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Identification of novel *BRCA* founder mutations in Middle Eastern breast cancer patients using capture and Sanger sequencing analysis

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Ethnic differences of breast cancer genomics have prompted us to investigate the spectra of *BRCA1* and *BRCA2* mutations in different populations. The prevalence and effect of *BRCA 1* and *BRCA 2* mutations in Middle Eastern population is not fully explored. To characterize the prevalence of *BRCA* mutations in Middle Eastern breast cancer patients, *BRCA* mutation screening was performed in 818 unselected breast cancer patients using Capture and/or Sanger sequencing. 19 short tandem repeat (STR) markers were used for founder mutation analysis. In our study, nine different types of deleterious mutation were identified in 28 (3.4%) cases, 25 (89.3%) cases in *BRCA 1* and 3 (10.7%) cases in *BRCA 2*. Seven recurrent mutations identified accounted for 92.9% (26/28) of all the mutant cases. Haplotype analysis was performed to confirm c.1140 dupG and c.4136_4137delCT mutations as novel putative founder mutation, accounting for 46.4% (13/28) of all *BRCA* mutant cases and 1.6% (13/818) of all the breast cancer cases, respectively. Moreover, *BRCA 1* mutation was significantly associated with *BRCA 1* protein expression loss ($p = 0.0005$). Our finding revealed that a substantial number of *BRCA* mutations were identified in clinically high risk breast cancer from Middle East region. Identification of the mutation spectrum, prevalence and founder effect in Middle Eastern population facilitates genetic counseling, risk assessment and development of cost-effective screening strategy.

Breast cancer is the most common female malignancy worldwide as well as in Saudi Arabia, accounting for 96 per 100,000 women in the Western countries and 22.7 per 100,000 women in Saudi Arabia.[1,2] The incidence rate has increased progressively over the last decade in Saudi Arabia.[2] Several studies have showed that median age of patients at diagnosis in Saudi Arabia is a decade younger and present with more advanced stages of malignancy compared to the Western population.[3,4] Similar observations have also been reported from other Middle Eastern countries.[5–8] These data indicate the differences of breast cancer epidemiology and biology between different ethnic populations.

Germline mutations of breast cancer susceptibility Gene 1 and 2 (*BRCA*1 and *BRCA*2) have been estimated to contribute to 5–10% of all breast cancer cases.[9] The proportion of familial breast cancer attributable to a mutation of *BRCA1* or *BRCA2* gene has been reported up to 40 and 25%, respectively.[10–13] The prevalence of *BRCA* mutations has also been shown to be strongly influenced by patient age, demonstrating highest frequency in younger age group cases.[14] Similar observations have been made with respect to triple-negative breast cancer (TNBC) phenotype.[15,16] However, genetic test conducted in different populations for the mutation in *BRCA* 1 and 2 reveals the ethnic diversity of prevalence, spectrum and founder effect of *BRCA1/BRCA2* mutations in breast cancer.[17] These differences can be attributed to several factors including lifestyle, reproduction and genetics.

To date, studies of *BRCA1* and *BRCA 2* mutations in Middle Eastern population are limited and only scarce data is available.[18–21] One study only focused on *BRCA1* mutations[21] while another study analyzed both *BRCA*1/2 but targeted only certain exons.[19] Thus, there is a lack of comprehensive screening of the two *BRCA* genes' mutations in this

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What's new?
Genetic testing for *BRCA* mutations reveals the ethnic diversity of prevalence and spectrum of *BRCA1/BRCA2* mutations in breast cancer. Compared with other populations, however, little is known about ethnic differences in breast cancer genomics in populations in the Middle East region. Here, *BRCA* mutation screening was carried out in 818 Middle Eastern breast cancer patients. The authors identify two putative founder mutations—together accounting for more than 46% of *BRCA* cases—and a particular spectrum of deleterious *BRCA* mutations, which may be unique to the population. The findings could impact genetic counseling in Middle Eastern populations.

population. In addition to the spectrum of BRCA1 and *BRCA 2* mutation, the founder mutations, as specific targets, can be used to make screening approach in order to reduce the cost and turnaround time of genetic testing. Since it has been shown that the penetrance of different mutations in *BRCA* genes differs,[22] identification of founder mutation may provide a large patients cohort to assess the accurate effectiveness of preventive measures. All these highlight the need to further elucidate the prevalence, spectrum and founder effect of *BRCA1* and *BRCA2* mutations in breast cancer from Middle East and develop the panel for screening approach in order to improve genetic testing strategy, which in turn should facilitate not only cancer risk assessment but also cancer prevention.

