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Novel BRCA1 and BRCA2 Deleterious Mutations and Unclassified Variants in Egyptian Female Breast Cancer Patients

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Abstract

Introduction: Breast cancer is the most common malignancy among females worldwide and the leading cause of cancer death in economically developing countries. The world's oldest documented cancer case hails from Ancient Egypt 3500 years BC and the Egyptian population shows high-degree of genetic diversity due to its complex ethnic origins. The contribution of *BRCA1*/*BRCA2* mutations to the burden of breast cancer in Egypt has not been extensively evaluated. **Methods:** A series of 103 Egyptian female breast cancer patients, unselected for age of onset or family history, were included in the study. Mutational screening of exons 2 and 20 of *BRCA1* and exons 9 and 11 of *BRCA2* genes was performed using HRM analysis followed by direct sequencing of detected variants. The potential clinical effect of the novel missense mutations on protein structure and function was evaluated using *In Silico* prediction tools. **Results:** Deleterious mutations were observed in 29 (28.15%) cases. Of those, 13 (44.8%) carried *BRCA1* mutations, 13 (44.8%) carried *BRCA2* mutations and 3 (10.34%) carried both *BRCA1* and *BRCA2* mutations. Thirteen carriers (44.8%) reported positive family history of breast cancer, 14 (48.27%) had early onset and 5 cases had bilateral disease. Twenty different sequence variants were identified: 5 novel frame shift mutations; 1 in *BRCA1* (c.5205delA) and 4 in *BRCA2* (c.3641delT, c.3291dupT, c.3292delA, and c.787dupA), 1 novel nonsense mutation (*BRCA2* c.3280A>T), 2 previously described missense mutations (*BRCA1* c.117T>G and c.110C>A), 1 silent mutation (*BRCA2* c.3396A>G) and 11 unclassified variants, 8 of which were novel. Novel mutations were submitted to NCBI Clinvar database. All the observed deleterious mutations were recurrent, except *BRCA1* c.110C>A mutation which was detected once. The *BRCA1* frame shift mutation c.5205delA was observed in high frequency (16/103, 15.5%) in our cohort. Unclassified variants were identified in 32 (31%) cases, 15 of them had a co-occurring deleterious mutation. Patients with BRCA mutations tended to have early onset breast cancer compared to non-carriers (P=0.002), more often premenopausal (P=0.006), with a familial history of breast cancer as well as other cancers (P=0.005).**Conclusion:** This study provides the results of our attempt to delineate the genetic aspect of breast cancer among the Egyptian population and emphasizes the necessity of implementing screening and preventive strategies as part of the national public health policy to facilitate early diagnosis and proper counseling for breast cancer patients in Egypt.

Keywords:

BRCA1;

BRCA2;

Breast Cancer;

Egypt;

HRM;

Germline Mutations;

Unclassified Variants.

Introduction

Breast cancer is the most commonly diagnosed cancer and the leading cause of death among females worldwide [1]. The world's oldest documented cancer case hails from Ancient Egypt 3500 years BC [2], and the Egyptian population shows high-degree of genetic diversity compared to other populations due to its complex and diverse ethnic origins

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[3,4]. Currently, breast cancer accounts for more than 34.4% of cancer cases among Egyptian females [5] and is responsible for 29.1% of cancer deaths [6]. Although incidence rates in Egypt are lower than other populations, rates are steadily increasing and mortality rates in Egypt are worse [7]. Furthermore, the current demographic trends favor the likelihood that breast cancer will become an even greater public health concern in Egypt in the future [8]. The age at diagnosis in Egypt is one decade younger than the corresponding age in Europe and North America with exceptionally high incidence in the age group 25 to 34 years [7]. In addition, 60-80% of breast cancers in Egypt present with advanced disease at time of diagnosis [9], and family history of breast cancer, possibly related to high rate of consanguineous marriage [10]. Taken together, this evidence may imply that a high proportion of breast cancer cases in Egypt may be attributable to genetic factors.

