Preliminary results of targeted sequencing of *BRCA1* and *BRCA2* in a cohort of breast cancer families: New insight into pathogenic variants in patients and at-risk relatives

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Abstract. Breast cancer (BC) is the most commonly diagnosed cancer worldwide and a major health concern in Egypt. There is a known association between pathogenic variants identified in breast cancer susceptibility gene (*BRCA)1* and *2* and the risk of developing BC. However, the number of studies investigating mutations in *BRCA1* and *BRCA2* in Egypt remains limited. Thus, the aim of the present study was to investigate the frequency of *BRCA1* and *BRCA2* variants in patients with BC and their relatives. For this purpose, 11 families (11 patients and 16 relatives) were included in the present study. *BRCA1* and *BRCA2* variants were investigated using the Ion S5 next-generation sequencer. It was found that pathogenic variants were more frequent in patients with familial BC (FBC) than in those with sporadic BC, with 71% of variants in *BRCA2,* including the first reported identification of c.9089del, c.5583_5584dup, c.8243G>A and c.7976G>A pathogenic variants in Egyptian patients with BC. Pathogenic variants in relatives were confined to FBC c.1278delA, c.1960_1961del, and c.1224delT, with a higher incidence of variants of uncertain significance (VUS), such as *BRCA2* intron 2 c.68-16delT. Of note, two cold spot benign variants, c.3113A>G and c.4837A>G, were repeatedly found (67%) in patients and relatives. In conclusion, to the best of our knowledge, novel pathogenic variants and VUS in Egyptian patients with BC and their high-risk relatives were identified for the first time in the present study. These findings should be integrated with other genomic data concerning Egyptian families and carefully interpreted during genetic counseling.

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Introduction

Breast cancer type 1 and type 2 susceptibility genes *(BRCA1* and *BRCA2)* are located at chromosomes 17q21.31 and 13q12-q13, respectively; these are tumor suppressor genes that share a common pathway in protecting cell fidelity (1). Both genes repair double-strand DNA breaks in homologous recombination patterns (2). Inheriting a germline mutation of a single copy of either gene increases the risk of developing several types of cancer, including breast cancer (BC). *BRCA1* and *BRCA2* genes have 24 and 27 exons, respectively. Exon 11 is the largest exon in both genes and is the location of the most common mutations found in patients with BC (3).

It is known that 4-5% of BC cases are hereditary (hereditary breast and ovarian cancer syndrome) (4), which is autosomal dominant and is characterized by an early onset of BC with the occurrence of multiple tumors successively, such as in the ovaries, pancreas and stomach (5). Furthermore, 5-10% of cases are familial BC (FBC), whereby BC affects family members usually <50 years of age, in clusters, and with a different pattern from that observed in hereditary BC (6). The majority of BC cases are sporadic (SBC), whereby mutations are acquired with an increased risk with age, environment, lifestyle or medical factors (7).

According to the American Society of Medical Genetics and Genomics, variants/mutations in cancer can be classified into five categories: i) Pathogenic; ii) likely pathogenic; iii) variant of uncertain significance (VUS); iv) likely benign; and v) benign (8). Individuals who carry pathogenic variants should undergo a follow-up scheme, including mammography at short time intervals, or possibly prophylactic surgeries, such as bilateral mastectomy or salpingo-oophorectomy (9). In addition, tamoxifen administration may be used as a protective strategy against tumor development in hormone-dependent BC (10).

Studies on *BRCA1* and *BRCA2* were previously conducted in various groups of Egyptian patients with BC. However, these studies were performed either at the genetic level targeting well-known variants (11-13), or at the genomic level in a limited number of patient families (14). Therefore, the present study aimed to include more BC families (patients and their first-degree, at-risk relatives) to investigate the frequency of *BRCA1* and *BRCA2* variants using targeted next-generation sequencing.

Patients and methods

Patients. The present study was based on residents of the city of Alexandria, located on the northern coast of Egypt. The majority of the included participants were educated, working women. A total of 11 families were included as follows: Six patients with FBC with a family history of BC (at least one first-degree relative affected before the age of 50 years) and 10 high-risk relatives. All the patients with FBC had a history of BC from the maternal side, apart from patient no. 4, who had a history of BC from both the maternal and paternal side. Additionally, there were five patients with SBC without a known family history of BC and six high-risk relatives. The clinicopathological characteristics of the patients are summarized in Table I. Patients were recruited between October 2018 and October 2019 from the Clinical Oncology Department, Faculty of Medicine and Medical Research Institute, Alexandria University (Alexandria, Egypt). There were no specific exclusion criteria, and all patients were included regardless of hormonal status and metastatic state. Patients and relatives signed a formal consent form to participate in the study. The present study was approved by the Ethical Committee, Faculty of Medicine, Alexandria University (approval no. 0304918).

