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Breast Cancer

BRCA1 and *BRCA2* Mutations in Ethnic Lebanese Arab Women With High Hereditary Risk Breast Cancer

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Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. Breast cancer • Young age • Family history • *BRCA* mutations • Haplotype • Lebanon • Arab countries

Abstract

Purpose. Breast cancer is the most common malignancy among women in Lebanon and in Arab countries, with 50% of cases presenting before the age of 50 years.

Methods. Between 2009 and 2012, 250 Lebanese women with breast cancer who were considered to be at high risk of carrying *BRCA1* or *BRCA2* mutations because of presentation at young age and/or positive family history (FH) of breast or ovarian cancer were recruited. Clinical data were analyzed statistically. Coding exons and intron-exon boundaries of *BRCA1* and *BRCA2* were sequenced from peripheral blood DNA. All patients were tested for *BRCA1* rearrangements using multiplex ligation-dependent probe amplification (MLPA). *BRCA2* MLPA was done in selected cases.

Results. Overall, 14 of 250 patients (5.6%) carried a deleterious *BRCA* mutation (7 *BRCA1*, 7 *BRCA2*) and 31 (12.4%) carried a variant of uncertain significance. Eight of 74 patients (10.8%) aged ≤40 years with positive FH and only 1 of 74 patients (1.4%) aged ≤40 years without FH had a mutated *BRCA*. Four of 75 patients (5.3%) aged 41–50 years with FH had a deleterious mutation. Only 1 of 27 patients aged >50 years at diagnosis had a *BRCA* mutation. All seven patients with *BRCA1* mutations had grade 3 infiltrating ductal carcinoma and triple-negative breast cancer. Nine *BRCA1* and 17 *BRCA2* common haplotypes were observed.

Conclusion. Prevalence of deleterious *BRCA* mutations is lower than expected and does not support the hypothesis that *BRCA* mutations alone cause the observed high percentage of breast cancer in young women of Lebanese and Arab descent. Studies to search for other genetic mutations are recommended. ***The Oncologist*** 2015;20:357–364

Implications for Practice: This study provides new data to support discussion and referral of patients of Lebanese and Arab ancestry with high-genetic-risk breast cancer for *BRCA* counseling and testing. The probability of carrying a deleterious *BRCA* mutation in this population seems low at 5.6%. The absence of family history in patients aged ≤40 years reduces the possibility of *BRCA* mutations to only 1.4%. Young age combined with a positive family history raises the prevalence to 10.8% and increases the yield of testing. Further clarification of the 12.4% of cases with variants of uncertain significance and searches for alternative gene mutations are needed. This study adds missing information to the international *BRCA* population maps.

Introduction

Breast cancer is the most common cancer seen in women [1]. Many differences with respect to age, stage at presentation, and biological characteristics exist among various countries and ethnic groups. In the U.S., approximately 65% of all women newly diagnosed with breast cancer are aged >55 years, whereas in most low- and middle-income countries, almost half of women with newly diagnosed breast cancer are aged <50 years [2]. Although early stage breast cancer has become most common in industrialized nations, locally advanced and metastatic disease at presentation remain very common in the developing countries [2], with only recent reports indicating a shift toward early stages because of active widespread awareness and early detection campaigns [3].

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Arab countries are home to >359 million people, according to the United Nations Development Program's Arab Human Development Reports, and studies, reviews, and available cancer registries have shown that breast cancer is the most commonly diagnosed cancer among Arab women [2, 4, 5]. Although the age-standardized incidence rates (ASRs) are still below those of industrialized nations, they are rising. ASR for breast cancer in Lebanon, a country of 4 million residents, increased from 20 of 100,000 women per year in 1964 to 69 of 100,000 women in 2004 [6].

In several studies from Lebanon and neighboring countries, median age at onset of breast cancer was shown to range between 48 and 52 years [2, 4, 5]. Hospital-based registries and national collection of data by the Lebanese Ministry of Health showed that almost half of cases are diagnosed in women aged <50 years, with a significant number of cases diagnosed at <40 years and even <35 years [2, 5–7]. This presentation at young age has been reported to confer worse prognosis [8, 9].