In this study, we sought to determine the spectrum, prevalence and founder effect of *BRCA1* and *BRCA2* mutations to identify the epidemiologic characteristics of these two genes' mutations in Middle Eastern breast cancer patients.

Material and Methods

Study population

A total of 818 unselected archival samples of patients from all over Saudi Arabia diagnosed with primary breast cancer between 1990 and 2011 were selected from the files of King Faisal Specialist Hospital & Research Centre, Riyadh. Among the first full gene screening set of 302 breast cancer cases, 88 non-tumor DNA samples were sent to a private vendor (Oxford Gene Technology, UK) for targeted capture sequencing of *BRCA 1* and *BRCA 2*, whereas 214 tumor-DNA samples with insufficient specifications for targeted capture were selected for traditional Sanger sequencing in house. Remaining 516 samples with limited amount of DNA were recruited for specific mutation screening using a panel of all nine mutations detected in first full gene screening set. High risk cases in this study were defined as those who: (*i*) had at least one first and/or second degree relative with breast cancer or ovarian cancer; (*ii*) were less than or equal to 40 years of age at diagnosis; (*iii*) had triple negative tumor; (*iv*) had bilateral breast cancer. Detailed clinicopathological parameters including age at diagnosis, ethnicity, history of malignancies and so forth were retrieved from case records and Integrated Clinical Information System (ICIS). Family history of possible breast and ovarian cancer in first and second degree relatives were also obtained. Patient follow-up data was obtained using clinical data chart and ICIS. All data collected was consented. The Institutional Review Board of the King Faisal Specialist Hospital and Research Centre approved the study under Project No. 2140-008.

DNA isolation

DNAs were isolated from formalin-fixed, paraffin-embedded (FFPE) breast cancer tumor tissues and/or non-tumor tissues using Gentra DNA isolation kit (Gentra, Minneapolis, MN) following the manufacturer's recommendations as described previously.[23,24]

Targeted capture sequencing of BRCA1/BRCA2 mutations

88 breast cancer non-tumor DNA samples with sufficient specifications for targeted capture were sent to Oxford Gene Technology (OGT, Oxfordshire, UK) for *BRCA1* and *BRCA2* mutation detection using Targeted Capture Sequencing technology with an average depth of coverage of 302X (Supporting Information Table 1). See Supporting Information Methods for details of enrichment, sequencing and mutation detection.

PCR and Sanger sequencing for detection of BRAC1/BRCA2 mutations

Direct sequencing of the entire coding/splicing region of *BRCA1* and *BRCA2* were performed on 214 samples, which were deemed of insufficient specifications for targeted capture. Detected mutations by Capture or Sanger sequencing were further confirmed for their germline origin by analyzing the corresponding non-tumor DNA from separate FFPE blocks. Finally, additional 516 samples were screened for the germline mutations using the panel comprising nine mutations detected in the first full gene screening set. Primer 3 software was used to design the primers for all coding exons and their flanking intronic sequences of *BRCA1* and *BRCA2* genes (available upon request). PCR and Sanger sequencing were performed as described in supplementary Methods.

Assessment of pathogenicity of variants

Previously reported pathogenic mutations were considered pathogenic as inferred from the Universal Mutation Database (UMD, http://www.umd.be/BRCA1/and http://www.umd.be/BRCA2/), Breast Cancer Information Core Database of National Institute of Health (BIC, http://research.nhgri.nih.gov/bic/) and literature evidence.

Genotype and haplotype analysis

Genotype analyses were performed if the recurrent mutation was observed in more than two cases from unrelated families. The individuals from different families sharing identical *BRCA1* mutation and 54 blood donor control samples were genotyped at 19 different highly polymorphic short tandem repeat (STR) markers in or

adjacent to *BRCA1* located on chromosome 17 including D17S1836, D17S1818, D17S1299, D17S800, D17S846, D17S1793, D17S855, D17S1320, D17S1322, D17S1323, D17S1325, D17S1326, D17S1327, D17S1789, D17S951, D17S930, D17S1334, D17S931 and D17S1859. PCR primer sequences for all STR markers were obtained from the genome database (http://genome.ucsc.edu). PCR was performed as described in supplementary Methods. Size fractionation of PCR products was performed on a 3130xl Genetic Analyzer (Applied Biosystems) using the LIZ 500 Size Standard and analyzed using Softgenetics GeneMaker software (SoftGenetics, LLC. State College, PA). Haplotype analysis and mutation age estimation analysis were performed using PHASE version 2.1.1 and DMLE+ version 2.3 as described in Supplementary Methods.

