Original Article
Middle East Journal of Cancer; October 2018; 9(4): 274-281

Identification of Three BRCA1/2 Mutations and a Study of the Likelihood of an Association with Certain Characteristics in Syrian Familial Breast Cancer Patients

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Abstract

Background: The main goal of the present study was to investigate BRCA1 and BRCA2 mutations in a number of Syrian familial breast cancer cases. We included 50 early onset invasive breast cancer patients from different Syrian families (48 females and 2 males) and 20 healthy women (control group) in the study. All participants were matched for age (28 to 49 years). There were 64% of breast cancer patients who had a significant family history of breast cancer.

Methods: DNA was isolated from blood samples and we performed polymerase chain reaction on the isolated DNA to amplify specific target regions (hotspots): exon 2 of the BRCA1 gene and exon 11 of the BRCA2 gene. Polymerase chain reaction products were then sequenced to investigate possible genetic variations that could be present in the examined regions.

Results: The sequenced polymerase chain reaction products revealed 3 point mutations that included two deletions and one substitution. An exon 2 mutation was found in 2% of the breast cancer patients. Mutations of exon 11 were each found in 4% of the patient group. We detected no founder mutations. The detected exon 2 mutation was previously mentioned by other researchers and classified as a harmful mutation.

Conclusion: To the best of our knowledge, the detected mutations in exon 11 of the BRCA2 gene were not previously identified. A significant association existed between those mutations and the triple negative subtype of breast cancer in Syrian familial breast cancer patients.

Keywords: Familial breast cancer, BRCA1, BRCA2, Syria, Substitution

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Introduction

Breast cancer is the most common cancer in women as it constitutes approximately 23% of all cancers that affect females worldwide. It is the most common cause of death in women around the world.[1,2] Breast cancer cases comprise an estimated

Received: October 25, 2017; Accepted: February 3, 2018

30% of all cancer cases among Syrian women.[3] The most important risk factors for breast cancer are sex, age, and family history. Breast cancer may be sporadic, hereditary or familial. A positive family history does not mean that an inherited genetic cause should always be present. The vast majority of affected individuals do not carry an inherited mutation in a specific breast cancer susceptibility gene.[4] A minority of breast cancer patients (5%-10%) have a strong family history, which suggests the presence of an inherited mutation in a highly penetrant gene; this type of breast cancer is referred to as hereditary breast cancer. Of breast cancer cases, 20% are considered to be familial,[5] which is the clustering of specific cancers in families and an unusually high number of affected family members compared to the general population. Familial breast cancers are characterized by the presence of a tumor of the same type or another related type in multiple close relatives on the same side of the family, an early onset breast cancer, and the presence of 2 or more primary tumors of the same type or different types in the same person.[4]

BRCA1 and BRCA2 are the most important high-penetrance breast cancer predisposition genes.[6] BRCA genes are extremely but not fully penetrant (about 80%) and more than 20 identified genes with low to moderate penetration have been shown to modify the penetrance of BRCA1/2 genes in mutation carriers (modifier genes).[7,8] Metcalfe et al., in 2017, found that the penetrance of breast cancer to age 80 for BRCA mutation carriers with no first-degree relative with breast cancer was 60.4% and 63.3% for those with at least one first-degree relative with breast cancer. These findings suggested an important role for family history in modulating the penetrance of breast cancer among BRCA mutation carriers.[9]

Germline mutations in these genes, which are dominantly inherited, predispose an individual to breast cancer.[4] BRCA gene testing and genetic counseling is recommended in patients with early onset or significant family history. BRCA1/2 mutations account for 5%-6% of all breast cancers[6] and are involved in 80% of hereditary breast cancer cases.[10]