Genetic susceptibility factors play important roles in predisposition to breast cancer. To date, mutations in *BRCA1* or *BRCA2* are the strongest known breast cancer predictors, and there is a wide variability in the specific types of mutations and their frequency in different geographic areas and ethnicities. Differences in study inclusion criteria and mutation-detection techniques can also partly explain differences in prevalence of *BRCA1* and *BRCA2* mutations between different populations [10].

Only a few small studies have looked at *BRCA* mutations in Arab countries. Data from Saudi Arabia, for example, showed a lack of association of *BRCA1* and *BRCA2* variants with100 breast cancer patients [11]. A study of 35 young patients from Sudan suggested an important role for *BRCA 1* and *BRCA2* genetic mutations [12]. Studies from North Africa looked at 36 selected patients found to have 4 *BRCA1* and 2 *BRCA2* frameshift mutations [13]. Moreover, 6%–36% *BRCA1* mutations were described in a cohort of familial patients from Algeria [14] and 31% from Morocco [15]. Four *BRCA1* exons and 1 *BRCA2* exon were assessed in 60 selected and referred Egyptian women and showed a mutation prevalence rate of 25% [16]. As for Lebanon, a cohort of 72 women with early onset breast cancer and/or positive family history who were referred for testing showed deleterious *BRCA1* and *BRCA2* mutations in 12.5% of patients [17]. The variability of results from the above-mentioned small studies depends on patient-selection criteria and referral patterns.

The aim of our study was to determine the prevalence and roles of previously known and unknown *BRCA1* and *BRCA2* mutations in Lebanese women with breast cancer who were at a high risk of harboring hereditary disease because of presentation at young age and/or positive family history. This project is an important milestone in determining the prevalence of genetic predisposition to breast cancer in Lebanese women and other ethnic Arab and Middle Eastern populations.

Patients and Methods

Study

We tested for *BRCA1* and *BRCA2* genetic mutations in a cohort of breast cancer patients who were considered to be at high risk of having a hereditary breast cancer by virtue of their young age at presentation and/or positive family history of breast and ovarian cancer. The study won a competitive grant from the Ethnic Research Initiative, funded by GlaxoSmithKline. The study was approved by the American University of Beirut Medical Center (AUBMC) institutional review board (IRB). All patients signed informed consent.

Participants

Lebanese women diagnosed with breast cancer and considered to be at high risk for carrying *BRCA1* or *BRCA2* mutations were recruited at AUBMC between 2009 and 2012. Eligibility for enrollment was considered when patients fulfilled any of the following criteria: diagnosis at age ≤40 years, diagnosis at age ≤50 years with at least one relative diagnosed with breast cancer at age ≤50 years or diagnosed with ovarian cancer, diagnosis at any age with 2 or more relatives with breast cancer, diagnosis at any age with 2 or more relatives with ovarian cancer, or diagnosis at any age with a personal history of ovarian cancer.

We recruited 250 Lebanese women. For every participant, medical records were completed for disease stage, pathology, grade, and receptor status. Family history of breast and ovarian cancer was collected to construct multigenerational pedigrees.

BRCA1 and *BRCA2* Genotyping

DNA was isolated from 0.6 mL peripheral blood using QIAamp DNA isolation kit (Qiagen, Venlo, The Netherlands, http://www.qiagen.com) and stored at −20°C. DNA was transferred to Centre Jean Perrin (CJP) in Clermont-Ferrand, France, for genotyping following AUBMC IRB approval and a material transfer agreement between the two institutions.

All coding exons and intron-exon boundaries of *BRCA1* and *BRCA2* were amplified using primers developed in the CJP laboratory and Fluidigm technology (primer sequences are available on request; Fluidigm, South San Francisco, CA, https://www.fluidigm.com). Bar codes for patient identification and universal sequencing primers were added by polymerase chain reaction before purification, quantification, and library preparation, according to the manufacturer's instructions for GS FLX amplicon sequencing (Roche, Basel, Switzerland, http://www.roche.com). In addition to the Amplicon Variant Analyzer software provided by Roche, in-house software was used for analysis of sequencing data. Parameters included a minimum coverage depth of 40 reads required to validate an amplicon and a minimum of 20% variant reads plus variant reads present for each strand for a sequence variant to be called. Insertions-deletions in homopolymer stretches were analyzed with an in-house software to verify mono- versus bimodality of base number calls [29]. Complementary sequencing to verify mutations and/or unvalidated amplicons was performed using standard Sanger sequencing on a 3130xL genetic analyzer (Life Technologies; Thermo Fisher Scientific, Rockford, IL, https://www.lifetechnologies.com) and analyzed with SeqMan software (DNASTAR, Madison, WI, http://www.dnastar.com). Variants were annotated and submitted to in silico analysis with Alamut (Interactive Biosoftware, Rouen, France, http://www.interactive-biosoftware.com).