The most well characterized genetic risk factors for breast and/or ovarian cancer to date are germline mutations of the breast-cancer susceptibility gene 1, *BRCA1* (MIM#113705) [11] and breast-cancer susceptibility gene 2, *BRCA2* (MIM#600185) [12]. Molecular analysis of *BRCA1/2* in different populations has demonstrated a very large mutational spectrum and variable mutation prevalence [13]. Identification of mutation carriers is important for early diagnosis and prevention of breast cancer as it provides the opportunity for healthy carriers to consider various risk-reducing options and intensive screening; and can also imply novel treatment strategies for affected patients such as poly (ADP-ribose) polymerase (PARP) inhibitors [14]. Unfortunately, cancer genetic services in developing countries such as Egypt are still lagging behind, due to lack of financial and practical resources, and so knowledge of the prevalence and spectrum of *BRCA1*and *BRCA2* mutations in the Egyptian population is sparse.

Mutation scanning analysis of *BRCA1/2* genes using high-resolution melting (HRM) analysis followed by direct sequence analysis is a sensitive, specific, cost- effective and reliable screening method for the identification of genetic variants while avoiding the unnecessary sequencing of wildtype genotypes, and is also capable of detecting new variants [15,16].

Few studies have addressed *BRCA1/2* mutations in the Egyptian population [17,18,19,20]. To the best of our knowledge, this is the first study on the frequency and profile of *BRCA1* and *BRCA2* mutations in a cohort of unselected Egyptian female breast cancer patients using HRM and direct sequencing on some exons of *BRCA1* and *BRCA2* genes. Our goal was to evaluate the burden of breast cancer in Egypt attributable to germline mutations in *BRCA1/2* genes using a rapid and cost-effective approach, as well as determine the profile of *BRCA1/2* mutations in this population; which can in turn guide breast cancer screening and thus help in reducing mortality and morbidity in this poor resource country. In the current study we describe novel deleterious mutations and unclassified variants (UVs) that were detected for the first time in the Egyptian population.

Methods

Recruitment of Subjects

All recruited patients provided a signed informed consent. The study protocol was approved by the Ethical Committee of Ain Shams University, Cairo, Egypt. The study was conducted in accordance with the regulations and recommendations of the Declaration of Helsinki.

A series of 103 unrelated Egyptian female breast cancer patients diagnosed with primary invasive breast cancer were recruited from the Breast Cancer Unit, Clinical Oncology Department, Ain Shams University, Cairo, Egypt. The enrolled patients were not selected on the basis of age of onset of breast cancer, family history or any other criterion that would enrich for *BRCA1/2* mutation carriers.

Eligibility criteria were female patients aged 18 years and above with histologically proven breast cancer, and the first line of treatment was surgery with curative intent followed by adjuvant therapy. Exclusion criteria were any previous history of malignancy other than breast cancer, and the evidence of distant metastases at the time of diagnosis.

All recruited patients agreed to genetic testing with the understanding that they would receive the results and would be counseled regarding the implications. The clinicopathological characteristics were extracted from hospital records.

Sample Collection and Storage

About 5 mL peripheral blood was collected from each case in blood vacutainer tubes having EDTA as anticoagulant. For storage, transportation and preservation, recommended guidelines were followed [21]. Genomic DNA was isolated from peripheral blood using QIAamp DNA Mini kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. The DNA concentration and quality for each extraction was determined by spectrophotometric measurement using Nanodrop ND2000 Spectrophotometer (Nanodrop Technologies, USA).

Mutational analysis of BRCA1 and BRCA2

The PCR and HRM were performed in a single run on Applied Biosystems 7500 Fast Real-Time PCR according to the manufacturer's instructions (Applied Biosystems, USA). The reaction mix contained 1 µl (20 ng) of genomic DNA, 5 µM of each primer and 10 µl MeltDoctor™ HRM Master Mix with PCR grade water adjusted to a total volume of 20 µl. The reaction conditions included an activation step at 95°C for 10 minutes followed by 40 cycles of 95°C for 15 seconds, a touch down of 60°C for 1 minute. Before the HRM step, the products were heated to 95°C for 10 seconds. The HRM was carried out over the range from 60°C to 95°C rising at 1°C per second with 30 acquisitions per degree. All reactions were performed in a 96-well microtiter plate. Upon completion of the run, HRM curve analysis was performed using the Applied Biosystems 7500 Software version 2.0.2 supplied with the Applied Biosystems 7500 fast.