DNA extraction. For patients with both FBC and SBC, formalin-fixed paraffin-embedded (FFPE) tissue blocks were collected; the fixative used for those archived blocks was 10% buffered formalin overnight at room temperature for breast lumpectomy and modified radical mastectomy specimens at room temperature. The immunohistochemistry data were collected from the pathological reports of the cases, and the previously obtained slides were reviewed to confirm the correct interpretation. Following a pathological examination, DNA extraction was performed using a QIAamp DNA FFPE Tissue kit (cat. no. 56404; Qiagen GmbH). For relatives, genomic DNA was extracted from the peripheral blood using a PureLink™ Genomic DNA Mini kit (cat. no. K1820-00; Invitrogen; Thermo Fisher Scientific, Inc.). DNA was quantified using a Qubit™ 1X dsDNA HS (High Sensitivity) Assay kit Q33231 with a Qubit Fluorometer version 4 (Thermo Fisher Scientific, Inc.), according to the manufacturer's instructions.

Library preparation and next-generation sequencing. A total of 10 ng DNA per sample was used for library preparation using the Ion AmpliSeq™ Library Kit Plus (cat. no. 4488990; Ion Torrent; Thermo Fisher Scientific, Inc.) and the Oncomine™ BRCA Research assay (cat. no. A32842; Ion Torrent; Thermo Fisher Scientific, Inc.), according to the manufacturer's instructions. Coding regions from *BRAC1* and *BRCA2* were amplified using 167 primer pairs divided into two pools. Each library was barcoded using the Ion Xpress™ Barcode Adapters 1-16 kit (cat. no. 4471250; Ion Torrent; Thermo Fisher Scientific, Inc.). An Ion Library TaqMan™ Quantitation kit (cat. no. 4468802; Ion Torrent; Thermo Fisher Scientific, Inc.) was used to quantify the libraries against a standard curve of serial dilutions of the *E. coli* DH10B Control Library provided (Thermo Fisher Scientific, Inc.). Barcoded libraries were pooled and diluted to a final concentration of 100 pM.

Template preparation by emulsion PCR on Ion Sphere Particles (ISPs) was performed using the Ion 520™ & Ion 530™ Kit-OT2 kit (cat. no. A27751) and the Ion OneTouch™ 2 System (Ion Torrent; Thermo Fisher Scientific, Inc.). The samples were loaded on Ion 520 Chip kit v2 (Ion Torrent; Thermo Fisher Scientific, Inc.). Sequencing primer and polymerase were added to the final enriched ISPs prior to loading onto an Ion 520 chip, according to the Ion 520 & Ion 530 kit-OT2 protocol.

Sequencing was performed using the Ion S5™ next generation sequencing system (Ion Torrent; Thermo Fisher Scientific, Inc.). Sequencing coverage for reporting were ≥20 unique reads (x20) for each base. Allelic frequency for somatic variants ranged from 0.05 to 1%, while for germline variants, the allele frequency was between 0.4 and 1%. Median coverage typically ranged between x200 and x300.

Statistical analysis. Sequencing data were processed using Torrent Suite software 5.12.1 (Ion Torrent; Thermo Fisher Scientific, Inc.) to analyze barcode reads; to align reads to the hg19 reference genome; and to generate run metrics, which include chip loading efficiency, total read counts and quality. Following statistical analysis, the annotation of single-nucleotide variants, insertions, deletions and splice site alterations, was performed using the Ion Reporter Server System (Oncomine BRCA Research Assay; Thermo Fisher Scientific, Inc.). The variants identified were further analyzed using Oncomine Reporter.

All mutations reported were confirmed in the Human Gene Mutation Database (HGMD; http://www.hgmd.cf.ac.uk/ac/index.php), Catalogue Of Somatic Mutations In Cancer (https://cancer.sanger.ac.uk/cosmic), the algorithms SIFT 6.2.1 (https://sift.bii.a-star.edu.sg/www/code.html) PolyPhen-2 (version 2) (http://genetics.bwh.harvard.edu/pph2/) and Align-GVGD (http://agvgd.hci.utah.edu/agvgd_input.php) and ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/), and reviewed by the BRCA Exchange (https://brcaexchange.org). The mutation function and mutation classes were further investigated using the Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA) (15).

Results

Patient characteristics and mutational status. The median age of patients with FBC and SBC was 35.5 and 66 years, respectively. Each patient had a history of unilateral BC, apart from FBC patient no. 6, who had a history of contralateral BC. With regards to first-degree relatives, no one had experienced a health issue, apart from the daughter of SBC patient no. 9, who had a history of fibroadenoma in the right breast (Tables I and II). The investigation of *BRCA1* and *BRCA2* variants in patients revealed 22 mutations, namely 9 in *BRCA1* and 13 in *BRCA2* (Table II).

All patients with FBC and SBC carried *BRCA1* and *BRCA2* mutations, apart from patients no. 6 and 11 who were carriers of *BRCA2*. Generally, no significant associations were detected between *BRCA1* and *BRCA2* mutations and patient age (data not shown). In total, ~82% of patients had infiltrating ductal carcinoma, 46% of patients had estrogen receptor (ER)- and progesterone receptor (PR)-positive BC of varying histological grades. In addition, three patients had triple-negative (ER, PR and Her2/neu) BC; two had FBC and one had SBC with a high histological grade (Table I). No significant association

Table I. Clinicopathological criteria for patients with FBC and SBC included in the present study.

