RESEARCH NOTE

Screening for genomic rearrangements at *BRCA1* locus in Iranian women with breast cancer using multiplex ligation-dependent probe amplification

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Introduction

Despite advances in cancer biology, breast cancer is presently the second most common cause of cancer death all over the world (Marcus *et al.* 1996; Parker *et al.* 1997). Genetic factors play major role in development of breast cancer. It has been proved recently that large genomic rearrangements account for a substantial proportion of breast-cancer-causing mutations with a wide range of frequencies among different populations (Ewald *et al.* 2009). We assessed the presence of *BRCA1* gene rearrangements in Iranian breast cancer patients using multiplex ligation-dependent probe amplification (MLPA). Among 50 women with early-onset breast cancer, who previously tested negative for point/indel mutations, a large deletion containing the whole exons 1 and 2 was found in three of the cases. Our results suggest that the *BRCA1* genomic rearrangements are responsible for about 6% of the studied population, and MLPA could be used as a robust screening method for breast cancer patients.

Among females of all ages in the Iranian population, breast, skin and colorectal cancers are diagnosed as the most prevalent cancers (Razavi *et al.* 2009). To date, several genes have been identified as breast cancer susceptibility factors, such as *BRCA1*, *BRCA2*, *CHECK2* and *PALB2* (Antoniou *et al.* 2003; Easton *et al.* 2004; Renwick *et al.* 2006; Seal *et al.* 2006; Rahman *et al.* 2007). Among these, *BRCA1*, located on chromosome 17 and containing 24 exons, is a major cause of early-onset breast cancer (Carter 2001). Its product is one of the components of a large protein complex which takes part in repairing double-strand breaks in DNA and controlling homologous recombination as well. This protein interacts with other proteins and factors such as TP53, RB, ATM and BRCA2 involved in DNA repair and transcriptional regulation (Carter 2001).

Mutations of the *BRCA1* gene could lead to about 50% of hereditary breast cancers (Zhang *et al.* 1998). According to the role of *BRCA* genes as tumour suppressors, they need to be mutated in both alleles for tumorigenesis. It should be noted that, when there is one inherited mutated allele, it is necessary that the remaining wild-type allele have a somatic mutation in the breast tissue, which results in the development of breast cancer. *BRCA1* and *BRCA2* genes contain segments of repetitive sequences that contribute to instability of the adjacent chromosomal locations. This instability is manifested as genomic rearrangements in the mentioned genes (Welcsh and King 2001). It has been indicated that the large genomic rearrangements (LGR) of *BRCA1* and *BRCA2* genes, not detectable by the commonly used point mutation screening assays, account for at least a part of cases that seem mutation-negative for *BRCA1* and *BRCA2*. High prevalence of large genomic rearrangements in the *BRCA1* gene has been reported among various populations such as German, French and UK (Petrij-Bosch *et al.* 1997; Gad *et al.* 2002; Montagna *et al.* 2003; Mayozer 2005; Walsh *et al.* 2006). Earlier studies indicated a low frequency of rearrangements in *BRCA1* (Hofmann *et al.* 2003), whereas subsequent studies suggested a more prominent role of *BRCA1* rearrangements in development of breast cancer (Hartmann *et al.* 2004). In addition, in Turkey, Czech Republic, Italy,

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Netherlands, Portugal, Canada and USA, different frequencies of *BRCA1* LGRs have been reported, ranging from 0% in Canada to 23% in Italy (Ewald *et al.* 2009). Hence, it is necessary to establish the frequency of *BRCA1* rearrangements in other populations.

The current study was planned to investigate the presence of LGRs in *BRCA1* gene among Iranian breast cancer patients using MLPA which is a novel method with high accuracy for rapid detection of copy number variations (Schouten *et al.* 2002).

Subjects and methods

A total of 50 women with early-onset breast cancer, plus 10 normal subjects as a control group, were investigated using MLPA for their *BRCA1* gene rearrangements. All participating families were given written detailed information about the study. Written informed consent was obtained from all participants and the medical history and pedigree information were obtained by questionnaire. Selected patients were included in this study based on following criteria: (i) pathologic confirmation, (ii) early onset of breast cancer (<45 years), and (iii) absence of any point and/or indel mutations according to our previous study (Yassaee *et al.* 2002).