To check and confirm the mutations detected by HRM analysis, aliquots of patients' DNA with suspected variants were subjected to PCR/direct sequencing analysis (Macrogen Lab, South Korea). Sequencing was performed using Big Dye® termination technology in both forward and reverse directions. Detected mutations and other sequence alterations are described at the cDNA level according to the *BRCA1* reference sequence (GenBank U14680) and *BRCA2* reference sequence (NM_000059.3), using the nomenclature recommendation guidelines used by Human Genome Variation Society (HGVS) (http://www.hgvs.org) using the A of the ATG translation initiation codon as nucleotide +1 [22]. Mutations are also provided using the BIC nomenclature, where the A of the ATG translation initiation codon is at position 120 of the *BRCA1* mRNA and at position 229 of the *BRCA2* mRNA, respectively. In the tables, all sequence variants are listed according to both BIC and HGVS. Potential clinical effect of UVs on protein structure and function was evaluated by analyses of the severity of the amino acid changes and their conservation across species. These analyses were performed using *In Silico* prediction analysis web tools: Alignment-Grantham variation Grantham deviation (Align GVGD; http://agvgd.iarc.fr/agvgd_input.php) [23], Polymorphism Phenotyping-2 (PolyPhen2); http://genetics.bwh.harvard.edu/pph2/) [24], and Sorting Intolerant From Tolerant (SIFT; http://blocks.fhcrc.org/sift/SIFT.html) [25] scores, and PMUT (http://mmb.irbbarcelona.org/PMut/).

Results

A total of 103 cases with invasive breast cancer were ultimately included in the study. The median age at diagnosis of breast cancer in the study cohort was 43 years (ranged from 24 to 66). Forty-three cases (41.75%) had early onset breast cancer (≤40 years). Forty-one cases (39.8%) reported a positive family history of breast cancer in first, second or third degree relative. The clinical characteristics of the study cohort are given in Table 1.

Table 1. The clinical characteristics of the study cohort.

Character	No. (%)	Median (Range)
Age at diagnosis		43 (24-66)
≤40	43 (41.75%)	
>40	60 (58.25%)	
Family history of cancer		
Breast cancer	41 (39.8%)	
Other cancers	23 (22.33%)	
No	39 (37.86%)	
Age at menarche, years		12 (10-16)
Age at first live birth, years		23 (15-33)
Parity		3 (0-8)
Hormonal contraception		
Yes	67 (65.05%)	
No	36 (34.95%)	
Onset of breast cancer		
Premenopausal	69 (67%)	
Postmenopausal	4 (33%)	

In total, 20 sequence variants were detected: 9 (45%) in *BRCA1* and 11 (55%) in *BRCA2*. The detected variants comprised 8 deleterious mutations, 11 UVs and one silent mutation. Deleterious mutations were observed in 29 (28.15%) cases. The clinicopathological characteristics of breast cancer patients carrying *BRCA1* or *BRCA2* deleterious mutations are shown in Table 2. Positive family history of breast cancer was observed in 13 mutation carriers (44.8%) and 9 patients reported family history of other cancers. Fourteen carriers had early onset breast cancer ≤40 years (48.27%) and 8 carriers were diagnosed between 41 and 50 years (27.5%). Seven patients (24.13%) had both early onset and family history of breast cancer. Bilateral breast cancer was diagnosed in 5 mutation carriers, 3 of them had early onset and

family history of cancer. Patients with *BRCA* mutations tended to have early onset breast cancer compared to non-carriers (P=0.002), more often premenopausal (P=0.006), with a familial history of breast cancer as well as other cancers (P=0.005).

