BRCA1 gene Molecular Alterations in Omani Breast Cancer Patients

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Abstract

Background:

Breast cancer (BC) is the most common cancer reported in females in Oman and usually occurs at a relatively younger age, presents at an advanced stage and behaves aggressively. BC occurs in hereditary and sporadic forms. Although germ-line mutations in BRCA1 and BRCA2 genes are rare in sporadic cases compared with hereditary cases, molecular alterations, such as loss of heterozygosity, and CpG methylation, are common. In this study, we investigated the types of molecular alterations associated with hereditary and sporadic BRCA1-associated BC in Omani patients.

Methods

We obtained clinical data and samples from 43 sporadic BC patients. The selection of cases was made based on the following criteria: aged ≤ 40 years, or bilateral breast cancer, or estrogen and progesterone receptor negative status, and HER-2/neu negative (Triple Negative phenotype) status. Screening for molecular alterations was performed by direct sequencing, multiplex ligation-dependent probe amplification (MLPA).

Results

Genomic deletions and duplication in the BRCA1 gene were identified in four female patients. Two patients carried exon 1 and 2 deletions and two showed exon 1 and 2 duplication. Screening for mutation by direct sequencing revealed three polymorphisms in exon 11. Two of these polymorphisms are non-synonymous (rs1800704, rs799917) and one is synonymous (rs1800740).

Conclusion

The current pilot study detected previously described gene rearrangements and polymorphisms involving the BRCA1 gene and no seemingly pathogenic missense mutations were elucidated.

Keywords

BRCA1, breast cancer, mutation, polymorphism, Omani, Arab

Introduction

Breast cancer (BC) is the most common cancer reported in females in Oman [1, 2]. It develops relatively at a younger age, presents at an advanced stage, and is associated with a low survival rate [1, 2]. Diagnosis of BC at a young age may be associated with various genetic defects—particularly, BRCA1 or BRCA2 gene mutations [3].

Breast cancer occurs in hereditary and sporadic forms. Although germ-line mutations in BRCA1 and BRCA2 genes are rare in sporadic cases compared with hereditary cases, molecular alterations, such as loss of heterozygosity and CpG methylation, are common[4, 5]. Published studies on BRCA1 or BRCA2 gene mutation in Arab populations remain very limited. El-Harith et al studied 29 Arab females and identified two mutations: a novel BRCA2 mutation

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(2482delGACT) and a BRCA1 mutation (Arg841Trp)[6]. Kadouri et al studied 31 women of Palestinian Arab (PA) origin with BC with four of them having a significant family history suggestive of genetic predisposition [7]. A novel BRCA1 mutation, E1373X in exon 12, was found in one patient [7].

In this study, we investigated various BRCA1 molecular alterations associated with high risk sporadic BC in Omani patients. We have employed two techniques, direct DNA sequencing of BRCA1 exons and multiplex ligation-dependent probe amplification (MLPA), to screen for major genomic rearrangements. To the best of our knowledge, this is the first study from Oman and one of a few studies involving Arab populations. Therefore, the results will provide a better understanding of the molecular alterations associated with BC carcinogenesis in Arab populations.

Materials and Methods

Study subjects

The study subjects consisted of high risk sporadic female BC patients who were diagnosed at Sultan Qaboos University Hospital. The selection of cases was made based on the following criteria: aged ≤ 40 years, or bilateral breast cancer, or estrogen and progesterone receptor negative status, and HER-2/neu negative (Triple Negative phenotype) status. Seven females had a family history of BC involving one first degree relative only but no patients had extensive family history of BC. The Medical Research and Ethics Committee of the College of Medicine of Sultan Qaboos University approved the study design. The study subjects gave informed consent prior to participation in the study.

Genotyping method

Ten milliliters of blood were collected in an EDTA tube and stored frozen until DNA extraction. DNA was extracted from whole blood using the commercially available QIAamp DNA blood Midi Kit (Qiagen, Germany) and stored until processed for genotyping.

Analysis of the BRCA1 mutations was performed using polymerase chain reaction (PCR) and DNA sequencing. Genomic DNA from whole blood was used as the PCR template in a total reaction volume of 25 µL containing 10 pmol of primers designed for exons 3, 5, 11, 13, and 20 of BRCA1 genes as shown in Table 1. PCR was performed as follows: one cycle at 94°C for 5 min, 35 cycles at 94°C for 30 sec, 50°C for 30 sec, and 72°C for 30 sec, followed by 72°C for 5 min. PCR products were electrophoresed on a 2% agarose gel and visualized under ultraviolet light (UV) light after ethidium bromide staining. After checking the PCR products for the presence of amplified DNA, the samples were purified to be sequenced. The first 5 μl of the PCR products were transferred into 96-wells plates and 2 μl