A, FBC

Patient ID	Age, years	ER	PR	Her2/neu	Ki67, %	Lymph node metastasis	Pathology	Tumorgrade	Distant metastasis
1	37	Positive, +++	Positive, ++	Negative	18	4/10	Infiltrating ductal carcinoma	II	-
2	34	Negative	Negative	Negative	60	5/11	Invasive mammary carcinoma NST (Invasive carcinoma of no special type) with focal apocrine differentiation	III	-
3	39	Positive, +++	Positive, ++	Negative	10	Multiple	Infiltrating ductal carcinoma	II	-
4	33	Positive, ++	Negative	Negative	90	5/15	Infiltrating ductal carcinoma	III/IV	-
5	47	Positive, ++	Positive, +	Negative	15	6/12	Infiltrating ductal carcinoma	II	Brain and bone metastasis
6	34	Negative	Negative	Negative	86	NA	Infiltrating ductal carcinoma/both sides	III/IV	Liver, bone and pulmonary metastasis

B, SBC

Patient ID	Age, years	ER	PR	Her2/neu	Ki67, %	Lymph node metastasis	Pathology	Grade	Distant metastasis
7	71	Negative	Negative	Negative	4	6/20	Infiltrating ductal carcinoma	III	-
8	44	Positive, ++	Negative	Negative	10	No lymph node metastasis	Mixed infiltrating ductal carcinoma	II	-
9	66	Positive, +++	Positive, +	Negative	10	3/10	Infiltrating ductal carcinoma	II	-
10	67	Positive, +++	Positive, +	Negative	12	2/11	Infiltrating ductal carcinoma	II/III	-
11	46	Positive, ++	Negative	Negative	13	NA	Infiltrating ductal carcinoma	II	-

FBS, familial breast cancer; SBC, sporadic breast cancer; ER, estrogen receptor; PR, progesterone receptor; Her2/neu, receptor tyrosine-protein kinase erbB-2. -, absent. Each patient had a history of unilateral BC except FBC patient no. 6, who had a history of contralateral BC.

Table II. *BRCA1* and *BRCA2* variants detected in patients with FBC and SBC.

A, FBC

Patient ID	Gene	Transcript	Amino acid change	Exon	Coding	Variant ID	Locus	Variant effect	ClinVar
1	*BRCA2*	NM_000059.3	p.(N372H)	10	c.1114A>C	rs144848	chr13:32906729	Missense	Benign
	BRCA2	NM_000059.3	p.(V2466A)	14	c.7397T>C	rs169547	chr13:32929387	Missense	Benign
	BRCA1	NM_007294.3	p.(S1613G)	16	c.4837A>G	rs1799966	chr17:41223094	Missense	Benign
	BRCA1	NM_007294.3	p.(G1471V)	13	c.4412G>T	rs587782708	chr17:41228577	Missense	Uncertain significance
	BRCA1	NM_007294.3	p.(K1183R)	11	c.3548A>G	rs16942	chr17:41244000	Missense	Benign
	BRCA1	NM_007294.3	p.(E1038G)	10	c.3113A>G	rs16941	chr17:41244435	Missense	Benign
	BRCA1	NM_007294.3	p.(P871L)	11	c.2612C>T	rs799917	chr17:41244936	Missense	Benign
2	*BRCA2*	NM_000059.3	p.S2186T	11	c.6556T>A	Unknown	chr13:32915048	Unknown	Uncertain significance
	BRCA2	NM_000059.3	p.(Thr3030LysfsTer32)	23	c.9089del	rs886040818	chr13:32954021	Frameshift	Pathogenic
	BRCA1	NM_007294.3	p.(A1588V)	15	c.4763C>T	rs151029675	chr17:41223168	Missense	Benign
	BRCA1	NM_007294.3	p.(K1183R)	11	c.3548A>G	rs16942	chr17:41244000	Missense	Benign
3	*BRCA1*	NM_007294.3	p.(T1685A)	16	c.5053A>G	rs80356890	chr17:41219646	Missense	Pathogenic/Uncertain significance
	BRCA2	NM_000059.3	p.(Thr3030LysfsTer32)	23	c.9089del	rs886040818	chr13:32954021	Frameshift	Pathogenic
	BRCA1	NM_007294.3	p.(S1613G)	16	c.4837A>G	rs1799966	chr17:41223094	Missense	Benign
	BRCA1	NM_007294.3	p.(K1183R)	11	c.3548A>G	rs16942	chr17:41244000	Missense	Benign
4	*BRCA2*	NM_000059.3	Unknown	Intron 13	c.7008-2A>T	rs81002823	chr13:32928996	Splice acceptor variant	Pathogenic
	BRCA1	NM_007294.3	Unknown	Intron 10	c.4097-1G>A	rs80358070	chr17:41243050	Splice acceptor variant	Pathogenic
	BRCA1	NM_007294.3	p.(E1038G)	10	c.3113A>G	rs16941	chr17:41244435	Missense	Benign
	BRCA1	NM_007294.3	p.(P871L)	11	c.2612C>T	rs799917	chr17:41244936	Missense	Benign
	BRCA1	NM_007294.3	p.(S1613G)	16	c.4837A>G	rs1799966	chr17:41223094	Missense	Benign
5	*BRCA2*	NM_000059.4	R2659K	17	c.7976G>A	rs80359027	13: 32362693	In-frame deletion	Pathogenic
	BRCA2	NM_000059.4	G2748D	18	c.8243G>A	rs80359071	13: 32936830	Missense	Pathogenic
	BRCA1	NM_007294.3	p.(E1038G)	10	c.3113A>G	rs16941	chr17:41244435	Missense	Benign
	BRCA1	NM_007294.3	p.(S1613G)	16	c.4837A>G	rs1799966	chr17:41223094	Missense	Benign
6	*BRCA2*	NM_000059.3	p.(Thr3030LysfsTer32)	23	c.9089del	rs886040818	chr13:32954021	Frame shift	Pathogenic
	BRCA2	NM_000059.3	p?	Intron 2	c.68-16delT	Unknown	chr13:32893197	Unknown	Conflicting interpretations of pathogenicity

