gene using MLPA in seventy patients without detectable point mutation or small deletions/insertions were investigated.

MATERIALS AND METHODS

Patients

The *BRCA1* rearrangements analysis was performed on seventy patients with breast cancer that completed the consent form [Table 1]. Our study was approved by the Ethics

Committee of Isfahan University of Medical Sciences. The criteria used for selection of patients were as follows: (1) early onset of breast cancer (under 35 years old), (2) two or more cases of breast cancer in the family, (3) bilateral breast cancer, or (4) men with breast cancer. All patients were negative for *BRCA1* point mutations or small deletions/insertions. This study was performed in breast cancer research center of Isfahan University of Medical Sciences.

DNA ISOLATION AND MULTIPLEX LIGATION-DEPENDENT PROBE AMPLIFICATION ANALYSIS

DNA was extracted from blood leukocytes using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The exact DNA concentration was determined using a NanoDrop instrument (Thermo 2000c) after dilution to 50 ng/ml. The *BRCA1*-MLPA analysis was carried out using the SALSA MLPA test kit P002-C2 (MRC Holland, The Netherlands) according to the manufacturer's instructions. P002-C2 *BRCA1* probemix contains probes for each exon of the *BRCA1* gene and nine reference probes that are included for normalization purposes. P087 MLPA kit was used for confirmation of the obtained data. This kit is designed to detect deletions/duplications of one or more sequences in the *BRCA1* gene in a DNA sample.

In brief, the ligation reaction was performed using 100 ng of target DNA in the following steps: Denaturation at 98°C for 5 min, hybridization using *BRCA1*-MLPA probemix at 60°C for 16 h, and ligation using Ligase-65 mix at 54°C for 15 min followed by ligase inactivation at 98°C for 5 min. After the ligation step, multiplex polymerase chain reaction was performed using the appropriate PCR primers, dNTPs, SALSA PCR buffer, and SALSA polymerase for thirty cycles (95°C for 30 s, 60°C for 30 s, and 72°C for 60 s) followed by one cycle at 72°C for 20 min. Probe amplification products were analyzed on 3130 capillary sequencer (Applied Biosystems, UK) with a 36 cm capillary array and POP-4™ polymer (Applied Biosystems) by mixing with 0.4 μ l of the GeneScan™-500 LIZ™ size standard (Applied Biosystems) and 9 μ l of Hi-Di Formamide (Applied Biosystems). The results (size and the peak area) were exported from the DNA sequencer and analyzed by GeneMarker software. It converts data from any sequencing system, offers two normalization and

Table 1: Characteristics of the patients

| Patients | Age at the diagnosis | Family history |
|------------------------------|----------------------|-----------------------------|
| Early onset of breast cancer | | |
| F434 | 29 | |
| F475 | 30 | |
| F579 | 28 | |
| F528 | 31 | |
| F567 | 34 | |
| F589 | 34 | |
| F538 | 32 | |
| F543 | 26 | |
| F559 | 32 | |
| F639 | 27 | |
| F217 | 31 | |
| F229 | 30 | |
| F239 | 27 | |
| F616 | 32 | |
| F681 | 24 | |
| F692 | 30 | |
| F709 | 31 | |
| F727 | 30 | |
| F489 | 27 | |
| F774 | 28 | |
| F821 | 30 | Br (M), Lu (MU) |
| F847 | 25 | Br (PC), Sto (MGM) |
| F835 | 37 | Br (M, MC) |
| F785 | 28 | Br (MA) |
| F498 | 27 | Br (MA) |
| F55 | 62 | Br (S) |
| F792 | 30 | Br (MGM) |
| F80 | 52 | Br (PC) |
| F458 | 39 | Br (PA) |
| F374 | 51 | Ov (MA) |
| F281 | 64 | Br (MA) |
| F193 | 30 | Br (PA) |
| F37 | 35 | Br (MC) |
| F276 | 52 | Ut (MA), Co (MA) |
| F69 | 32 | Br (MGM, MC) |
| F126 | 31 | Br (MA, MC) |
| F180 | 34 | BilatBr (M), PA (Ut) |
| F205 | 29 | Br (MA, PA) |
| F642 | 35 | Br (S, M) |
| F147 | 34 | Br (M), Lv (MU) |
| F257 | 39 | Sto (PU, PGF, PGM), Co (PA) |
| F296 | 56 | Br (N, PA) |
| F307 | 26 | Br (M, MGM, 2MC) |
| F442 | 59 | Br (PA, 2PC) |
| F44 | 43 | Br (S), Co (M) |
| F99 | 62 | Br (S, N, PC) |
| F158 | 50 | BilatBr (2S, M, MGM) |
| F246 | 50 | Br (MA, 2MC) |
| F317 | 52 | Br (A, MC), BilatBr (MC) |
| F167 | 50 | Br (PA, MA) |
| F678 | 40 | Br (M, MA) |

Contd...