Tissue microarray construction and Immunohistochemistry

For each tumor, representative areas were selected, mapped and tissue microarray was constructed. The tissue microarrayer (Semiautomated Arrayer, CM1 Mirlacher, Neuenburg, Germany) was used to prepare a tissue microarray block as described previously.[25] Sections from TMA blocks were subjected to immunohistochemical staining using routine protocol as done before.[24] Antibody clone, manufacturer and dilution used for each antibody are given in Supporting Information Table 2.

Triple Negative Breast Cancer (TNBC) was defined as ER, PR negative (cut off of nuclear positivity $\geq$1%) and Her-2 negative.[26] Her 2-overexpression was assessed according to ASCO/CAP guidelines.[27] Cases with Her-2 Score 2 were subjected to Her-2 FISH analysis. Her-2 FISH was performed utilizing the PathVysion Vysis LSI HER-2/neu Kit from Vysis (Abott Molecular, IL) according to the manufacturers recommendations. Slides were screened using the BX51 Olympus fluorescence microscope (Olympus, Richardson, TX) and a SenSysW CCD camera (Photometrics, Tucson, AZ) was used to capture selected images. Twenty to thirty tumor nuclei per core, with both red and green signals were selected for scoring. The ratio of Her-2 to chromosome 17 centromeric region of $>$2.2 was considered as amplified.

BRCA 1 and *BRCA 2* immunostaining was assessed qualitatively in breast cancer cells and nuclear staining was taken into consideration. Intensity of nuclear *BRCA* protein expression was graded as strong (Score 3), moderate (Score 2), weak (Score 1) and absent staining (Score 0). Tumor cells showing Score 0 were considered negative for *BRCA* expression and Score 1 and above were considered positive for *BRCA* expression.[28]

IHC scoring was done by two pathologists, blinded to the clinicopathological characteristics. Discordant scores were reviewed together to achieve agreement.

Statistical analysis

The JMP10.0 (SAS Institute, Cary, NC) software package was used for data analyses. Contingency table analysis and χ^2 tests were used to study the relationship between clinicopathological variables and biomarker. The limit of significance for all analyses was defined as a *p* values of 0.05; two-sided tests were used in all calculations.

Results

Sample characteristics

In this study, sequencing of all coding exons and their splicing sites of *BRCA1* and *BRCA2* gene was carried out by Capture or Sanger sequencing on 302 archival breast cancer samples. Clinicopathological details of 818 patients are as follows: Majority of the patients (81.6%) was in Stage II and Stage III. Grade 2 constituted most common grade (50.4%) followed by Grade 3 (40.9%). 20.3% of cases had positive family history of either breast or ovarian cancer in either first or second degree relatives. 32.5% of cases were early-onset (age at diagnosis $\leq$40 years). 15.3% of cases were TNBC.

Identification of BRCA mutations and development of validation screening assay for BRCA mutation

Extensive sequencing of all coding exons and their splicing regions in *BRCA 1* and *BRCA 2* of 302 samples out of 818 patients were performed using either Capture or Sanger sequencing. We identified nine different types of deleterious mutation in 14 (4.6%) cases. Of 14 mutant cases, 12 (85.7%) were in *BRCA 1* and two (14.3%) were in *BRCA 2* (Table 1). Among the early-onset cases, seven (7/102, 6.9%) had a *BRCA 1* mutation, two (2/102, 2.0%) harbored a *BRCA 2* mutation. Of the cases with positive family history, six (6/60, 10%) carried a *BRCA 1* mutation. Of the TNBC cases, eight (8/56, 14.3%) had a *BRCA 1* mutation. None of *BRCA 2* mutations was found in TNBC cases or in cases with positive family history (Table 2 and Supporting Information Table 3). The only one bilateral breast cancer case in this group didn't show carrying any mutations in both *BRCA 1* and *2* genes. The spectra of the nine types of identified deleterious mutations are present in Table 1.