DNA was obtained from peripheral whole blood using PROMEGA kit (Cat. #LA1620) (Karachi, Pakistan), followed by MLPA analysis using P002-C1 kit from MRC-Holland (Amsterdam, The Netherlands). The kit includes probes for each exon of the *BRCA1* gene, except exon 4, plus probes for a number of control regions across the genome. Products were separated on an ABI3100 genetic analyzer (New York, USA) and interpreted using GeneMarker v. 1.97 (www.softgenetics.com/GeneMarker.html/). The sample is considered as normal sample if the dosage quotient is 1.0; and dosage quotient for deletion or duplication is 0.5 and 1.5, respectively. An abnormal dosage quotient was scored

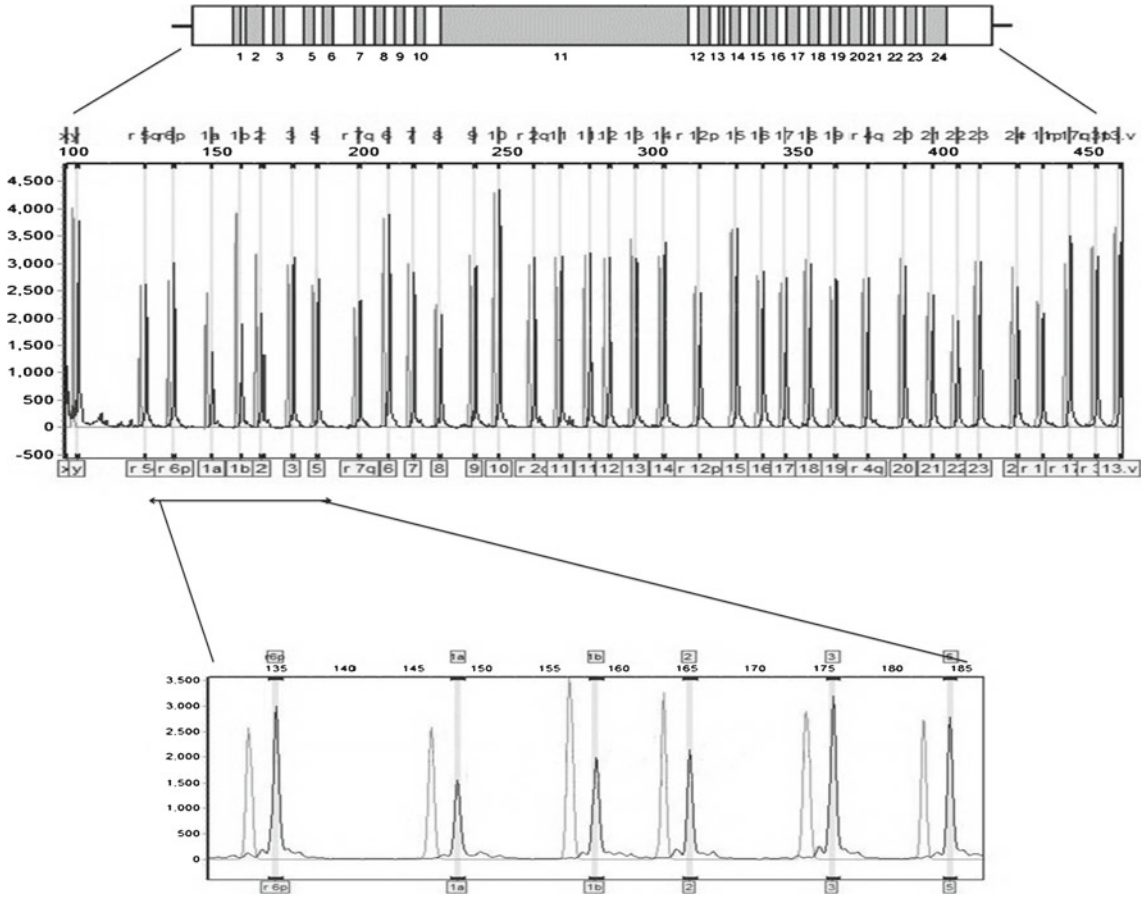

Figure 1. Detection of large deletion including exons 1 and 2 using P002C1 MLPA kit. The whole exons of the *BRCA1* gene are shown at the top of the figure and the peaks for each exon achieved by MLPA method below it. At the bottom of the figure, the three peaks showing heterozygous deletions are depicted with the half size of the sample compared to normal control peak for probes 1a, 1b and 2. In addition, one normal peak in the left side and two in the right side are shown with equal sample and control peak sizes. Gray and black peaks refer to normal reference and sample respectively.

as deletion if the mean dosage quotient of the test to internal control peaks was less than 0.7 and as duplication if the mean dosage quotient was 1.3 or greater.