Table 2. Clinicopathological characteristics of breast cancer patients carrying *BRCA1* or *BRCA2* deleterious mutations

Patient ID	Mutation	Age (years)	Laterality	Family history of cancer	Affected family members	
					Breast Cancer	Other cancers
1025	*BRCA1* c.110C>A, c.117T>G, c.5205delA	44	Unilateral	+	Sister	-
1006	*BRCA1* c.117T>G, c.5205delA	39	Unilateral	-	-	-
1002	*BRCA1* c.5205delA	53	Unilateral	+	Daughter	Son (brain)
1003	*BRCA1* c.5205delA	36	Bilateral	+	Aunt	-
1008	*BRCA1* c.5205delA	39	Unilateral	-	-	-
1011	*BRCA1* c.5205delA *BRCA2* c.3280A>T	34	Unilateral	+	Sister, 2 Aunts	-
1018	*BRCA1* c.5205delA	40	Unilateral	+	Sister	-
1024	*BRCA1* c.5205delA	43	Bilateral	-	-	-
1029	*BRCA1* c.5205delA	24	Unilateral	+	Aunt, Grandmother	Grandfather (colon)
1032	*BRCA1* c.5205delA	61	Unilateral	+	-	Mother (colon)
1033	*BRCA1* c.5205delA	41	Unilateral	+	Aunt	-
1035	*BRCA1* c.5205delA *BRCA2* c.787dupA	40	Unilateral	+	Cousin	-
1036	*BRCA1* c.5205delA	56	Unilateral	+	Sister	-
1040	*BRCA1* c.5205delA	39	Unilateral	-	-	-
1052	*BRCA1* c.5205delA *BRCA2* c.3641delT	40	Unilateral	+	Aunt, 2 cousins	-
1099	*BRCA1* c.5205delA	42	Bilateral	+	Mother, Sister, Aunt	
1023	*BRCA2* c.787dupA	40	Unilateral	-	-	-
1056	*BRCA2* c.787dupA	38	Bilateral	+	-	Mother (abdomen)
1082	*BRCA2* c.787dupA	63	Unilateral	+	-	Mother (liver)
1083	*BRCA2* c.787dupA	60	Unilateral	-	-	-
1087	*BRCA2* c.787dupA, c.3280A>T	40	Unilateral	+	-	Brother (leukemia)
1084	*BRCA2* c.3280A>T	61	Unilateral	+	-	Niece, Son (leukemia)
1085	*BRCA2* c.3280A>T	36	Bilateral	+	Mother	-
1061	*BRCA2* 3291dupA	50	Unilateral	-	-	-
1089	*BRCA2* c.3291dupA	40	Unilateral	+	Aunt, Grandmother	-
1093	*BRCA2*c.3291dupA	50	Unilateral	+	-	Uncle (colon)
1031	*BRCA2* c.3292delA	47	Unilateral	-	-	-
1034	*BRCA2* c.3292delA	35	Unilateral	-	-	-
1009	*BRCA2* c.3641delT	49	Unilateral	+	-	Son (colon)

Age, age of breast cancer diagnosis

The spectra of the deleterious mutations identified are illustrated in Table 3. They included 2 previously described missense mutations, 1 novel nonsense mutation and 5 novel frameshift mutations. The novel mutations were submitted to NCBI Clinvar database and accession numbers obtained:

NM_007294.3(*BRCA1*):c.5205delA (SCV000172260)
NM_000059.3(*BRCA2*):c.3280A>T (SCV000191885)
NM_000059.3(*BRCA2*):c.3291dupT (SCV000191886)
NM_000059.3(*BRCA2*):c.3292delA (SCV000191887)
NM_000059.3(*BRCA2*):c.3641delT (SCV000191888)
NM_000059.3(*BRCA2*):c.787dupA (SCV000191884)

Table 3. Spectrum of *BRCA1* and *BRCA2* deleterious mutations identified in the study cohort.