We screened for large deletions and duplications using reagents from MRC-Holland (kit P001 version C2 for *BRCA1* and

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Table 1. Sequence mutations identified in the *BRCA1* gene

Nucleotide change	AA change	Exon	Number of patients[a]	Frequency (%)
Deleterious				
c.34C>T	p.Gln12*	2	1	0.2
c.131G>T	p.Cys44Phe	3	2	0.4
c.485_486delTG	p.Val162Glufs*19	8	1	0.2
c.2158G>T	p.Glu720*	11	1	0.2
c.2410_2411del	p.Gln804Valfs*5	11	1	0.2
c.5030_5033del	p.Thr1677Ilefs*2	17	1	0.2
VUS				
c.1703C>T	p.Pro568Leu	11	2	0.4
c.1772T>C	p.Ile591Thr	11	1	0.2
c.3367G>T	p.Asp1123Tyr	11	1	0.2
c.3526G>A	p.Val1176Ile	11	1	0.2
c.4132G>A	p.Val1378Ile	12	2	0.4
c.4985T>C	p.Phe1662Ser	16	1	0.2

[a]All patients with deleterious mutations were heterozygous.
Abbreviation: VUS, variants of uncertain significance.

Table 2. Sequence mutations identified in the *BRCA2* gene

Nucleotide change	AA change	Exon	Number of patients[a]	Frequency (%)
Deleterious				
c.674delC	p.Thr225Ilefs*5	8	1	0.2
c.1310_1313del	p.Lys437Ilefs*22	10	1	0.2
c.3971del	p.Tyr1324Leufs*11	11	1	0.2
c.5576_5579del	p.Ile1859Lysfs*3	11	1	0.2
c.9257-1G>A	p.?	IVS24	3	0.6
VUS				
c.517G>T	p.Gly173Cys	7	1	0.2
c.670G>A	p.Asp224Asn	8	1	0.2
c.752C>G	p.Thr251Arg	9	1	0.2
c.1627C>A	p.His543Asn	10	1	0.2
c.1861_1863delinsAGC	p.Glu621Ser	10	1	0.2
c.1951G>T	p.Asp651Tyr	11	1	0.2
c.3131G>T	p.Cys1044Phe	11	1	0.2
c.3743G>A	p.Ser1248Asn	11	1	0.2
c.4446_4451dup	p.Glu1482_Thr1483dup	11	1	0.2
c.5612G>A	p.Ser1871Asn	11	1	0.2
c.6322C>T	p.Arg2108Cys	11	6	1.4
c.6550C>G	p.Gln2184Glu	11	1	0.2
c.6726T>A	p.Asp2242Glu	11	1	0.2
c.7415A>G	p.Lys2472Arg	14	1	0.2
c.7669G>A	p.Ala2557Thr	16	2	0.4
c.8377G>A	p.Gly2793Arg	19	2	0.4
c.8398C>A	p.Pro2800Thr	19	1	0.2
c.9613_9614delinsCT	p.Ala3205Leu	26	1	0.2
c.9628G>A	p.Gly3210Ser	26	1	0.2

[a]All patients with deleterious mutations were heterozygous.
Abbreviation: VUS, variants of uncertain significance.

kit P090 version A4 for *BRCA2*; MRC-Holland, Amsterdam, The Netherlands, http://www.mlpa.com), according to the manufacturer's instructions, and resolved on a 3130xL genetic analyzer (Life Technologies; Thermo Fisher Scientific). Coffalyser software (MRC-Holland) was used for normalization and quantitative analysis of peak data. All patients were tested for *BRCA1* rearrangements using multiplex ligation-dependent probe amplification. *BRCA2* was tested in 39 selected cases