Annealing Temperature	Product size (base pairs)	Forward and reverse primers	Exon/gene
55°C	250	3'-gcaactccatatcccctgct-5' 3'-tcctgtgcctccctaccc-5'	Exon 3/BRCA1
57°C	189	3'-cacttgctgagtgtgtttctca-5' 3'-caaacttcctgagttttcatgg-5'	Exon 5/BRCA1
56°C	286	3'-tgaattatcactatcagaacaaagca-5' 3'-aaaaactggagaaagtatggtgaaa-5'	Exon 13/BRCA1
55°C	173	3'-ttcccctgtcccctctctctt-5' 3'-ccatcgtgggatcttgctta-5'	Exon 20/BRCA1
54°C	993	3'-gaattttctgagacggatgtaa-5' 3'-tgttttattctcatgaccactatta-5'	Exon 11A/BRCA1
62°C	917	3'-aacggagcagaatggtcaag-5' 3'-ttgtttcccgactgtggtta-5'	Exon 11B/BRCA1
58°C	952	3'-tggcactcaggaaagtatct-5' 3'-atagacctcaggttgcaaaa-5'	Exon 11C/BRCA1
58°C	923	3'-tgatgaaaacattcaagcag-5' 3'-aaaacctggttccaatacct-5'	Exon 11D/BRCA1

Table 1. Polymerase chain reaction primers, product sizes in base pairs, and annealing temperatures

of EXOSAP-IT were added to each well. The plates were covered with a heat-proof sticky lid and placed in a thermocycler with the following program: 37°C for 15 min and 80°C for 15 min for one cycle.

The sequencing reaction was carried out after this step and was performed for both the forward and reverse directions in different tubes. Each tube contained 3 μl of MilliQ water, 1 μl of 3 pmol/μl of primers, 2 μl of BigDye buffer, 2 μl of BigDye terminator, and 2 μl of PCR Exosap products.

We also tested BC cases using MLPA for genomic rearrangements. The commercially available P002 BRCA1 kit (MRC-Holland) was used for MLPA. Target DNA per 5 microliters (50–200 ng) was denatured for 5 min at 98°C, after which 1.5 µl of the probe mix and 1.5 µl of MLPA buffer were added. The mixture was heated at 95°C for 1 min and incubated at 60°C overnight (16 h). Ligation was performed using Ligase-65 mix (32 µl) for 15 min at 54°C. Next, the ligase was inactivated by incubation for 5 min at 98°C. Ten microliters of this ligation mix was premixed with 30 µl of PCR buffer and placed in a PCR machine at 60°C. Subsequently, a 10-µl polymerase mix was added. PCR was conducted for 35 cycles (30 s at 95°C, 30 s at 60°C, and 60 s at 72°C). The fragments were analyzed on an ABI 3130 XL sequencer (Applied Biosystems) using Genescan-ROX 500 size standards (Applied Biosystems). Fragment analysis was performed using Genemarker software (v1.85, Demo).

Results

Clinical and pathological characteristics of patients

Forty-three patients with a pathologically confirmed diagnosis of invasive BC were included. The mean age of all patients was 45.3 [standard deviation (SD), ±12.9] years. Most of the patients (65.1%) were pre-menopausal and 53.5% were aged ≤45 years.

The mean clinical tumor size was 5.3 cm (±2.4 cm), whereas the mean pathological size was 3.9 cm (±2.8 cm). Axillary lymph nodes were found to be involved in 51.1% of patients who had surgical intervention (n=32). All patients had invasive ductal carcinoma except for two patients, each with lobular carcinoma and carcinosarcoma. Hormone receptor status was available for 40 patients and, among those, 24 (55.8%) and 24 (55.8%) patients expressed estrogen and progesterone receptors, respectively. Information regarding Her2/neu status was available for 30 patients, with three having Her2-positive disease.

Thirty-six patients underwent surgical intervention and, of these, 20 patients (46.5%) had a mastectomy. Thirty-eight patients received chemotherapy either as neoadjuvant (10 patients) or adjuvant (28 patients) regimens. External beam radiotherapy was administered to 74.4% of patients (n=32). All patients who expressed hormone receptors received hormonal treatment (28 patients). At the time of analysis

Exon	Rs #	Substitution	Site	Type	Sequence	Comment
11	rs1800704	M→I	1008	Non-synonymous	ATG⇒ATA	Common polymorphism
11	rs1800740	G→G	911	Synonymous	GGA⇒GGG	
11	rs799917	P→L	871	Non-synonymous	CCG ⇒ CTG	Common polymorphism

Table 2. BRCA1 polymorphisms detected in 43 high-risk patients

(May 2012), 27 patients were alive, 13 died, and 3 were lost to follow-up. With a mean follow-up duration of 54 months, the mean survival time was 87.1 months [95% confidence interval (CI): 74.5–99.8 months] and the median survival time was 112.1 months (95% CI: 70.1–154.2 months) with a 5-year survival rate of 77.5 %.