Gene	BIC Nomenclature	HGVS Nomenclature	Amino acid change	Type of Mutation	No. Observations	Reference
BRCA1	229C>A	c.110C>A	p.T37K	Missense	1	BIC, HGMD, LOVD
BRCA1	236T>G	c.117T>G	p.C39W	Missense	2	UMD-*BRCA1*, HGMD
BRCA1	5324delA	c.5205delA	p.(Val1736Serfs*29)	Frameshift	16	Novel
BRCA2	1014insA	c.787dupA	p.(Ser263Lysfs*13)	Frameshift	6	Novel
BRCA2	3509A>T	c.3280A>T	p.K1094*	Nonsense	4	Novel
BRCA2	3519insT	c.3291dupT	p.(Asn1098*)	Frameshift	3	Novel
BRCA2	3520delA	c.3292delA	p.(Asn1098Ilefs*6)	Frameshift	2	Novel
BRCA2	3869delT	c.3641delT	p.(Val1214Glyfs*14)	Frameshift	2	Novel

***BRCA1*/*BRCA2*, breast cancer-susceptibility genes 1 and 2, respectively; BIC, Breast Cancer Information Core Database; HGVS, Human Genome Variation Society; HGMD, Human Gene Mutation Database; LOVD, Leiden Open Variation Database; UMD, Universal Mutation Database; T, threonine; K, lysine; C, cysteine; W, tryptophan; del, deletion; ins, insertion; dup, duplication.**

These mutations were predicted to be deleterious because each generated a stop codon in the open reading frame leading to premature termination of translation at the same positions as other mutations reported to be of clinical significance in BRCA mutational databases including ClinVar [26], BRCA exchange [27], Human Gene Mutation Database (HGMD-Professional) [28], Universal Mutation Database (UMD) [29] or Leiden Open Variation Database (LOVD) [30]. The *BRCA1* frameshift mutation c.5205delA (p.Val1736Serfs*29) was observed in high frequency (15.5%) in the study group. Most of the carriers of this mutation (9 out of 16) had early onset breast cancer, and 4 patients were diagnosed between 40 and 45 years. This novel mutation occurs within the *BRCA1* C-terminal (BRCT) repeats. The clinicopathological characteristics of the patients harboring this mutation are given in Table 4.

Table 4. Clinical characteristics of Egyptian female breast cancer patients carrying the *BRCA1* mutation c.5205delA p.(Val1736Serfs*29).

Sample	Age (years)	Tumor size	Histo- pathology	Grade	Lymph node involvement	Family history of breast cancer	Family members with breast cancer	
							Number	Degree
1025	44	T2	IDC	2	-	+	1	1st
1006	39	T3	ILC	2	+	-	-	-
1002	53	T2	IDC+ILC	3	+	+	1	1st
1003	36	T2	IDC	2	+	+	1	2nd
1008	39	T3	IDC	2	-	-	-	-
1011	34	T2	IDC	2	+	+	3	1st, 2nd
1018	40	T2	IDC	2	+	+	1	1st
1024	43	T2	IDC	2	+	-	-	-
1029	24	T2	IDC	2	-	+	1	1st, 2nd
1032	61	T2	IDC	2	+	-	-	-
1033	41	T2	IDC	2	-	+	1	2nd
1035	40	T2	IDC	2	-	+	1	3rd
1036	56	T2	ILC	2	-	+	1	1st
1040	39	T3	IDC	2	+	-	-	-
1052	40	T2	IDC	2	+	+	3	2nd, 3rd
1099	42	T2	IDC	2	-	+	3	1st, 2nd

Age, age of breast cancer diagnosis; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma.

The *BRCA1* missense mutation c.110C>A (p.T37K) was observed only once in this study and c.117T>G (p.C39W) mutation was observed in 2 patients. They both lie within 100% conserved cysteine residues of the *BRCA1* RING domain.

Five novel deleterious mutations were detected in *BRCA2* gene, 4 of them occur in the ovarian cancer cluster region spanning nucleotides 3035 to 6629. However, the precise family history of ovarian cancer was not available for the patients harboring these mutations. They comprised the nonsense mutation c.3280A>T (4 patients), the frameshift mutations c.3291dupT (3 patients), c.3292delA (2 patients), c.3641delT (2 patients), and c.787dupA (6 patients).

Of note, 3 of the 29 mutation carriers (10.34%) had deleterious mutations in both *BRCA1* and *BRCA2* genes. The first case was found to carry both *BRCA1* c.5205delA and *BRCA2* c.3280A>T mutations; the second case had both *BRCA1* c.5205delA and *BRCA2* c.3641delT mutations and the third case carried both *BRCA1* c.5205delA and *BRCA2* c.787dupA mutations. Interestingly, the 3 cases had early onset and positive family history breast cancer.