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Table 3. Characteristics of patients with *BRCA1* deleterious variants and variants of uncertain significance

ID	Variant	Deleterious	Age (years)	Family history	Patients in family history (*n*)	Ductal vs. lobular	ER	PR	HER2	TNBC	Stage	Grade
144	c.34C>T	Yes	37	Yes	2	D	Neg	Neg	Neg	Yes	II	3
88	c.131G>T	Yes	36	Yes	4	D	Neg	Neg	Neg	Yes	II	3
161	c.131G>T	Yes	34	Yes	2	D	Neg	Neg	Neg	Yes	II	3
154	c.485_486delTG	Yes	39	Yes	1	D	Neg	Neg	Neg	Yes	II	3
234	c.2158G>T	Yes	39	Yes	2	D	Neg	Neg	Neg	Yes	II	3
131	c.2410_2411del	Yes	48	Yes	1	D	Neg	Neg	Neg	Yes	II	NA
204	c.5030_5033del	Yes	32	Yes	2	D	Neg	Neg	Neg	Yes	II	3
84	c.1703C>T	No	35	No	0	L	Pos	Pos	Neg	No	II	2
91	c.1703C>T	No	31	Yes	2	D	Pos	Pos	Neg	No	IV	NA
179	c.1772T>C	No	37	No	0	L	Pos	Pos	Neg	No	II	1
148	c.3367G>T	No	37	Yes	1	D	Pos	Neg	Neg	No	I	2
61	c.3526G>A	No	31	No	0	D	Neg	Neg	Pos	No	II	2
22	c.4132G>A	No	38	No	0	D	Pos	Pos	Neg	No	III	3
90	c.4132G>A	No	33	No	0	D	Pos	Pos	Neg	No	IV	2
253	c.4985T>C	No	35	Yes	2	D	Neg	Neg	Pos	No	III	2

Abbreviations: D, ductal; L, lobular; NA, not available; Neg, negative; Pos, positive; TNBC, triple-negative breast cancer.

that were characterized by family history of ovarian cancer or male breast cancer. This selection was considered cost-effective based on the rarity of this type of mutation in the *BRCA2* gene.

Analysis

Power Analysis

Deleterious mutations in *BRCA1* and *BRCA2* are rare in the general population but are much higher in hereditary breast and ovarian cancer families [10, 18]. It is estimated that mutations in these genes account for ~20% of families with large clusters of breast cancers and 12.4% of young breast cancer patients (aged <35 years) [18]. Using a conservative estimate of 12% of patients with early onset and/or familial breast cancer carrying a *BRCA* mutation, a cohort of at least 250 unrelated subjects was calculated to measure the prevalence of *BRCA1* and *BRCA2* alleles in Lebanon with a 95% confidence interval.

Variant and Haplotype Analysis

Sequences were compared with the cDNA reference sequence of *BRCA1* and *BRCA2* (accession numbers NM_007294.2 and NM 000059.3, respectively), and nucleotide numbering of all variants is in reference to the coding sequence with the A of the start codon being 1. The significance of variants was verified using the Breast Cancer Information Core (BIC) [19] and the *UMD-BRCA1* and *UMD-BRCA2* mutations databases [20, 21]. Variants were classified into three categories: deleterious variants, variants of uncertain significance (VUS), and neutral variants. In silico prediction analysis was performed for VUS using several prediction algorithms (supplemental online Table 1). Deleterious mutations were confirmed on a second blood sample. Single nucleotide polymorphisms (SNPs) and common variants present in the exonic and near-exonic sequence were used to manually determine *BRCA1* and *BRCA2* haplotypes.

Data Analysis

Data were entered and analyzed using SPSS version 20.0.0 (IBM Corp, Armonk, NY, http://www-01.ibm.com/software/analytics/spss/). Patient and tumor characteristics were compared among patients who were positive for deleterious mutations, those with VUS, and those who were negative (i.e., have neutral or no variants). Nonparametric Mann-Whitney, Kruskal-Wallis, and Fisher's exact tests were used as appropriate. A p value of $<.05$ was considered statistically significant.