Since only nine different types of deleterious mutation were identified in the full gene screening set, we thereafter developed specific mutation screening assay using the panel comprising these nine mutations in order to further screen additional 516 cases. Among the 516 cases, six types of deleterious mutation were identified in 14 (2.7%) cases, 13 in *BRCA 1* and one in *BRCA 2* (Table 1). Compared to full gene screening set, the specific mutation screening assay using this panel could identify 58.7% (2.7 *vs.* 4.6%) of all mutant cases. Taken all together, BRCA mutations were detected in 3.4% (28/818) of all breast cancer cases. Seven recurrent mutations were identified in 26 cases in our cohort. The overall frequency of the cases carrying recurrent mutation was 3.2% (26/818) and recurrent mutations accounted for 92.9% (26/28) of all the mutant cases (Table 1). Interestingly, three mutations (*BRCA 1* c.1140dupG, c.4136_4137delCT and c.5530delC) were reported only once in UMD database and not reported in BIC database, accounting for 60.7% (17/28) of all mutant cases. All these three mutations were recurrent frameshift *BRCA 1* mutation, as shown in Table 1.

Founder mutation confirmation and age estimation

In our study, a total of three recurrent *BRCA 1* mutations (c.1140dupG, c.4136_4137delCT and c.5530delC) observed in

Table 1. Spectrum of BRCA deleterious mutations identified

Gene	Exon	Mutation	No. of Cases		BIC Database		UMD Database Entries
			First Set[1]	Second Set[2]	Entries	Ethnicity	
BRCA1	11	c.1140dupG; p.Lys381Glufs	1	4	0	NA	1
	11	c.4065_4068delTCAA; p.Asn1355Lysfs	2	0	133	Worldwide[3]	59
	12	c.4136_4137delCT; p.Ser1379X	4	4	0	NA	1
	15	c.4524G>A; p.Trp1508X	1	1	23	Europe, Caucasian/Native American, Ashkenazi	0
	18	IVS18+1G>C	1	0	2	W. Europe	0
	20	c.5251C>T; p.Arg1751X	2[4]	1[4]	34	Worldwide[5]	15
	24	c.5530delC; p.Leu1844SerfsX11	1	3	0	NA	1
BRCA2	11	c.6025C>T; p.Gln2009X	1	0	3	W. Europe, Ashkenazi	1
	13	c.7007G>A; p.Arg2336His[2]	1	1	19	Europe, Canadian	0

[1]: Full Gene Screening Assay.
[2]: Specific Mutation Screening Assay.
[3]: European, Ashkenazi, African, Canadian, Irish, Latin-American, Hispanic Mexican, Native American and Chinese.
[4]: 1st and 3rd degree relatives;
[5]: European, African, Indian, Greek, Asian, Latin-American and Native American.

Table 2. Correlation of BRCA-1 mutation with clinic-pathological parameters in breast cancer in full gene screening set

	Total		Positive		Negative	
	N	%	*N*	%	*N*	%
Total Number of Cases	302		12	4.0	290	96.0
Age Groups						
≤ 40	102	33.8	7	6.8	95	93.2
> 40	200	66.2	5	2.5	195	97.5
Family History						
No	199	76.8	3	1.5	196	98.5
Yes	60	23.2	6	10.0	54	90.0
Triple Negative						
No	234	80.7	4	1.7	230	98.3
Yes	56	19.3	8	14.3	48	85.7

more than two cases from unrelated families were identified (Table 1). To determine if these mutations arose from a common founder, haplotype analysis was carried out for all cases harboring these mutations with 54 control samples. Our results revealed that all the carriers of c.1140 dupG and c.4136_4137delCT mutation shared the same haplotype with length of 1.04 and 1.46 MB, respectively suggesting that these two recurrent mutations are putative founder mutation derived from common ancestor (Supporting Information Tables 4 and 5). To the best of our knowledge, we are the first to report these two mutations as founder mutation. Furthermore, the results of age estimation showed the average age of c.1140dupG and c.4136_4137delCT mutation as 21 generations (16–27 generations; 95% CI) and 25 generations (20–33 generations; 95% CI), respectively. In our cohort, the two founder mutations accounted for 46.4% (13/28) of all *BRCA* mutant cases, giving an overall rate of 1.6% (13/818) of all the breast cancer cases in this cohort.

Clinicopathological characteristic and mutational status

As shown in the Table 3, the mean age of onset for *BRCA1* mutation carriers was 40.1 years, about 6.6 years younger than that of non-carriers (46.7, $p = 0.011$). However, no significant difference was found in age at diagnosis between *BRCA2* mutation carriers and non-carriers due to only three cases harboring *BRCA 2* mutations ($p = 0.102$) in our cohort.

In our cohort, the *BRCA1* mutations were observed to be significantly associated with early-onset (5.6 *vs.* 1.8%, $p = 0.0042$), family history (9.8 *vs.* 0.9%, $p < 0.001$) and TNBC cases (12.2 *vs.* 1.3%, $p < 0.0001$), respectively (Table 4). However, the *BRCA2* mutations were significantly associated with early-onset cases only (1.1% *vs.* 0, $p = 0.0093$) (Supporting Information Table 6).