Furthermore, 11 UVs (6 in *BRCA1* 5 in *BRCA2*) were detected in 32 cases, 15 of them had a co-occurring deleterious mutation. Twelve cases (37.5%) had a family history of breast cancer. The spectrum of the UVs identified is given in Table 5. Among these UVs, 8 were novel. The functional effects of the novel UVs were predicted using the prediction analysis tools SIFT, PolyPhen-2 and PMUT (Table 6). Align-GVGD categorized 1/8 (12.5 %) as C65, 0/8 as C55, C45, C35 or C25, and 7/8 (87.5 %) as C0.

Table 5. Spectrum of *BRCA1* and *BRCA2* unclassified variants (UVs) identified in Egyptian female breast cancer patients.

Gene	BIC Nomenclature	HGVS Nomenclature	Amino acid change	Type of Mutation	No. observations	Reference
BRCA1	150G>T	c.31G>T	p.V11L	Missense	5	Novel
BRCA1	161C>A	c.42C>A	p.V14V	Silent	1	BIC
BRCA1	162A>T	c.43A>T	p.I15F	Missense	2	Novel
BRCA1	235G>T	c.116G>T	p.C39F	Missense	8	Novel
BRCA1	5387G>T	c.5268G>T	p.Q1756H	Missense	1	Novel
BRCA1	5394A>G	c.5275A>G	p.K1759E	Missense	1	Novel
BRCA2	968T>C	c.740T>C	p.I247T	Missense	1	BIC
BRCA2	967A>T	c.739A>T	p.I247F	Missense	9	Novel
BRCA2	3520 A>T	c.3292A>T	p.N1098Y	Missense	5	Novel
BRCA2	3744 G>A	c.3516G>A	p.S1172S	Silent	1	BIC
BRCA2	3868 G>T	c.3640G>T	p.V1214L	Missense	2	Novel

***BRCA1/BRCA2*, breast cancer-susceptibility genes 1 and 2, respectively; BIC, Breast Cancer Information Core Database; V, valine; L, leucine; I, isoleucine; F,phenylyalanine; C, cysteine; Q, glutamine; H, histidine; K, lysine; E, glutamic acid; T, threonine; N, asparagine; Y, tyrosine; S, serine, W, tryptophan.**

Table 6. Predicted effect of novel unclassified missense variants of *BRCA1* and *BRCA2*.

Gene	BIC	HGVS	Amino acid	GV	GD	Align-	SIFT	Polyphen2	PMUT

	nomenclature	nomenclature	Change	GVGD					
BRCA1	150G>T	c.31G>T	p.V11L	28.68	4.86	C0	0.00 (not tolerated)	0.993 (probably damaging)	Neutral
BRCA1	162A>T	c.43A>T	p.I15F	4.86	21.28	C0	0.00 (not tolerated)	0.068 (benign)	Neutral
BRCA1	235G>T	c.116G>T	p.C39F	0	204.39	C65	0.00 (not tolerated)	0.996 (probably damaging)	Pathological
BRCA1	5387G>T	c.5268G>T	p.Q1756H	152.42	0	C0	0.03 (not tolerated)	0.129 (benign)	Pathological
BRCA1	5394A>G	c.5275A>G	p.K1759E	164.69	20.36	C0	0.00 (not tolerated)	0.01 (benign)	Neutral
BRCA2	967A>T	c.739A>T	p.I247F	242.23	0	C0	0.05 (Tolerated)	0.838 (possibly damaging)	Neutral
BRCA2	3520 A>T	c.3292A>T	p.N1098Y	231.68	79.14	C0	0.1 (Tolerated)	0.868 (possibly damaging)	Pathological
BRCA2	3868 G>T	c.3640G>T	p.V1214L	172.62	12.95	C0	0.33 (Tolerated)	0.006 (benign)	Neutral