Table 1: Contd...

| Patients | Age at the diagnosis | Family history |
|-------------------------|----------------------|------------------|
| F445 | 59 | Br (PA, 2PC) |
| F409 | 47 | BilatBr (2PC) |
| F417 | 53 | Br (S, MA) |
| F427 | 43 | Br (MA, PC) |
| F593 | 26 | Br (MGM), Ov (M) |
| F513 | 45 | Br (S, MA) |
| F717 | 54 | Br (M, MC) |
| F736 | 35 | |
| F748 | 54 | |
| F752 | 61 | |
| F769 | 49 | |
| F669 | 57 | |
| Bilateral breast cancer | | |
| F395 | 65 | BilatBr (PA) |
| F356 | 60 | |
| F571 | 59 | |
| F609 | 48 | |
| Men with breast cancer | | |
| F368 | | BilatBr (54) |
| F328 | 49 | |
| F622 | 54 | Br (B) |
| F652 | 59 | |
| F463 | 34 | Br (PC) |
| F509 | 51 | Br (S, B) |

Type/site of cancer-Br = Breast; Bilat = Bilateral; Co = Colon; Lu = Lung; Lv = Liver; Ov = Ovarian; Sto = Stomach; Ut = Uterus; Affected relatives of the patient in parentheses-B = Brother; M = Mother; MA = Maternal aunt; MC = Maternal cousin; MGM = Maternal grandmother; MU = Maternal uncle; N = Niece; PA = Paternal aunt; PC = Paternal cousin; PGF = Paternal grandfather; PGM = Paternal grandmother; PU = Paternal uncle; S = Sister

analysis methods. A ratio under 0.7 was taken as a sign of the presence of only one copy of a sequence in the *BRCA1* gene.

RESULTS

MLPA analysis of genomic DNA for seventy high-risk individuals who were found negative for point mutations or small insertions/deletions in the *BRCA1* revealed two different rearrangements [Figure 2]. We detected a deletion of exons 1A/1B-2 in a patient (F307) with a strong family history of breast cancer, with the mother, grandmother, and two other relatives affected with breast cancer. A deletion of exon 24 was found in a woman (F792) with early onset breast cancer (age 30) and with the grandmother affected with breast cancer. The obtained results were confirmed using confirmation MLPA kit (P087).

DISCUSSION

In this study, we carried out MLPA analyses of seventy patients with breast cancer to identify the prevalence and spectrum of LGRs in the *BRCA1* gene. Our results indicate