BRCA1 gene rearrangement and polymorphisms

Genomic deletions and duplication in BRCA1 were identified in four female cases. Two cases carried exon 1 and 2 deletions and two showed

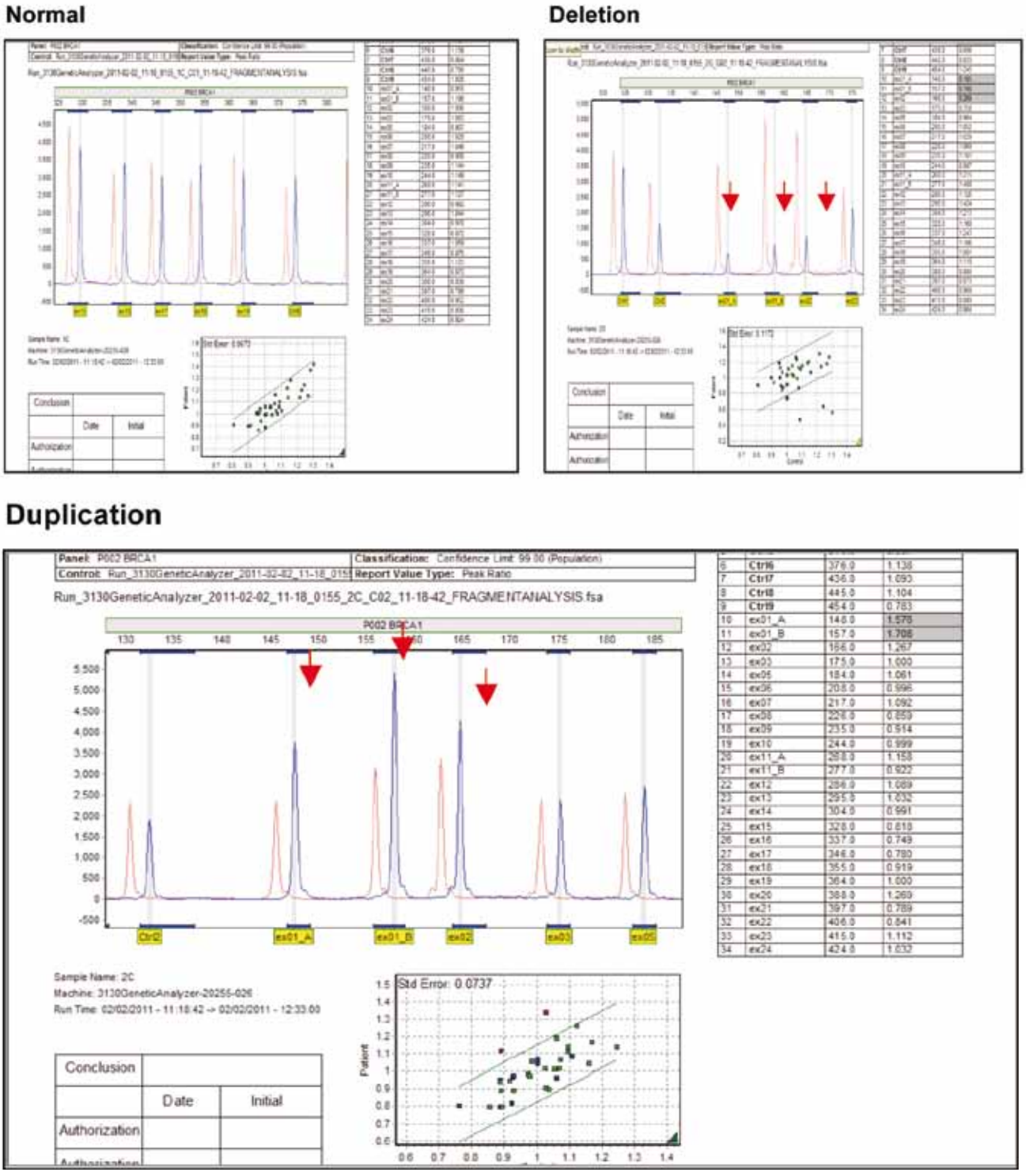

Figure 1. BRCA1 MLPA analysis results for normal samples, samples with BRCA1 deletion, and samples with BRCA1 duplication. Profiles corresponding to BRCA1 exons and control probes are obtained from the overlap of a normal sample (red) with a test sample (blue). Exons are indicated at the bottom of the figure refer to the BRCA1 exons recognized by each MLPA probe. Arrows mark the deleted BRCA1 exons. 'c' indicates the control peaks resulting from the amplification of probes located in different chromosomes.

exon 1 and 2 duplication as shown in Figure 1. Interestingly, none of the four cases with genomic rearrangement was known to have a family history of BC, but two are young and the other two have a triple-negative status.