Among 423 high-risk cases, *BRCA* mutations were present in 26 cases (6.2%). Moreover, *BRCA* mutation were detected in eight of 49 cases (16.3%) with early-onset and TNBC, 19 of 58 of cases (17.2%) with family history and early–onset, nine of 25 cases (36.0%) with family history and TNBC, six of 16 cases (40.0%) with early-onset, family history and TNBC. The difference between low-risk cases and each subset of high-risk cases was significant ($p < 0.0001$). Interestingly, in 341 low risk cases, none carried *BRCA1* or *2* mutations (Table 5).

In our study, we were able to collect family history for six out of 13 cases carrying the two founder mutations. In the two cases carrying the founder mutation c.1140 dupG, the first case had one first-degree relative diagnosed with breast cancer and two first-degree relatives developed ovarian cancer. The second case had one first-degree relative with breast cancer. In the remaining four cases harboring founder

Table 3. Correlation of BRCA mutation with onset age in breast cancer

818	BRCA 1 Positive Mean ± SD	BRCA 1 Negative Mean ± SD	BRCA 2 Positive Mean ± SD	BRCA 2 Negative Mean ± SD
Age	40.12 ± 11.52	46.68 ± 11.90	34.33 ± 7.37	46.52 ± 11.93
p values	0.011		0.102	

Table 4. Correlation of BRCA-1 mutation with clinicopathological parameters in breast cancer

	Total		Positive		Negative		
	N	%	*N*	%	*N*	%	*p* values
Total Number of Cases	818		25	3.1	793	96.9	
Age Groups							
≤ 40	266	32.5	15	5.6	251	94.4	0.0042
> 40	552	67.5	10	1.8	542	98.2	
Family History							
No	562	79.7	5	0.9	557	99.1	< 0.0001
Yes	143	20.3	14	9.8	129	90.2	
Triple Negative							
No	683	84.7	9	1.3	674	98.7	< 0.0001
Yes	123	15.3	15	12.2	108	87.8	

Table 5. Prevalence of BRCA mutation according to the combination of different risk factors to carry BRCA mutation in breast cancer

	Total		Positive		Negative		
	N	%	*N*	%	*N*	%	*p* values
Total Number of Cases	818		28	3.5	790	96.5	
Any-1-Positive							
Low Risk	341	44.6	0	0	341	100.0	<0.0001
Yes	423	55.4	26	6.2	397	93.8	
FH + Age ≤ 40							
Low Risk	341	85.5	0	0	341	100.0	<0.0001
Yes	58	14.5	10	17.2	48	82.8	
FH + TNBC							
Low Risk	341	93.2	0	0	341	100.0	<0.0001
Yes	25	6.8	9	36.0	16	64.0	
TNBC + Age ≤ 40							
Low Risk	341	87.4	0	0	341	100.0	<0.0001
Yes	49	12.6	8	16.3	41	83.7	
FH+ TNBC +Age ≤ 40							
Low Risk	341	95.8	0	0	341	100.0	<0.0001
Yes	16	4.2	6	40.0	9	60.0	

mutation c.4136_4137delCT, two had one first-degree relative with breast cancer, the third case had one second-degree relative affected with ovarian cancer, and the last case had one third-degree relative and two fourth degree-relatives diagnosed with breast cancer.

BRCA immunohistochemistry

Loss of *BRCA 1* expression was seen in 17.9% (143/797) of unselected breast cancer cases. This *BRCA 1* protein expression loss was significantly associated with *BRCA 1* mutation ($p = 0.0005$), triple negative phenotype ($p = 0.0003$) and a trend was

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seen with BRCA-2 mutation ($p = 0.0626$). *BRCA2* loss of protein expression was seen in 10.4% (83/794) of the cases. No association was detected between *BRCA 2* IHC loss and *BRCA 2* mutation, however both *BRCA 1* and *BRCA 2* protein expression loss were significantly associated with each other ($p = 0.0012$). Interestingly *BRCA 2* protein loss was significantly associated with large tumor size of >5 cm ($p = 0.0395$).