BRCA1 and BRCA2 are classified as tumor suppressor genes. The BRCA1 gene is located on 17q21 and contains 22 coding exons, whereas BRCA2 gene is located on 13q12 and contains 26 coding exons.[11] Numerous mutations detected in both genes are distributed along the entire coding region. More than 1600 mutations in BRCA1 and 1900 mutations in BRCA2 have been reported.[12,13] Frame shift mutations contribute to about 70% of all mutational spectra while each of the nonsense and missense mutations contributes to nearly 10% of all detected mutations. In addition to frame shift and point mutations, large genomic rearrangements in the BRCA genes have been reported. The spectra of BRCA1/2 mutations are population specific although founder mutations have been reported in different populations.[11,13] 185delAG and 5382insC mutations are considered to be the most common founder mutations that account for approximately 10% of all mutations detected in the BRCA1 gene. There are significantly higher frequencies of these mutations in some ethnic groups such as Ashkenazi Jews where above 90% of BRCA mutation carriers have one or more of 3 founder mutations: 185 delAG and 5382 inserC in BRCA1 and 6174 delT in BRCA2.[14,15] In addition to founder mutations, large numbers of additional random genetic variants have been reported in different populations. High risk families tend to have their own mutation.[16,17] Data analysis of breast cancer families has shown that germline mutations of BRCA1 are responsible for 15%-45% of hereditary breast cancer cases.[18] The absence of disease that causes the mutation in an individual with breast cancer does not necessarily mean that a mutation does not exist. It should be considered that first degree relatives have a 50% chance to inherit the same mutation.[4] Immunohistochemistry profiling of breast cancers provides significant therapeutic and prognostic information. Breast tumors are classified into subgroups according to immunohistochemistry profile; the presence or absence of endogenous expression of hormone receptors in tumor tissue; estrogen receptors,

Table 1. 5′-3′sequences of primers 2F, 2R for exon 2 of the BRCA1 gene and primers 11F and 11R for exon 11 of the BRCA2 gene.

Primer	5′-3′ sequence	GC content	Annealing temperature (°C)	Product size (bp)	Reference
Exon 2F	GAAGTTGTCATTTTATAAACCTTT	25%	50	250	1
Exon 2R	ACATACTAGGGAAGAAAAGACA	36.36%			
Exon 11F	AATGATGAATGTAGCACGC	42.1%	56	341	
Exon 11R	GTCTGAATGTTCGTTACT	38.9%			Designed

progesterone receptors, and human epidermal growth factor receptor type 2.[19] Estrogen and/or progesterone receptors are expressed in nearly 60% of breast tumors, which makes them responsive to the growth and proliferation stimulating effects of estrogen.[20] Triple-negative breast cancers (TNBC) as well as little or no expression of estrogen receptors, progesterone receptors and human epidermal growth factor receptor type 2 in the tumor tissue account for 12% to 15% of all breast cancers.[21] Patients with this subtype often have a worse outcome than patients with other breast cancer subtypes.[22] Germline mutations in the BRCA1 breast cancer susceptibility gene have been related to TNBC, with 60% to 80% of breast cancer patients that are BRCA1 mutation carriers show a TNBC phenotype. Inherited mutations in BRCA1 and BRCA2 account for a high proportion of TNBCs.[23] In 2017, Lang et al. have reported that BRCA mutations showed a significant relationship to clinical features of family history, invasive carcinoma, a higher tumor grade, and negativity of human epidermal growth factor type 2 receptors.[24]

Genetic investigations for exons 2 and 11 of the BRCA genes have not been achieved in Syria. This study aimed to investigate possible mutations in exons 2 and 11 of the BRCA genes in Syrian familial breast cancer patients.

Materials and Methods

This prospective cross-sectional study enrolled 50 familial breast cancer patients (48 females and 2 males) from different Syrian families. An age-matched (28-49 years) control group of 20 healthy women without cancer or a family history of any type of cancer were also included. We have obtained an informed consent from all participants and got an approval by the ethical committee in Damascus University in order to achieve our research. Patients were included after meeting one or more of the following criteria: i. early onset age at diagnosis (before the age of 50), ii. bilateral breast cancer, iii. presence of breast and/or ovarian cancer in 2 or more relatives (on the same side of the family, either father or mother), and iv. male breast cancer.[25] All breast cancer patients in this study had early onset invasive breast carcinoma, 64% (32/50) had a positive family history of breast cancer, and 36% (18/50) had no family history. There were 40% (20/50) of patients diagnosed with breast cancer at ≤40 years of age and 60% (30/50) were diagnosed at >40 years of age. One of our male probands was diagnosed with an invasive ductal carcinoma at the age of 48; he had a significant family history with 2 affected first degree relatives (mother and aunt). We obtained patients' breast cancer clinical data from their medical records at the Syrian Arab Red Crescent Hospital. Participants provided 5 ml blood samples collected in EDTA tubes. All samples were frozen at -80°C and stored until the time of DNA isolation.