B, SBC

Patient ID	Gene	Transcript	Amino acid change	Exon	Coding	Variant ID	Locus	Variant effect	ClinVar
7	*BRCA2*	NM_000059.3	p.(N372H)	10	c.1114A>C	rs144848	chr13:32906729	Missense	Benign
	BRCA2	NM_000059.3	p.(V2466A)	14	c.7397T>C	rs169547	chr13:32929387	Missense	Benign

Table II. Continued.

B, SBC

Patient ID	Gene	Transcript	Amino acid change	Exon	Coding	Variant ID	Locus	Variant effect	ClinVar
	BRCA1	NM_007294.3	p.(S1613G)	16	c.4837A>G	rs1799966	chr17:41223094	Missense	Benign
	BRCA1	NM_007294.3	p.(K1183R)	11	c.3548A>G	rs16942	chr17:41244000	Missense	Benign
	BRCA1	NM_007294.3	p.(E1038G)	10	c.3113A>G	rs16941	chr17:41244435	Missense	Benign
	BRCA1	NM_007294.3	p.(P871L)	11	c.2612C>T	rs799917	chr17:41244936	Missense	Benign
8	*BRCA2*	NM_000059.3	p.(?)	UTR 5	c.-26G>A	rs1799943	chr13:32890572	Unknown	Benign
	BRCA2	NM_000059.3	p.(V2466A)	14	c.7397T>C	rs169547	chr13:32929387	Missense	Benign
	BRCA2	NM_000059.3	p.(S2835P)	20	c.8503T>C	rs11571746	chr13:32945108	Missense	Benign/Likely benign
	BRCA1	NM_007294.3	p.(S1613G)	16	c.4837A>G	rs1799966	chr17:41223094	Missense	Benign
	BRCA1	NM_007294.3	p.(A1588V)	15	c.4763C>T	rs151029675	chr17:41223168	Missense	Benign
	BRCA1	NM_007294.3	p.(K1183R)	11	c.3548A>G	rs16942	chr17:41244000	Missense	Benign
	BRCA1	NM_007294.3	p.(E1038G)	10	c.3113A>G	rs16941	chr17:41244435	Missense	Benign
	BRCA1	NM_007294.3	p.(P871L)	10	c.2612C>T	rs799917	chr17:41244936	Missense	Benign
9	*BRCA2*	NM_000059.3	p.(Ala3029=)	23	c.9087G>A	rs368576266	chr13:32954020	Synonymous	Likely benign; Benign/ Uncertain significance
	BRCA1	NM_007294.3	p.(Ser694=)	10	c.2082C>T	rs1799949	chr17:41245466	Synonymous	Benign
	BRCA2	NM_000059.3	p.?	Intron 21	c.8755-66T>C	rs4942486	chr13:32953388	Unknown	Benign
	BRCA2	NM_000059.3	G2748D	18	c.8243G>A	rs80359071	chr13:32937582	Missense	Pathogenic; Likely pathogenic/ Pathogenic/Uncertain significance
10	*BRCA1*	NM_007294.4	T1685A	15	c.5053A>G	rs879254205	chr17:41219646	Missense	Pathogenic
	BRCA2	NM_000059.4	G2748D	18	c.8243G>A	rs80359071	chr13: 32937582	Missense	Pathogenic
	BRCA2	NM_000059.3	p.(N372H)	10	c.1114A>C	rs144848	chr13:32906729	Missense	Benign
	BRCA1	NM_007294.3	p.(P871L)	11	c.2612C>T	rs799917	chr17:41244936	Missense	Benign
11	*BRCA2*	NM_000059.3	p.(V1862Sfs*11)	11	c.5583_5584insA		chr13:32339938	Frameshift insertion	Pathogenic
	BRCA2	NM_000059.3	p.(N372H)	10	c.1114A>C	rs144848	chr13:32906729	Missense	Benign
	BRCA2	NM_000059.3	p.(V2466A)	14	c.7397T>C	rs169547	chr13:32929387	Missense	Benign

FBS, familial breast cancer; SBC, sporadic breast cancer; BRCA1, breast cancer type 1 susceptibility gene; BRCA2, breast cancer type 2 susceptibility gene.

was observed between the hormonal status of the tumor, and *BRCA1* and *BRCA2* mutations (data not shown).