Results

Patients and Disease Characteristics

Of the total of 250 patients, with a mean age of 40.8 ± 9 years, 148 patients were aged ≤40 years, and of those, only 74 had a positive family history. All patients aged >40 years were required to have a positive family history. Family history was obtained from medical records during the process of screening for eligibility. Once the patients were considered eligible for the study, they were thoroughly and reliably interviewed for family history by our assigned study research physicians. We have breast cancer histological confirmation on all patients enrolled in the study. Family members who were reported to have a history of breast or ovarian cancer were treated at various hospitals in Lebanon, and some were treated overseas; this made it very difficult to check histopathology. This was true for the 17 patients who had a positive family history of ovarian cancer and for whom we had details about history of surgery and treatment for ovarian cancer, but histological confirmation was available on only the 3 who happened to have been treated at the American University of Beirut Medical Center.

Stages at diagnosis for the whole group were as follows: stage I, 20.4%; stage II, 46.9%; stage III, 24.9%; stage IV, 7.8%. The majority of patients (92.3%) had infiltrating ductal carcinoma, and 64.5% had positive hormone receptors, whereas 19.2% were HER2 overexpressive.

Mutations

For the *BRCA1* gene, seven patients had deleterious mutations and six had VUS (Table 1). For the *BRCA2* gene, seven patients had deleterious mutations and 19 had VUS (Table 2). The

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Table 4. Characteristics of patients with *BRCA2* deleterious variants and variants of uncertain significance

ID	Variant	Deleterious	Age (years)	Family history	Patients in family history (*n*)	Ductal vs. lobular	ER	PR	HER2	TNBC	Stage	Grade
60	c.674delC	Yes	40	Yes	2	D	Pos	Pos	Neg	No	III	3
23	c.1310_1313del	Yes	46	Yes	2	D	Pos	Pos	Neg	No	III	1
183	c.3971del	Yes	48	Yes	2	D	Pos	Neg	Neg	No	III	3
106	c.5576_5579del	Yes	51	Yes	1	NA	Pos	Pos	Neg	No	NA	2
180	c.9257-1G>A	Yes	27	No	0	D	Pos	Pos	Neg	No	II	3
72	c.9257-1G>A	Yes	39	Yes	12	D	Pos	Pos	Pos	No	III	2
132	c.9257-1G>A	Yes	43	Yes	5	D	Pos	Pos	Pos	No	I	2
96	c.517G>T	No	39	Yes	1	D	Pos	Pos	Neg	No	I	3
27	c.670G>A	No	34	Yes	1	D	Neg	Neg	Neg	Yes	IV	2
6	c.752C>G	No	34	No	0	D	Pos	Neg	Pos	No	IV	3
198	c.1627C>A	No	43	Yes	1	D	Pos	Pos	Pos	No	IV	3
179	c.1861_1863delinsAGC	No	37	No	0	L	Pos	Pos	Neg	No	II	1
228	c.1951G>T	No	58	Yes	2	D	Pos	Pos	Neg	No	I	1
163	c.3131G>T	No	40	Yes	3	D and L	Pos	Pos	Neg	No	I	2
14	c.3743G>A	No	38	Yes	2	D	Neg	Neg	N/A	N/A	III	2
121	c.4446_4451dup	No	38	No	0	D	Neg	Neg	Neg	Yes	III	3
182	c.5612G>A	No	28	Yes	3	D	Pos	Pos	Pos	No	II	3
23	c.6322C>T	No	46	Yes	2	D	Pos	Pos	Neg	No	III	1
25	c.6322C>T	No	38	No	0	D	Pos	Pos	Pos	No	I	3
27	c.6322C>T	No	34	Yes	1	D	Neg	Neg	Neg	Yes	IV	2
32	c.6322C>T	No	36	Yes	1	D	Pos	Pos	Neg	No	II	2
111	c.6322C>T	No	41	Yes	1	L	Pos	Pos	Pos	No	II	NA
248	c.6322C>T	No	50	Yes	2	D	Pos	Pos	Neg	No	II	1
191	c.6550C>G	No	38	Yes	1	D	Neg	Neg	Pos	No	II	2
41	c.6726T>A	No	41	No	0	D	Pos	Pos	Neg	No	I	1
31	c.7415A>G	No	39	Yes	1	D	Pos	Pos	Pos	No	II	2
187[a]	c.7669G>A	No	68	Yes	2	D	Pos	Pos	Neg	No	I	2
188[a]	c.7669G>A	No	63	Yes	2	D	Neg	Neg	Pos	No	III	3
95	c.8377G>A	No	30	Yes	1	D	Pos	Pos	Neg	No	III	3
134	c.8377G>A	No	39	Yes	3	D	Pos	Pos	Neg	No	II	1
5	c.8398C>A	No	28	No	0	D	Pos	Pos	Neg	No	II	2
232	c.9613_9614delinsCT	No	42	Yes	1	D	Pos	Pos	Neg	No	III	3
82	c.9628G>A	No	38	Yes	1	D	Pos	Pos	Neg	No	IV	2