DNA extraction and amplification

Genomic DNA was isolated from peripheral leukocytes using a blood DNA isolation kit (Fermentas/Thermo Fisher Scientific, MA, USA). Isolated DNA was checked for both purity and yield by a spectrophotometric assay. Polymerase chain reaction (PCR) was performed after validation on isolated DNA by using specific primers (Eurofins, UK). The primers 2F and 2R were adopted from Bensam et al.,[1] whereas primers 11F and 11R were designed by using the Oligo Analyzer 3.1 tool

Table 2. Phenotype characteristics and genetic analysis of the breast cancer patients.

Patient	Age at diagnosis (years)	Tumor		Receptor status			Family history	Exon 2	Exon 11
		Grade (1-3)	Size (mm)	ER	PR	Her2			
1	47	1	10	+	+	+	-	Normal	Normal
2	30	2	25	-	-	+	+	Normal	Normal
3	39	1	23	-	-	-	+	Normal	Mutant
4	49	3	35	-	-	-	+	Normal	Normal
5	44	2	30	-	+	+	+	Normal	Normal
6	29	2	27	+	+	+	+	Normal	Normal
7	33	3	15	+	-	+	-	Normal	Normal
8	35	3	70	-	-	+++	+	Normal	Normal
9	42	2	40	-	-	-	+	Normal	Mutant
10	35	2	55	+	+	-	-	Normal	Normal
11	45	3	65	+	+	-	+	Normal	Normal
12	48	2	25	-	-	+	+	Normal	Normal
13	44	3	57	+	+	+	+	Normal	Normal
14	32	3	27	-	-	++	-	Normal	Normal
15	46	2	29	-	-	-	-	Normal	Normal
16	39	1	12	+	+	++	-	Normal	Normal
17	49	3	30	+	-	+	+	Normal	Normal
18	37	1	19	-	-	-	-	Normal	Normal
19	41	3	40	-	+	++	+	Normal	Normal
20	48	3	20	+	+	+	-	Normal	Normal
21	37	3	33	-	-	-	-	Normal	Normal
22	31	2	28	-	+	++	+	Normal	Normal
23	42	2	21	+	+	+++	-	Normal	Normal
24	49	3	31	+	+	-	+	Normal	Normal
25	39	1	18	++	++	-	-	Normal	Normal
26	45	3	43	+	+	+	+	Normal	Normal
27	47	3	51	++	++	+++	+	Normal	Normal
28	36	2	26	+	-	++	+	Mutant	Normal

(http://eu.idtdna.com/calc/analyzer) to amplify the region from 32,340,082 to 32,340,423 in exon 11 of the BRCA2 gene. Table 1 shows the primer sequences of the targeted regions in this study.

Polymerase chain reaction was performed in a final volume of 25 µL that contained 100 ng of genomic DNA, 10× TBE buffer, DNTPs, $MgCl_2$, Taq polymerase, and primers at 95°C for 5 min; 35 cycles of 30 s at 95°C; 20 s at annealing temperature; 30 s at 72°C; and one cycle for a final extension at 72°C for 7 min. Polymerase chain reaction products were sequenced using a Big Dye Terminator v3.1 Cycle Sequencing Kit and ABI PRISM® 310 Genetic Analyzer (Applied Biosystems, USA). Sequencing was achieved using 2F, 2R, 11F and 11R primers and products were read using Geneious program and resultant sequences were analyzed and compared to the published sequences of target regions in GenBank using BLAST tool in Center for Biotechnology Information (NCBI). Detected variations were documented and analyzed using database concerning the previously identified mutations of BRCA genes (BRCA Mutation Database). IBM SPSS Statistics version 18 was used for statistical analysis. A P-value<0.05 was considered statistically significant.

Results

We identified 2 mutations in exon 11 of the BRCA2 gene. Each of the detected mutations in exon 11 was found in 2/50 (4%) of breast cancer patients. A point mutation was also identified in exon 2 of the BRCA1 gene in one of the 50

Table 3. Phenotype characteristics and genetic analysis results of breast cancer patients.