Pathogenic variants in patients are more frequent in BRCA2 than in BRCA1. The analysis of pathogenic variants in patients revealed two variants in *BRCA1* and five variants in *BRCA2* with no preference to a specific exon (Table II). Pathogenic variants were mainly found in patients with FBC (five with FBC and three with SBC) (Table II). A pathogenic variant c.9089del *BRCA2*, located in exon 23 was shared by three patients with FBC (nos. 2, 3 and 6) with a median age of 34 years and who presented with early-onset BC: Two of them had triple-negative BC of high histological grade (III/IV) (Tables I and II). The variant c.9089del is a frameshift class 1 mutation that is predicted to encode a truncated non-functional protein (15). This variant was previously detected in Chinese patients with early-onset BC (16). Additionally, a missense pathogenic variant, c.8243G>A *BRCA2,* located in exon 18, was shared by FBC patient no. 5 and two patients with SBC (nos. 9 and 10) with medium to high-histological grades (II/III). Variant c.8243G>A is a class 2 mutation with a stable mutant protein (17). A class 2 missense pathogenic variant c.5053A>G *BRCA1,* located in exon 16, was found in FBC patient no. 3 and SBC patient no. 10 (grade II/III). This variant is linked to an increased risk of breast and ovarian cancer (18,19). Other pathogenic variants were detected, such as a class 1 frameshift c.5583_5584insA *BRCA2* variant, located in exon 11 and observed in SBC patient no. 11 (grade II). Two intronic variants (*BRCA2* c.7008-2A>T intron 13 with non-functional transcript, *BRCA1* c.4097-1G>A intron 10) were identified in FBC patient no. 4 with a very high histological grade (III/IV). Both variants have been found to have splicing aberrations (20,21). In addition, one in-frame class 2 c.7976G>A *BRCA2* located in exon 17 was found in FBC patient no. 5 (grade II). This variant has been previously reported in patients with BC and/or ovarian cancer (19,22).

Furthermore, as presented in Table II, four VUS were identified in four patients: Three variants in patients with FBC (two in *BRCA2* and one in *BRCA1*) and one variant in *BRCA2* in a patient with SBC. The variants were distributed among different exons: 11, 13, 20, 23 and intron 2. The BRCA exchange revealed that the synonymous substitution variant c.9087G>A had a low bioinformatic likelihood of resulting in a splicing aberration. Variant c.68-16delT has no existing functional assay data. The algorithms SIFT, PolyPhen-2 and Align-GVGD that predict the effect of missense changes on protein structure and function, all suggested that the variants c.4412G>T and c.6556T>A were likely to be tolerated.

One patient with SBC (patient no. 8) had a probable benign variant c.8503T>C *BRCA2* in exon 20. This variant has previously been identified in two North African countries: Tunisia and Algeria (23).

Cold spot benign variants identified in both BRCA1 and BRCA2. Missense benign mutations with amino acid replacement were also identified: Six variants in *BRCA1* and four variants in *BRCA2*. These benign variants were observed in both FBC and patients with SBC (Table II). Benign missense mutations were found in exon 10 (40%) and exon 11 (20%) followed by 10% each in exons 13, 14, 15 and 16. These benign variants located in exon 10 and exon 11 are considered 'cold spots' since they are tolerant to variations (24).

Pathogenic variants are confined to relatives of patients with FBC. The investigation of *BRCA1* and *BRCA2* variants in first-degree relatives revealed three pathogenic variants, with all of these found in relatives of patients with FBC (Table III). Variant c.1278delA (GA>A) *BRCA2* in exon 10 was detected in a daughter of patient no. 2, where the G allele is predicted to cause a frameshift deletion mutation with an amino acid change. Both relatives of FBC patient no. 4 had pathogenic variants: The mother had c.1960_1961delA, (CT>C, CTT) and the daughter had c.1224del, (CT>C). The last two variants were frameshift mutations located in *BRCA1* exon 10. Variant c.1960_1961delA was also found in a sister of FBC patient no. 3. No pathogenic variants were identified in the relatives of patients with SBC.

Moreover, VUS, likely benign, or conflicting interpretation of pathogenicity variants were identified in 9/16 relatives: Six relatives with FBC and three with SBC. Three variants were detected in *BRCA1* (exons 10 and 16) and four variants in *BRCA2* (exons 10, 11 and intron 2). Notably, *BRCA2* intron 2 c.68-16delT was observed in six at-risk relatives in four families (familial and sporadic). This variant was repeated in family no. 6 (patient and two relatives) and in both relatives of patient no. 4. The A allele in c.68-16delT (AT>A), is predicted to cause an unknown/non-coding mutation in the *BRCA2* gene. This variant was previously selected as one of the specific deletions in BC microarray analyses (25). In addition, a daughter of FBC patient no. 1 had a likely benign *BRCA1* exon 10 missense mutation. This locus is linked to adenocarcinoma in the Catalogue Of Somatic Mutations In Cancer (https://cancer.sanger.ac.uk/cosmic). Furthermore, one sister of FBC patient no. 3 had a *BRCA1* exon 10 c.2733A>G synonymous variant that was previously detected only in patients with unilateral BC (24). One daughter of SBC patient no. 11 had a missense *BRCA2* mutation c.5640T>G with a conflicting interpretation of pathogenicity that was previously identified as an unclassified variant in both contralateral and unilateral BC (26). Finally, both daughters of SBC patient no. 9 shared a *BRCA2* exon 10 c.1011C>T synonymous variant. One of these daughters had a fibroadenoma in one breast and a history of pregnancy and lactation.