[a]Patients 187 and 188 are sisters.
Abbreviations: D, ductal; L, lobular; NA, not available; Neg, negative; Pos, positive; TNBC, triple-negative breast cancer.

prevalence of deleterious *BRCA* mutations in high-risk Lebanese women with breast cancer was 5.6% (2.8% for *BRCA1* and 2.8% for *BRCA2*), and the prevalence of VUS was 13.2% (3.2% for *BRCA1* and 10% for *BRCA2*). No large deletions or duplications in *BRCA1* or *BRCA2* were observed. VUS are described in supplemental online Table 1.

All patients with deleterious *BRCA1* mutations had triple-negative grade 3 ductal breast carcinoma (Table 3).

Patients with deleterious *BRCA1* mutations were characterized by triple-negative grade 3 disease and positive family history (Table 3), whereas none of the *BRCA2*-positive patients had triple-negative breast cancer (Table 4).

One *BRCA2* VUS (c.6322C>T) was recurrent ($n = 6$) and co-occurred with a deleterious mutation (patient 23) (Table 4), suggesting this variant is benign. Patients with *BRCA1* VUS were more likely nonmutated except for those who were slightly younger at diagnosis (the number of such cases was small) (Table 5).

Of the 148 patients who were aged ≤40 years at diagnosis, 9 (6%) harbored deleterious mutations (6 *BRCA1* and 3 *BRCA2*). When stratified by family history, 8 of 74 patients (10.8%) who were aged ≤40 years with positive family history had deleterious *BRCA* mutations (6 *BRCA1* and 2 *BRCA2*). In contrast, only 1 of all 74 patients aged ≤40 years with negative family history (1.4%) had a deleterious *BRCA2* mutation. Of the 75 patients aged 41–50 years with positive family history of ovarian and/or breast cancer, 4 (5.3%) had a deleterious mutation (1 *BRCA1* and 3 *BRCA2*). Only 1 of 27 patients aged >50 years at diagnosis with positive family history had a *BRCA2* deleterious mutation.

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Table 5. Comparison of patient characteristics among negative, deleterious mutation, and VUS groups