Screening for BRCA1 mutation by direct sequencing revealed three polymorphisms in exon 11. Two of these polymorphisms are non-synonymous (rs1800704, rs799917) and one is synonymous (rs1800740) as shown in Table 2.

Discussion

MLPA has considerably facilitated the study of BRCA genomic rearrangements simply and efficiently [8]. Using MLPA, we identified genomic deletions and duplications in BRCA1 in four female cases. Two cases harbored exon 1 and 2 deletions and two showed exon 1 and 2 duplication. Therefore, we report a BRCA1 deletion/duplication detection rate of 9.7% in this cohort of patients. Previously, an Italian study reported a rate of genomic rearrangements of the BRCA1 gene of 20% and suggested routine BRCA1 mutational analysis in patients with no family history [9]. However, other studies reported lower rates of BRCA1 rearrangements. An American study that included 71 American families reported a rate of 8% of BRCA1 rearrangements, which is consistent with our study [10]. Similarly, a French study reported a BRCA1 rearrangement rate of 9.6% when they found five gene rearrangements in 52 mutation-negative families [11]. Rearrangements of the BRCA1 gene varied in the German population from 1.7% to 5.7% and appear to be lower than other populations [12-14]. Overall, our results are consistent with other studies despite the differences in sample size and selection criteria. Several reports regarding different ethnic groups reported rearrangements involving BRCA1 exons 1 and 2 [9, 14-16]. In our study, these alterations were found in non-familial cases; therefore, a pathogenic role cannot be assumed. Furthermore, these deletions were reported in Italian families with breast/ovarian cancer [9]. Deletion of exons 1 and 2 are thought to result from recombination between sequences located in intron 2 of the pseudo-BRCA1 gene and the corresponding region of the BRCA1 gene [9, 16].

We also performed direct sequencing of BRCA1 exons 3, 5, 13, and 11, which constitute 60% of the entire gene. Screening for mutation by direct sequencing revealed three polymorphisms in exon 11. Two of these polymorphisms are non-synonymous (rs1800704 and rs799917), and one is synonymous (rs1800740) as shown in Table 2. The single-nucleotide polymorphism found in exon 14 (rs 169547) results in a change from alanine to valine in codon 2466 (Ala2466Val). However, the Ala2466Val substitution conserves the chemical and physical proprieties at this site as both amino acids are aliphatic and non-polar and, therefore, probably has no functional significance. In the present study, a missense mutation in BRCA1 (M1008I) was detected in one patient, a finding that has been reported previously in different populations. The M1008I missense mutation is a conservative mutation due to a G>A transition at nucleotide 3143 in exon 11 changing codon 1008 (ATG) encoding the non-polar Met into ATA-encoding Ile, another non-polar residue [17]. This polymorphism has been classified as neutral and could be found in trans with a clearly deleterious BRCA1 mutation [18, 19]. Therefore, this variation has been suggested to lack a deleterious effect on BRCA1 protein function [17].

The BRCA1 Pro871variation is a common polymorphism and has a high variant allele frequency of 45% in the current study and about 36% in Caucasians as reported previously [20]. The function of BRCA1 Pro871Leu has not been clearly elucidated. The breast and ovarian cancer risk predisposition of this polymorphism has been inconsistent [21, 22]. Two studies observed an increased risk associated with the variant allele [22, 23]. However, large case-control studies found no association between heterozygosity or homozygosity of the BRCA1 Pro871Leu polymorphism and the risk of breast and/or ovarian cancers [5, 21, 24].

The current study is limited by it being a single-institution study and where small number of high risk patients was included. Therefore, the possibility that other mutations may exist in Arab Omani population and a larger study is required for validation. Moreover, confirmatory

tests such as RT-PCR or southern blotting are needed for confirmation of MLPA findings.

In conclusion, the current pilot such study detected previously described gene rearrangements and polymorphisms involving BRCA1 and no seemingly pathogenic missense mutations were elucidated. An extension and validation of these preliminary results is highly warranted.

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Author's contributions

1. Aliya Al-Ansari: Conduct and supervision of all molecular work and review of manuscript.
2. Kamla Al-Mawali: Technical support from department of pathology
3. Bassim Al-Bahrani: Involved in patient enrollment and review of manuscript.
4. Mansour Al-Moundhri: Originator of study concept and leader of funded research project and writing of manuscript.

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