Common benign missense variants identified in relatives of patients with FBC and SBC. Seven out of 11 families had repeated benign *BRCA* missense mutations found in both patients and their relatives. Two benign mutations, c.3113A>G *BRCA1* in exon 10 and c.4837A>G *BRCA1* in exon 16, were detected in 8/16 relatives (50%). Variants c.3113A>G and c.4837A>G were previously reported as candidate founder mutations in an Iranian population (27).

Discussion

The present study was conducted on 11 families: Six with FBC and five with SBC. A similar previous study on *BRCA1* and *BRCA2* in five Egyptian families with a history of BC was previously performed using exome sequencing (14), where the families were selected from a more rural population in the

Table III. Variants detected in relatives of patients with FBC and SBC.

A, FBC

Relative ID	Relationship	Gene	Transcript	Amino acid change	Exon	Coding	Variant ID	Locus	Variant effect	ClinVar
1	Daughter	*BRCA1*	NM_007294.3	p.(Ala622=)	10	c.1866G>T	rs1805324	chr17:41245682	Missense	Likely benign; Likely benign/Uncertain significance
		BRCA1	NM_007294.3	p.(E1038G)	10	c.3113A>G	rs16941	chr17:41244435	Missense	Benign; Benign/no known pathogenicity/ not provided
		BRCA1	NM_007294.3	p.(S1613G)	16	c.4837A>G	rs1799966	chr17:41223094	Missense	Benign; Benign/no known pathogenicity/ not provided
2	Daughter	*BRCA2*	NM_000059.3	NP_000050.2: p.(Asp427ThrfsTer3)	10	c.1278delA	rs80359274	chr13:32906888	Frameshift	Pathogenic
		BRCA1	NM_007294.3	NP_009225.1:p.(E1038G)	10	c.3113A>G	rs16941	chr17:41244435	Missense	Benign; Benign/no known pathogenicity/ not provided
		BRCA1	NM_007294.3	NP_009225.1:p.(S1613G)	16	c.4837A>G	rs1799966	chr17:41223094	Missense	Benign; Benign/no known pathogenicity/ not provided
3	Sister 1	*BRCA1*	NM_007294.3	p.(Lys654ValfsTer18)	10	c.1960_1961delA	rs80357522	chr17:41245586	Frameshift	Pathogenic
		BRCA1	NM_007294.3	p.(S1613G)	16	c.4837A>G	rs1799966	chr17:41223094	Missense	Benign
		BRCA1	NM_007294.3	p.(E1038G)	10	c.3113A>G	rs16941	chr17:41244435	Missense	Benign
	Sister 2	*BRCA1*	NM_007294.3	p.(Gly911=)	10	c.2733A>G	rs1800740	chr17:41244815	Synonymous	Benign/Likely benign
		BRCA1	NM_007294.3	p.?	Intron 5	c.441+18C>T	rs371973519	chr17:41256121	Unknown	Benign; Benign/no known pathogenicity/ not provided
		BRCA1	NM_007294.3	p.(Glu1038Gly)	10	c.3113A>G	rs16941	chr17:41244435	Missense	Benign; Benign/no known pathogenicity/ not provided
		BRCA1	NM_007294.3	p.(Ser1613Gly)	16	c.4837A>G	rs1799966	chr17:41223094	Missense	Likely benign/ Uncertain significance
		BRCA1	NM_007294.3	p.(Asn1592Lys)	15	c.4776C>G	rs761925468	chr17:41223155	Missense	Benign/Likely benign
4	Sister	*BRCA1*	NM_007294.3	p.(Val409Ter)	10	c.1224delT	rs879255320	chr17:41246323	Frameshift	Pathogenic
		BRCA2	NM_000059.3	p.?	Intron 2	c.68-16delT	rs276174878	chr13:32893197		Benign, Uncertain significance

Table III. Continued.

A, FBC

Relative ID	Relationship	Gene	Transcript	Amino acid change	Exon	Coding	Variant ID	Locus	Variant effect	ClinVar
	Mother	*BRCA1*	NM_007294.3	p.(Lys654SerfsTer47)	10	c.1960_1961delA	rs80357522	chr17:41245586	Frameshift	Pathogenic; Pathogenic/Uncertain significance
		BRCA1	NM_007294.3	p.(Glu1038Gly)	10	c.3113A>G	rs16941	chr17:41244435	Missense	Benign; Benign/no known pathogenicity/ not provided
		BRCA1	NM_007294.3	p.(Ser1613Gly)	16	c.4837A>G	rs1799966	chr17:41223094	Missense	Benign; Benign/no known pathogenicity/ not provided
		BRCA2	NM_000059.3	p.?	Intron 2	c.68-16delT	rs276174878	chr13:32893197	Unknown	Benign/Uncertain significance
5	Daughter 1	*BRCA2*	NM_000059.3	p.(V2466A)	14	c.7397T>C	rs169547	chr13:32929387	Missense	Benign
		BRCA1	NM_007294.3	p.(S1613G)	16	c.4837A>G	rs1799966	chr17:41223094	Missense	Benign
		BRCA1	NM_007294.3	p.(K1183R)	11	c.3548A>G	rs16942	chr17:41244000	Missense	Benign
		BRCA1	NM_007294.3	p.(S1040N)	10	c.3119G>A	rs4986852	chr17:41244429	Missense	Benign
		BRCA1	NM_007294.3	p.(E1038G)	10	c.3113A>G	rs16941	chr17:41244435	Missense	Benign
		BRCA1	NM_007294.3	p.(P871L)	11	c.2612C>T	rs799917	chr17:41244936	Missense	Benign
		BRCA1	NM_007294.3	p.(D693N)	10	c.2077G>A	rs4986850	chr17:41245471	Missense	Benign
		BRCA2	NM_000059.3	p.(V2466A)	14	c.7397T>C	rs169547	chr13:32929387	Missense	Benign
	Daughter 2	*BRCA2*	NM_000059.3	p.(V2466A)	14	c.7397T>C	rs169547	chr13:32929387	Missense	Benign
6	Sister 1	*BRCA2*		p?	Intron 2	c.68-16delT	Unknown	chr13:32893197	Unknown	Conflicting interpretations of pathogenicity
	Sister 2	*BRCA2*		p?	Intron 2	c.68-16delT	Unknown	chr13:32893197	Unknown	Conflicting interpretations of pathogenicity