		BRCA1		*BRCA2*		All	
Variable	**Negative[a]**	**Deleterious**	**VUS**	**Deleterious**	**VUS**	**Deleterious**	**VUS**
Number of patients	205	7	8	7	24[b]	14	31[c]
Age (years)	39 [35, 46]	37 [34, 39]	35 [31.5, 37][d]	43 [39, 48]	38.5 [36.25, 41.75]	39 [35.5, 46.5]	38 [34, 41]
Family history	1 [0, 2]	2 [1, 2][e]	0 [0, 1.75]	2 [1, 5]	1 [0.25, 2]	2 [1, 2.5][f]	1 [0, 2]
Family history, yes	137 (66.8)	7 (100.0)[e]	3 (37.5)	6 (85.7)	18 (75.0)	13 (92.9)	21 (67.7)
ER positive, yes	159 (79.5)	0 (0.0)[f]	6 (75.0)	7 (100.0)	19 (79.2)	7 (50.0)[d]	24 (77.4)
PR positive, yes	137 (68.5)	0 (0.0)[f]	5 (62.5)	6 (85.7)	18 (75.0)	6 (42.9)	22 (71.0)
HER2 positive, yes	50 (25.6)	0 (0.0)	2 (25.0)	2 (28.6)	8 (34.8)	2 (14.3)	10 (33.3)
TNBC, yes	19 (9.5)	7 (100.0)[f]	0 (0.0)	0 (0.0)	2 (8.7)	7 (50.0)[f]	2 (6.7)
Stage							
I	42 (20.9)	0 (0.0)	1 (12.5)	1 (16.7)	6 (25.0)	1 (7.7)	7 (22.6)
II	96 (47.8)	7 (100.0)	3 (37.5)	1 (16.7)	9 (37.5)	8 (61.5)	11 (35.5)
III	50 (24.9)	0 (0.0)	2 (25.0)	4 (66.7)	5 (20.8)	4 (30.8)	7 (22.6)
IV	13 (6.5)	0 (0.0)	2 (25.0)	0 (0.0)	4 (16.7)	0 (0.0)	6 (19.4)
Grade							
1	30 (15.8)	0 (0.0)	1 (14.3)	1 (14.3)	5 (21.7)	1 (7.7)	5 (17.2)
2	81 (42.6)	0 (0.0)	5 (71.4)	3 (42.9)	9 (39.1)	3 (23.1)	14 (48.3)
3	79 (41.6)	6 (100.0)[f]	1 (14.3)	3 (42.9)	9 (39.1)	9 (69.2)	10 (34.5)

[a]Negative includes the presence of neutral variants or no variants.
[b]Patient 23 had both a deleterious variant and a variant of uncertain significance and was counted among the deleterious group; patient 27 had two variants of uncertain significance was counted once.
[c]Patient 179 had two variants of uncertain significance, one *BRCA1* and one *BRCA2*, and was counted once.
[d]Statistically significant difference from negative patients.
[e]Statistically significant difference between patients who had deleterious mutations and those who had VUSs.
[f]Statistically significant difference among patients who had deleterious mutations and those who had VUSs and those who were negative.
Data are presented as median [Q2, Q3] or *n* (%) as appropriate, using Mann-Whitney, Kruskal-Wallis, and Fisher's exact nonparametric tests as appropriate. A p value $<.05$ was considered statistically significant.
Abbreviations: TNBC, triple-negative breast cancer; VUS, variant of uncertain significance.

Eight patients reported family history of pancreatic cancer, and none were among those with *BRCA2* mutations. Of the 21 patients with family history of prostate cancer, one was a *BRCA1* carrier (c.34C>T) and one was a *BRCA2* carrier (c.9257-1G>A).

Haplotypes

Eight *BRCA1* haplotypes were identified at a frequency of ≥1% In addition to the most frequent haplotype (50% of alleles) that presented the default nucleotide at all positions (H1) (supplemental online Table 2), we observed 17 haplotypes with a frequency of >1%, with the most common at 27.5% (supplemental online Table 3). Fourteen major types accounted for 99% of all haplotypes; the remaining 1% consisted of 6 different rare combinations of alleles.

Discussion

This report is the first large study of *BRCA* mutations in high-risk hereditary breast and ovarian cancer patients in Lebanon and Arab countries. We observed 6 different deleterious mutations in *BRCA1* and 5 in *BRCA2* for a total of 14 mutation carriers using direct sequencing. The types of mutations observed were similar to those observed elsewhere, including stop codons, frame shifts, and splice-site mutations. No gene rearrangement and founder mutations were detected. Nine deleterious mutations were not reported previously, whereas five had been described previously by others: c.131G>T [22], c.485_486delTG [23], and c.5030_5033del [24] in *BRCA1* and c.1310_1313del [25] and c.5576_5579del [24] in *BRCA2*. None of the 14 deleterious mutations were previously reported in Arab participants, including the *BRCA*2c.9257-1G>A variant that was detected in three families [10–17, 26–30]. Several of the detected VUS were novel because they were absent from the BIC database.

It has been estimated that the frequency of *BRCA1* and *BRCA2* mutations is around 1 in 800 in the general white population and reaches 1 in 40–50 in the Ashkenazi Jewish population [31]. Women carrying these mutations typically present at a young age (<40 years), especially carriers of *BRCA1* mutations, and have a lifetime risk of developing breast and/or ovarian cancer in the range of 45%–85% for *BRCA1* carriers and 40%–70% for *BRCA2* carriers.