Patient	Age at diagnosis (years)	Tumor		Receptor status			Family history	Exon 2	Exon 11
		Grade (1-3)	Size (mm)	ER	PR	Her2			
29	44	3	10	+	+	-	+	Normal	Normal
30	37	2	22	-	-	++	-	Normal	Normal
31	37	3	20	+	+	-	+	Normal	Normal
32	44	3	50	-	+	+	-	Normal	Normal
33	39	3	40	-	-	+	-	Normal	Normal
34	47	2	35	++++	++++	+++	+	Normal	Normal
35	42	2	23	-	-	+	-	Normal	Normal
36	41	3	30	-	-	-	+	Normal	Normal
37	43	1	20	-	-	+++	-	Normal	Normal
38	48	2	44	-	-	+	+	Normal	Normal
39	42	1	20	-	-	++	+	Normal	Normal
40	49	3	75	++	++	-	-	Normal	Normal
41	28	3	40	-	-	-	+	Normal	Mutant
42	36	1	21	-	++	+	+	Normal	Normal
43	38	1	14	-	+	+	+	Normal	Normal
44	43	2	27	-	-	+	+	Normal	Normal
45	48	3	45	-	-	-	+	Normal	Mutant
46	44	3	65	+	+	++	+	Normal	Normal
47	45	2	40	+	+	+	+	Normal	Normal
48	35	1	24	-	-	-	-	Normal	Normal
49	41	1	17	+++	+++	+++	+	Normal	Normal
50	48	1	10	+	+	-	+	Normal	Normal

studied breast cancer patients; 2% of breast cancer patients had a mutation in exon 2 of the BRCA1 gene. The detected mutations involved 2 deletions in exon 11 of the BRCA2 gene (6205 delA and 6284 delA) and one substitution mutation 184(T>C) in exon 2 of the BRCA1 gene. There were no founder mutations in our studied population. Tables 2 and 3 show the phenotype

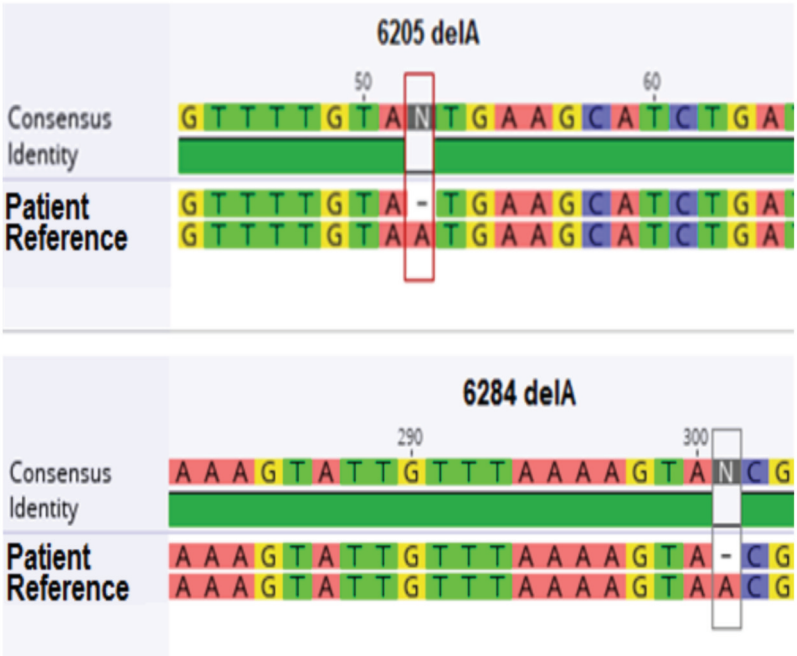

Figure 1. 6205 delA and 6284 delA mutations detected in exon 11 of the BRCA2 gene.

characteristics and results of analysis of exons 2 and 11 in the breast cancer patients.
Figures 1 and 2 show mutations 6205 delA and 6284 delA detected in exon 11 and the 184(T>C) mutation detected in exon 2.