***BRCA1/BRCA2*, breast cancer-susceptibility genes 1 and 2, respectively; BIC, Breast Cancer Information Core Database; HGVS, Human Genome Variation Society; GV, Grantham Variation score; GD, Grantham Deviation score; Align-GVGD, Align Grantham Variation Grantham Deviation; SIFT, Sorting Intolerant from Tolerant; PolyPhen; Polymorphism Phenotyping; V, valine; L, leucine; I, isoleucine; F, phenylyalanine; C, cysteine; Q, glutamine; H, histidine; K, lysine; E, glutamic acid; N, asparagine; Y, tyrosine.**

The *BRCA1* c.116G>T (p.C39F) mutation which was shown to be deleterious according to SIFT, Polyphen and PMUT and is placed in class C65 in Align-GVGD, was observed in 8 cases in our study cohort. It lies within the RING domain of *BRCA1* protein. Additionally, *BRCA1* c.5268G>T (p.Q1756H) and c.5275A>G (p.K1759E) mutations occurring within *BRCA1* BRCT repeats were predicted to be deleterious according to SIFT. Both mutations were identified only once in the same patient with early onset breast cancer. Noteworthy, this case was found to harbor the deleterious mutation *BRCA2* c.787dupA.

Discussion

The Egyptian population has many unique genetic features developed during its evolution history [3]. Breast cancer patients in Egypt have not been extensively studied for *BRCA1/2* mutations; knowledge of which can lead to better understanding of genetic risk factors of this disease. In the present study, we have determined the spectrum and frequency of *BRCA1/2* mutations in a cohort derived from hospital-based Egyptian female breast cancer population not selected on the basis of age of onset or family history, aiming to elucidate the contribution of mutations in these genes to the burden of breast cancer in Egypt. We have performed HRM analysis and direct sequencing of exons 2 and 20 of *BRCA1* gene and exons 9 and 11 of *BRCA2* gene. These exons contain frequently recurring mutations described worldwide, but the type of mutations identified can differ considerably in different populations.

In this study, *BRCA1* and *BRCA2* deleterious mutations were identified in 29 patients representing a prevalence of 28.15%. The frequency of *BRCA1/2* mutations display considerable variation among populations that coincides with ethnic and geographical diversity [13]. A high prevalence of 28% has been reported in the Levant region [31]. The Moroccan, Tunisian and Algerian populations have reported frequencies of 25.64%, 19.4% and 20%, respectively [32,33,34].

Our data are in line with prior studies in the Egyptian population that reported high mutation rate, supporting enrichment for genetic risk factors among Egyptian breast cancer patients. Ibrahim *et al* [17] have detected *BRCA1/2* mutations in 86% among 60 breast cancer patients and 120 cases of their asymptomatic female first degree relatives. The study reported five mutations, previously described in other populations, 2 in *BRCA1* (5454delC, 185delAG) and 3 in *BRCA2* (999del5, 4446C--T and 738C--A) using the combination of SSCP and heteroduplex analysis. A similar rate was detected by Fattoh *et al* [18]. El-Debaky *et al* [19] identified *BRCA1* mutations in 73.35% of breast cancer patients with family history (15 patients), 68.35% of breast cancer patients without family history (15 patients) and 55% of healthy controls (20 cases). They used primers for detection of 185delAG, 5382insC and C61G mutations using multiplex mutagenically separated PCR and RFLP. On the other hand, Saied *et al* [20] found a carrier frequency of 2.5% for heterozygous mutation of *BRCA1* AG185del using pyrosequencing technique.

In the current study, the spectrum of mutations identified differs from that previously reported in the Egyptian population. The aforementioned studies used primers designed for detection of specific mutations. In contrast, HRM analysis in our study allowed the detection of any sequence variations which were then confirmed and checked for by sequencing, allowing the detection of novel mutations as well as UVs. Taken together, these findings underline the importance of the development of Egyptian breast cancer mutational database.

Our findings show that all mutation carriers had either early onset, family history of breast cancer or bilateral disease; which are all suggestive of genetic predisposition. Thirteen mutation carriers reported positive family history of breast cancer, representing 31.7% of all familial cases. This is in agreement to that reported in Moroccan, Algerian and Tunisian hereditary breast cancer patients [33,35,36]. Fourteen mutation carriers had early onset breast cancer, providing a frequency of 32.5% of all early onset cases, which is similar to that detected in Ashkenazi Jews (30- 35%) [37,38]. Five mutation carriers had bilateral breast cancer, representing 31.2% of all cases with bilateral disease, which is consistent to that reported elsewhere [39]. This data highlights the need to use these clinical criteria for routine testing for *BRCA* genes mutations in Egyptian breast cancer patients.