At 5.6%, the prevalence of deleterious mutations in our cohort of patients with high-genetic-risk breast cancer is lower than expected when compared with studies from industrialized nations. Using the same or similar case selection criteria, routine screening of cases at the CJP suggests 10%–25% of families carry a *BRCA* mutation if family history or young age at diagnosis is present. This finding suggests that we need to look for alternative gene mutations and other risk factors that may contribute to the development of breast cancer in these high-risk patients [32]. In addition, long-term follow-up of patients' families is needed to estimate prognosis and penetrance. This aspect is important because penetrance and occurrence of

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breast cancer may be modified by other genetic mutations, reproductive factors, environmental factors, exercise, or diet. Recent increases in incidence rates in many countries, for example, have reflected changes showing that *BRCA* mutation carriers born after 1940 have higher breast cancer incidence than those born before 1940 (67% vs. 24%) [33].

The association of *BRCA1* mutations with triple-negative grade 3 breast cancer was confirmed in our cohort, with all such mutation carriers having this particularly aggressive type of cancer. Only one patient had a positive family history of male breast cancer, but she did not carry a *BRCA2* mutation.

Although this study did not include an extensive haplotype analysis, few different *BRCA1* haplotypes were present in this cohort of Lebanese patients. Comparison with other published haplotypes of Moroccan populations showed that this haplotype accounted for 63% of haplotypes in Tunisia [13] and 59% of those in Algeria [14]; however, these studies did not include markers outside of a certain core. If we exclude those same markers from our estimate, grouping the L4 and L5 haplotypes with the canonical H1, 58% of Lebanese haplotypes correspond to the H1 reference. Both North African population studies observed the H7 and H10 haplotypes at 2%–5%, and these were present in the Lebanese sample at 1% and 3%, respectively [13, 14]. In addition to H1, H7, and H10, *BRCA1* in our Lebanese sample can be described by five haplotypes. One of these, L1, represented 26% of haplotypes. Fifteen additional Tunisian and 12 additional Algerian *BRCA1* haplotypes were absent from the Lebanese sample, despite the much larger population studied.

Very few publications are available for comparison of *BRCA2* haplotype analysis using common SNPs in the coding region. A large study of Jewish populations looked at SNPs within and surrounding the *BRCA2* locus, but only two markers were common with our data [34]. *BRCA2* markers were not published for the Tunisian and Algerian populations described for *BRCA1* above. As with *BRCA1*, using such a limited set of SNPs may oversimplify the haplotype structure present.

Conclusion

This report is the first large modern study of a cohort of Lebanese Arab women with breast cancer considered to be at a high risk for harboring hereditary disease. We showed a prevalence rate of 5.6% for having deleterious *BRCA1* or *BRCA2* mutations among this group of women. Prevalence is 10.8% in patients aged ≤40 years with positive family history (6 *BRCA1* and 2 *BRCA2* mutations) but is only 1.4% in patients aged ≤40 years with negative family history. Our study showed that *BRCA* mutations were lower than expected when compared with other populations and hence does not support the hypothesis that *BRCA* mutations alone are the cause of the high percentage of young women with breast cancer in Lebanon and in Arab countries. These results should help guide clinicians and policy makers with regard to genetic counseling and testing in Lebanon and Arab countries and in the Americas, Europe, and Australia, where large numbers of immigrants and citizens of Lebanese and Arab descent reside. Additional studies are needed to look for other mutations such as *PALB2*, *CHEK2*, and *TP53*.

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Disclosures

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Implications for Practice:

This review illustrates that breast cancer (BC) among Latin American women is a growing burden throughout the region. The increased proportion of BC cases in young women is important because their diagnoses and tumor behavior are usually more aggressive than in their older counterparts. The findings of this study reveal that there is scarce information regarding this matter in Latin American countries, especially concerning the particular effects and complications that this group of women face during and after treatment. Also, there are no specific clinical or educational programs that focus on this population. A call to action from health policy planners, medical providers, researchers, BC patients, families, and the community in general is deserved for better care of this emergent challenge.

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