B, SBC

Relative ID	Relationship	Gene	Transcript	Amino Acid Change	Exon	Coding	Variant ID	Locus	Variant effect	ClinVar
7	Niece	*BRCA2*	NM_000059.3	p.(?)/protein identifier NP_000050.2	2	c.-26G>A	rs1799943	chr13:32890572	Unknown	Benign/Little clinical significance
		BRCA2	NM_000059.3	p.(N372H)	10	c.1114A>C	rs144848	chr13:32906729	Missense	Benign
		BRCA2	NM_000059.3	p.(V2466A)	14	c.7397T>C	rs169547	chr13:32929387	Missense	Benign

Table III. Continued.

B, SBC

Relative ID	Relationship	Gene	Transcript	Amino acid change	Exon	Coding	Variant ID	Locus	Variant effect	ClinVar
		BRCA1	NM_007294.3	p.(S1613G)	16	c.4837A>G	rs1799966	chr17:41223094	Missense	Benign
		BRCA1	NM_007294.3	p.(K1183R)	11	c.3548A>G	rs16942	chr17:41244000	Missense	Benign
		BRCA1	NM_007294.3	p.(E1038G)	10	c.3113A>G	rs16941	chr17:41244435	Missense	Benign
		BRCA1	NM_007294.3	p.(P871L)	11	c.2612C>T	rs799917	chr17:41244936	Missense	Benign
8	Daughter	*BRCA1*	NM_007294.3	S1613G	16	c.4837A>G	rs1799966	chr17:41223094	Missense	Benign; Benign/no known pathogenicity/ not provided
		BRCA1	NM_007294.3	E1038G	10	c.3113A>G	rs16941	chr17:41244435	Missense	Benign; Benign/no known pathogenicity/ not provided
		BRCA2	NM_000059.3	p.?	Intron 2	c.68-16delT	rs276174878	chr13:32893197	Unknown	Benign/Uncertain significance
9	[a]Daughter 1	*BRCA2*	NM_000059.3	p.(Asn337=)	10	c.1011C>T	rs41293473	chr13:32906626	Synonymous	Likely benign; Benign/Likely benign/Uncertain significance
		BRCA2	NM_000059.3	p.(Val2171=)	11	c.6513G>C	rs206076	chr13:32915005	Synonymous	Benign
		BRCA2	NM_000059.3	p.(Val2171=)	11	c.6513G>T	rs206076	chr13:32915005	Synonymous	Likely benign; Benign/Likely benign/Uncertain significance
		BRCA1	NM_007294.3	p.(S1613G)	16	c.4837A>G	rs1799966	chr17:41223094	Missense	Benign; Benign/no known pathogenicity/ not provided
		BRCA1	NM_007294.3	p.(E1038G)	10	c.3113A>G	rs16941	chr17:41244435	Missense	Benign; Benign/no known pathogenicity/ not provided
		BRCA2	NM_000059.3	p.?	Intron 2	c.68-16delT	rs276174878	chr13:32893197	Unknown	Conflicting interpretations of pathogenicity
	Daughter 2	*BRCA2*	NM_000059.3	p.(Asn337=)	10	c.1011C>T	rs41293473	chr13:32906626	Synonymous	Likely benign; Benign/Likely benign/Uncertain significance

Table III. Continued.