Discussion

In this study, we have reported the contribution of deleterious mutations of *BRCA 1* and *BRCA 2* to breast cancer from Saudi Arabia. Since the incidence rate of breast cancer has increased in the last decade[2], it has become a matter of great concern to the population in Middle East. Therefore, it is important to better understand the cause of breast cancer among Middle Eastern population in order to improve the prevention and assessment of breast cancer risk. To the best of our knowledge, this is the first large cohort study in Middle Eastern population comprising 818 breast cancer cases. Our finding revealed that the proportion of *BRCA* deleterious mutation is 3.4% in breast cancer patients and TNBC was the most important high-risk factor for BRCA mutations, following by family history and onset age. The prevalence of BRCA mutation in cases with two or more high-risk factors was significantly higher than that in low risk cases. Our result is comparable to the results from western populations.[12,29–32]

Unlike some Asian populations in which *BRCA 2* mutations are more common,[33–37] we found a predominance of *BRCA1* mutations (89.3%) in our cohort, similar to that reported in Western populations.[14] These could possibly be due to similarity of genetic makeup between Middle Eastern population and Western population.

In this study, we discovered three recurrent deleterious *BRCA 1* mutations (c.1140dupG, c.4136_4137delCT and c.5530delC) that were rarely reported previously, but interestingly, the proportion of these mutations are relatively high in our cohort of breast cancer cases. This shows that the spectrum of *BRCA* mutation in our breast cancer patients from Middle East region is different from the rest of world. Notably, we identified two (c.1140dupG and c.4136_4137delCT) out of these three recurrent mutations as putative founder mutations. Interestingly, these two mutations have not been reported as founder mutation previously. Due to the Middle Eastern conservative culture,[38,39] we were not able to collect the family history for all cases involved in this study. Among the six founder mutation carriers in which family history were available, we observed strong hereditary history of breast cancer as well as ovarian cancer suggesting that these two novel founder mutation were not only associated with breast cancer but also with ovarian cancer. The third recurrent mutation which was not found to be a founder mutation could be attributed to the fact that this recurrent mutation and STR markers are no longer in linkage disequilibrium. Since founder mutations can provide larger patient cohort not only for the penetrance study of *BRCA* mutations but also for the assessment of breast cancer risk in a specific population, a larger scale study is required for evaluation of association between the founder mutation and breast cancer risk.

Since *BRCA1* and *BRCA 2* are large genes, coding a protein of 1,863 and 3,418 amino acids, respectively, the discovery of the spectrum of mutation and founder effect in a specific population may provide a cost effective approach for rapid screening in this population. In this study, our finding revealed that 58.7% of all mutant cases of breast cancer could be identified by the panel of nine mutations. Hence, we recommend that this panel can be used for diagnostic setting in breast cancer from Middle Eastern population and greatly reduce the cost and turnaround time of genetic testing. However, a large cohort of unselected breast cancer cases is required to further determine the sensitivity of this panel in unselected patients from Middle East region. In addition, we extracted BRCA1&2 genes data from Saudi Arabian whole genome database of around 207 healthy females, in which no *BRCA* mutations were identified. However, a large population based study is required to further investigate the contribution and characteristics of *BRCA 1 & 2* mutations in Middle Eastern population.

Loss of *BRCA 1* protein expression was significantly correlated with *BRCA 1* mutational status in our patients. Majority of *BRCA 1* mutations in our cohort are either frameshift or stop gain that cause protein truncation. These findings could partially explain the significant association between *BRCA* 1 mutation and loss of protein expression. However, we could not find similar association between loss of expression and mutation in *BRCA 2* gene. This data is in concordance with similar results previously shown by other authors.[40]

In conclusion, we conducted a *BRCA1* and *BRCA 2* gene mutation analysis in a large cohort from Middle Eastern population. Two novel founder mutations were identified and these two novel founder mutations accounts for 46.4% of all *BRCA* mutant cases and 1.6% of all breast cancer cases, respectively. Knowledge about mutation distribution in Middle Eastern ethnic population is extremely important for cost-effective genetic testing strategy for *BRCA 1* and *BRCA 2* gene mutations. Since all of the founder and recurrent mutations identified in this study are detected in the high risk breast cancer patients, we recommend the use of this panel of mutations in all high risk breast cancer patients defined as those who: (*i*) had at least one first and/or second degree relative with breast cancer or ovarian cancer; (*ii*) were less than or equal to 40 years of age at diagnosis; (*iii*) had triple negative tumor; (*iv*) had bilateral breast cancer. Using this panel will not only help in identifying patients with hereditary breast cancer, but it will also allow us to identify family members of the patients who carry these mutations. Hopefully, through a national strategy for genetic testing, genetic counseling will eventually lead to prevention and decrease of the incidence of breast cancer in this ethnic population.

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