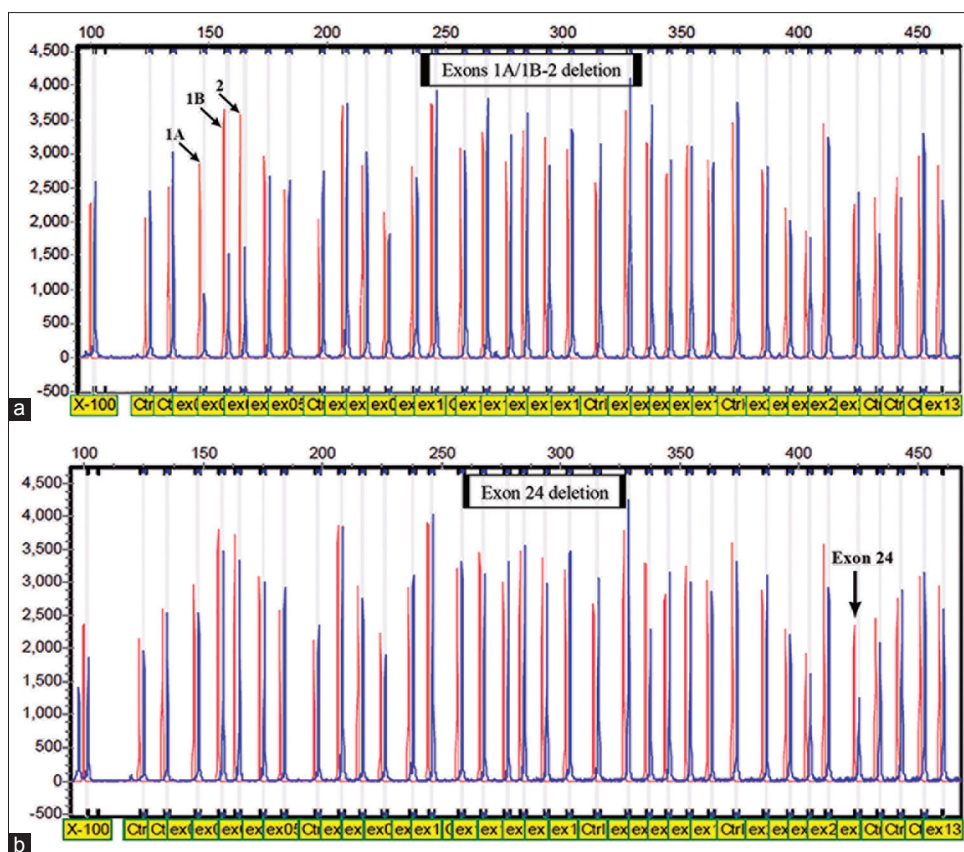


Figure 2: Multiplex ligation-dependent probe amplification electropherogram. (a) Analysis of individual carrying deletion of exons 1A/1B-2. (b) Analysis of individual with deletion of exon 24

that LGRs account for 2.8% of high-risk families without point mutations and small insertion/deletion.

The frequency of LGRs in our study (2.8% of mutation-negative families) was comparable with the results reported in Singapore (3%),^[18] Malaysia (8%),^[19] and in various European populations.^[20,21] However, it is not in agreement with previous study in the Iranian population.^[22] Based on the previous study, the frequency of LGRs in the Iranian population was reported 0%. In this study, eight Iranian high-risk breast cancer families without point mutations in *BRCA1/2* were investigated by semiquantitative multiplex PCR method. However, in our study, seventy patients negative for point mutations in *BRCA1* were analyzed by MLPA analysis. Nevertheless, the comparison between two studies is rather inappropriate because the sample size and the criteria for family selection are largely different.

MLPA analysis of the *BRCA1* gene led to the identification of two *BRCA1* deletions, exons 1A/1B-2 and exon 24 deletions. To date, six different exons 1A/1B-2 deletions, with different break points, in different populations have been identified.^[23] Two different types of exons 1A/1B-2 deletions are considered founder mutations in the Dutch population.^[14] Exon 24 deletion is identified in the Greek^[24]

and German populations. This exon is involved in four more deletions in combination with other exons, exons 21–24 deletion,^[25,26] and exons 23–24 deletion.^[23,27] Since deletion of exons 1A/1B-2 and exon 24 removes two essential regions for RNA expression, promoter, polyA tail, and 3'-untranslated region, respectively, the possible result of these deletions is loss of RNA transcript.^[24,28]

Our data verify that large *BRCA1* deletions or duplications comprise a relatively small proportion of mutations in hereditary breast cancer in the Iranian population. Since the identification of large rearrangements in the *BRCA1* gene is important for clinical management of individuals affected with breast cancer^[29] and for preventive measures for healthy carriers of a familial *BRCA1* mutation,^[30] MLPA analysis should be considered for screening of LGRs in high-risk individuals.

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Nil.

Conflicts of interest

There are no conflicts of interest.

AUTHORS' CONTRIBUTION

- MS contributed in conception of the work, conducting the study, revising the draft and agreed for all aspects of the work
- EE contributed in conception of the work, conducting the study and agreed for all aspects of the work
- EN contributed in conducting the study, writing the draft and agreed for all aspects of the work
- AS contributed in drafting and revising the draft and agreed for all aspects of the work
- MS contributed in conducting the study and agreed for all aspects of the work
- NN contributed in drafting and agreed for all aspects of the work
- SF contributed in conception of the work and agreed for all aspects of the work
- LD contributed in revising the draft and agreed for all aspects of the work
- FM contributed in conception of the work, conducting the study, revising the draft and agreed for all aspects of the work.

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