B, SBC

Relative ID	Relationship	Gene	Transcript	Amino acid change	Exon	Coding	Variant ID	Locus	Variant effect	ClinVar
		BRCA2	NM_000059.3	p.(Val2171=)	11	c.6513G>C	rs206076	chr13:32915005	Synonymous	Benign
		BRCA2	NM_000059.3	p.(Val2171=)	11	c.6513G>T	rs206076	chr13:32915005	Synonymous	Likely benign; Benign/Likely benign/Uncertain significance
		BRCA1	NM_007294.3	p.(S1613G)	16	c.4837A>G	rs1799966	chr17:41223094	Missense	Benign; Benign/no known pathogenicity/ not provided
10	Daughter	*BRCA2*	NM_000059.3	p.?/protein identifier NP_000050.2	utr_5	c.-26G>A	rs1799943	chr13:32890572	Unknown	Benign/Little clinical significance
		BRCA2	NM_000059.3	p.(V2466A)	14	c.7397T>C	rs169547	chr13:32929387	Missense	Benign
11	Daughter	*BRCA2*	NM_000059.3	p.(N372H)	10	c.1114A>C	rs144848	chr13:32906729	Missense	Benign
		BRCA2	NM_000059.3	p.(V2466A)	14	c.7397T>C	rs169547	chr13:32929387	Missense	Benign
		BRCA2	NM_000059.3	p.(N1880K)	11	c.5640T>G	rs11571657	chr13:32914132	Missense	Conflicting interpretations of pathogenicity

FBS, familial breast cancer; SBC, sporadic breast cancer; BRCA1, breast cancer type 1 susceptibility gene; BRCA2, breast cancer type 2 susceptibility gene. [a]This relative had a history of fibroadenoma in the right breast.

Gharbiah district of the Delta region of Egypt. In the present study, various benign missense variants that were identified either in patients or their relatives, have been previously reported in Egyptian patients with BC (14) and in other BC populations, such as Indian, Greek, Turkish and Italian (3). However, in contrast to the findings of the present study, pathogenic/likely pathogenic variants were not observed using exome sequencing of the five patients with FBC (14), and where the median age for FBC was 48.5 years, which was higher than the median age of 35.5 years for FBC in the present study.

In the current study, no significant associations were found between the clinicopathological characteristics of the patients and *BRCA* mutations. Nevertheless, a broad-scale research including a greater number of patients is required to improve the characterization of *BRCA1* and *BRCA2* mutation carriers among the Egyptian population.

Certain pathogenic variants in the present study were repeated in more than one patient, such as c.8243G>A, c.9089del and c.5053A>G. However, no inherited pathogenic variant was found in the same family. Those recurrent loci may be used as a suggested targeted panel for genetic testing in Egyptian patients (28).

In the present study, only one pathogenic variant was found in exon 11 and the remaining variants were located outside exons 10 or 11, such as in exons 16, 17, 18 and 23, and introns 3 and 10. Therefore, screening for pathogenic variants in exons beyond exons 10 and 11 is crucial (29).

Additionally, one VUS: *BRCA2* intron 2 c.68-16delT was repeated in the relatives of both the sporadic and familial cases. This variant has not been previously reported in Egyptian patients (14), at least to the best of our knowledge. A very recent screening of *BRCA1* and *BRCA2* in 103 Egyptian female patients with BC using high resolution melting analysis revealed novel VUS that were different from the results of the present study, as they screened only exons 2 and 20 of *BRCA1*, and exons 9 and 11 of *BRCA2* (30). Nevertheless, the observation of the present study that VUS were frequent in patients with FBC and their relatives drew attention to the potential role of VUS in FBC. This necessitates further genetic analyses in order to fully elucidate the role of these variants in BC and to determine guidelines for the follow-up of relatives who are at a high risk (31).

Moreover, pathogenic variants that were detected in the relatives of patients with FBC, have been previously reported in other populations. For example, c.1278delA and c.1224delT were previously detected in the Japanese population (32,33) and c.1960_1961delA was observed in the Brazilian population (0.3% of a total 441 variants identified) (34). Variant c.1960_1961delA was also repeated in two relatives and may thus be added to the suggested targeted panel for genetic screening, as aforementioned. The association between FBC and pathogenic variants in *BRCA* or even in other BC predisposition genes, such as *P53* and *ATM*, has been previously elucidated (35).

The data presented herein demonstrated that there was no specific pattern of inheritance of variants between patients with FBC and their relatives. However, the presence of pathogenic variants only in relatives of patients with FBC confirmed a genetic predisposition to the familial type of the tumor and thus, these *BRCA* pathogenic carriers must undergo close surveillance. Similarly, for the family of FBC patient no. 4, who has a history of BC from the paternal and maternal side, and the identification of *BRCA1* pathogenic variants in both the unaffected mother and sister, genetic counseling and follow-up are mandatory (36).

In conclusion, to the best of our knowledge, the present study was the first to identify pathogenic variants in *BRCA1* and *BRCA2* in an Egyptian population, with a spotlight on pathogenic variants and VUS in at-risk relatives of patients with FBC. The present data and the results of previous studies on Egyptian families must be integrated in order to further understand the characteristics of *BRCA1* and *BRCA2* mutations in Egyptian families with BC.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

MHS conceived the study, participated in its design and coordination, carried out the molecular genetics and sequencing studies, analyzed the data, and drafted the manuscript. DEK and ET participated in conception, design, molecular genetics, sequencing studies and revised the manuscript. RF, RAH, AR, AEK and MT participated in conception, design and practical sequencing work, and revised the manuscript. II participated in design and performed the pathological examination of the FFPE sections. MS and ET confirm the authenticity of all the raw data. All the authors read and approved the final manuscript.

Ethics approval and consent to participate

This research was conducted on human participants. The study was approved by the Ethics Committee of Alexandria University (approval no. 0304918; Alexandria, Egypt). Patients and relatives signed a formal consent form to participate in the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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