In all cases detected with LGRs, we were able to obtain a satisfactory result on repeat analysis. Usually, deletions of a single exon need confirmation, due to possibility of occurrence of variations or small-scale mutations in the probe binding site. But deletions of more than one exon that are adjacent are very unlikely to be false positives and caused by chance in the MLPA assay. For greater certainty, we repeated the MLPA analysis for all cases showing deletions and all of the results that were repeated.

Results

The MLPA kit used to screen *BRCA1* gene in our samples had probes for each exon of *BRCA1* gene except exon 4. In addition, exons 1 and 11 had two probes due to large size.

Fifty samples were analysed and three of them suffered from the same rearrangement including deletion of exons 1 and 2 of *BRCA1*, resulting in the half peak size for probes 1a, 1b and 2. Fragment analysis of this deletion is shown in figure 1. No rearrangement was identified in the rest of the samples analysed. The peaks of probes 1a, 1b and 2 depict carrying heterozygous deletion of exons 1 and 2 of *BRCA1* gene. The three probes for this region show an approximately 50% reduced signal.

Discussion

Fifty samples collected from women, diagnosed with breast cancer at age <45 years, were analysed for LGR in *BRCA1*. Three samples were positive for *BRCA1* rearrangement which account for 6% of samples those previously have been tested and negative for point and/or small indel mutations in *BRCA1*. Our results are simillar to other reported results from some other countries such as German (6%) (Hartmann *et al.* 2004), French (5.9%) (Gad *et al.* 2002) and UK (4.4%) (Bunyan *et al.* 2004) in *BRCA1*/*BRCA2*-variation-negative families. On the other hand, there are several studies which suggest a spectrum of *BRCA1* LGR frequencies ranging from 0% in Finland and Canada (Lahti-Domenici *et al.* 2001) to 23% in Italy (Montagna *et al.* 2003). The similarities and differences in different populations could be due to different genetic backgrounds including various founder mutations and different or identical origins of populations. Moreover, another reason for this variation is the heterogeneity of clinical inclusion criteria used for selection of breast cancer patients in each study. Besides, inclusion of *BRCA*-mutation-negative samples or previously untested samples will influence the results. The presence of LGRs is more likely in the mutation-negative samples.

The LGR found is located at the 5′ end of the *BRCA1* gene. It involves exons 1 and 2 or, in other words, three adjacent probes including probes 1a, 1b and 2. Due to the presence of the exon 3 in normal copy number, it could be assumed that the right-hand breakpoint occurred somewhere in intron 2, and because of the absence of any probes in the region upstream of exon 1, we could not locate the left-hand site of the breakpoint. However, this deletion removes the translation initiation codon, which is present in the middle of exon 2 and results in disability of this allele to produce any BRCA1 protein. In three samples which possess this LGR, It is suggested that a somatic mutation in the other allele could lead to loss of heterozygosity for *BRCA1* and develop the breast cancer. Identification of the second mutation in the breast cancer cells is required to complete the molecular diagnosis process.

The possibility of occurrence of common variants at or near the binding site of MLPA probes is a source of MLPA false positives (as identified by the company in the assay inserts). However, when two or more adjacent probes illustrate decrease in quantity, the chance of false positive result becomes negligible, since the chance of occurrence of two or more variations in such a short distance is very trivial.

In summary, three out of 50 samples which were analysed had a deletion in the *BRCA1* gene given that 6% frequency of rearrangements within this region. The remaining samples might have rearrangements in *BRCA2* or mutations of several other predisposing genes, which could be examined in future studies. Although more samples would require analysing *BRCA1* for LGR detection, the MLPA method is quite in hand to use in *BRCA1*/*BRCA2* analysing processes.

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