Some of the mutations identified in our study were previously described in other populations. The *BRCA1* missense mutation c.110C>A (p.T37K) was detected before in the American population. The *BRCA1* missense mutation c.117T>G (p.C39W) was reported in the UMD-*BRCA1*and HGMD databases in the French population. The *BRCA2* silent mutation c.3396A>G (p.K1132K), observed in 2.9% of the studied cases, was cited in BIC database (232 reports), LOVD database and UMD database (6 reports) where it is described as polymorphism.

An important finding in our study is the detection of 6 novel deleterious mutations, and all of them were recurrent. The *BRCA1* frameshift mutation c.5205delA was observed in 15.5% of the study cohort, mainly in cases with early onset and positive family history of breast cancer. Interestingly, 3 patients had double mutations in both *BRCA1* and *BRCA2* genes, which is a rare condition in most populations. The 3 patients of them had early onset and positive family history of breast cancer. Frank *et al* [40] found 10 patients with both mutations in *BRCA1* and *BRCA2* out of 10,000 patients. On the other hand a higher frequency was observed by Choi *et al* [41], who detected 2 patients out of 9 carriers in a sample size of 60 young Korean breast cancer females.

To our knowledge, this report is the first to include information on the prevalence of missense mutations of unknown significance in the Egyptian population. About one-third of the genetic variants in *BRCA1* and 50% of those found in *BRCA2* reported by the BIC are considered genetic variants of unknown clinical significance, because of the uncertainty about their cancer risks [42]. We identified 11 different *BRCA1* and *BRCA2* UVs, 8 of them are novel. They may have a functional role in breast cancer development, which deserves further elucidation.

A major strength of the current study was the detection of novel deleterious mutations and UVs, which may imply that the Egyptian population has genetic peculiarity. Screening for these mutations in extended breast cancer families could help in the identification of specific effect. Further studies on larger sample size are warranted to better elucidate the role of *BRCA1/2* in breast cancer in Egypt. Studying the association of *BRCA1/2* mutational status and clinicopathological characteristics as well as its impact on clinical outcome is ongoing in another study.

Conclusions

This study provides the results of our attempt to delineate the genetic aspect of breast cancer among the Egyptian population. For the first time, we used HRM and direct sequencing to identify *BRCA1/2* mutations in Egyptian patients, providing a rapid, reliable and cost-effective approach for *BRCA* mutation detection in this resource constraint country. The accumulating evidence from this study and previous studies about the profile and frequency of *BRCA1/2* mutations in the Egyptian population emphasize the necessity of implementing screening and preventive strategies as part of the national public health policy in Egypt to facilitate early diagnosis and proper counseling for breast cancer patients.

List of Abbreviations

BRCA1: breast cancer susceptibility gene 1; *BRCA2*: breast cancer susceptibility gene 2; HRM: High-resolution melting; UVs: Unclassified variants; BIC: Breast Cancer Information Core Database; HGMD-Professional: Human Gene Mutation Database; UMD: Universal Mutation Database; LOVD: Leiden Open Variation Database; HGVS: Human Genome Variation Society; Align GVGD: Alignment-Grantham variation Grantham deviation; PolyPhen2: Polymorphism Phenotyping-2; SIFT: Sorting Intolerant From Tolerant; IARC: International Agency for Research on Cancer; BRCT: *BRCA1* C-terminal.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

HOE: Participated in study design, participated in analysis of clinical data and helped in the revision of the manuscript. SGA: Participated in study design, performed mutational analyses, analyzed clinical data and drafted the manuscript. HAA: Participated in the study design, revised clinical and pathological data and helped in the revision of the manuscript. ANZ: Participated in the study design, participated in sequence alignment and the analysis of the mutational data and revised the manuscript. All authors read and approved the final manuscript.

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