Discussion

To the best of our knowledge, the detected mutations in exon 11 of the BRCA2 gene were not mentioned in any previous study. They might be novel and specific for the Syrian population. The detected mutation in exon 2 of the BRCA1 gene was previously identified by Sweet et al. in 2010[26] and Tavtigian et al. in 2008.[27] This mutation was classified as a definitely pathogenic missense mutation. We did not expect to locate any founder mutations because the current study population was not a candidate for this type of search. The mean age at diagnosis for breast cancer patients that carried the BRCA gene mutations (36 years) was significantly lower than the mean age at diagnosis for the BRCA mutation negative patients (42.94 years; P=0.034). We investigated the likelihood of a correlation between the detected mutations with some phenotype characteristics – family history of breast cancer, age at diagnosis of disease, and triple negative molecular subtype. There were no significant correlations found between exon 11 mutation and family history of breast cancer (Pearson chi-square, df=1, P=0.269). We observed no significant association between mutations of exon 11 and younger age at diagnosis (≤40 years) of breast cancer (Pearson chi-square, df=1, P=0.763). Mutations of exon 11 in the BRCA2 gene had a significant association with the triple negative subtype in Syrian familial breast cancer patients (Pearson chi-square, df=1, P=0.003). This finding was consistent with Yeh et al.[28] who found that the presence of a BRCA1 or BRCA2 mutation seemed to be positively associated with TNBC. Our result came consistent with Yeh et al. (2017)[28] who found that having a BRCA1 or BRCA2 mutation seemed to be positively associated with TNBC. On the other hand, they found that BRCA1 mutations were more frequent than BRCA2 mutations in patients with the triple negative subtype of breast cancer which contrasted our results. Luporsi et al.[29] also found that BRCA mutations were more frequent in the TNBC group. However, they also found more BRCA1 mutations compared to BRCA2 mutations in the triple negative group. These

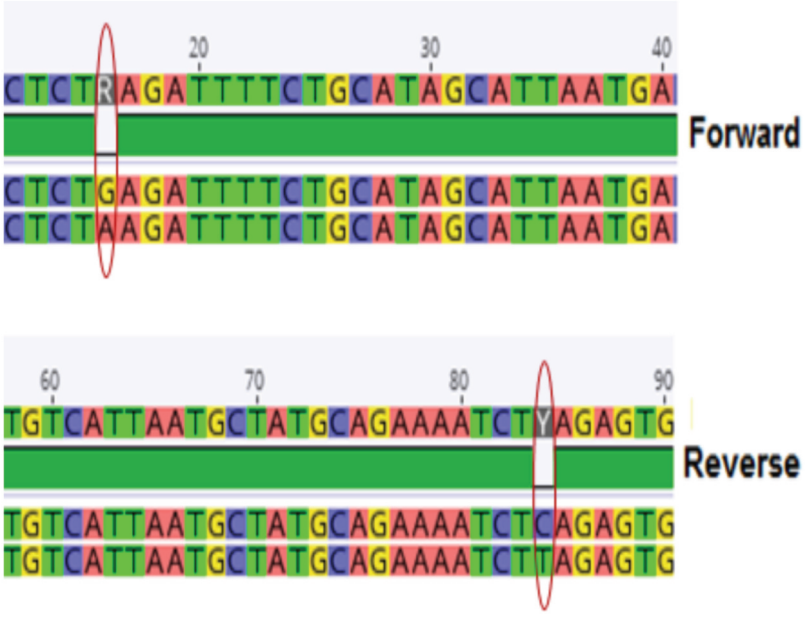

Figure 2. 184(T>C) mutation detected in exon 2 of the BRCA1 gene.

differences may be attributed to the variation in the overall frequencies of BRCA1 and BRCA2 detected mutations between our study and their studies. A mutation of exon 2 in the BRCA1 gene was found in one of the breast cancer patients with bilateral breast cancer who was diagnosed at the age of 36 and had a significant family history of two first degree relatives with breast cancer. The patient underwent a bilateral surgical mastectomy at the age of 37. This result was consistent with the result obtained by de Juan Jiménez et al. in 2013.[30] They found that probands with family histories of breast cancer in at least two first degree relatives were more likely to have BRCA1/BRCA2 mutations. These researchers observed that most BRCA1mutations occurred in probands with early-onset breast cancer.

Conclusion

To the best of our knowledge, this study was the first that investigated mutations in selected regions of the BRCA1 and BRCA2 genes in breast cancer cases considered to be familial in Syria. The mutations 6205 delA and 6284 delA detected in exon 11 of the BRCA2 gene and 184(T>C) mutation detected in exon 2 of the BRCA1 gene might enrich our growing genetic database for Syrian breast cancer patients. Our results have suggested a possible association of mutations of exon 11 in the BRCA2 gene with the triple negative molecular subtype of breast cancer. This association needs to be confirmed by future studies that include larger numbers of Syrian familial breast cancer patients.

Conflict of Interest

None declared.

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