RESEARCH ARTICLE

Editorial Process: Submission:06/29/2025 Acceptance:01/30/2026 Published:02/06/2026

Identification of *BRCA1* and *BRCA2* Germline Mutations in Female Breast Cancer Patients Using Next Generation Sequencing

Menha Swellam[1,2]*, Mohamed K. Khalifa[3,4], Amira M Nageeb[1,2], Magda Sayed Mahmoud[1,2], Maha Hashim[1,2], Aliaa Alisslam I. A. Alshazly[5], Magdy E. Mahfouz[5], Lobna R Ez ElArab[6], Madyha H. Mahmoud[7], Mohamed Emam Sobeih[8]

Abstract

Background: Breast cancer is the most common cancer affecting females according to WHO 2020 report, with *BRCA1* and *BRCA2* identified as the major tumor suppressor genes linked to disease susceptibility. Hence, the purpose of the current study was to investigate germline mutations among these genes in a group of Egyptian female breast cancer patients. **Methods:** Blood samples from primary breast cancer patients (n=22), benign breast lesions (n=5) and healthy controls (n=7) were sequenced for BRCA genes by next generation sequencer. **Results:** A total of 135 genetic variations were detected, 59 in *BRCA1* and 76 *BRCA2*, 2 indels (insertion-deletion) and 133 SNV (single nucleotide variation), nearly 55% of those variants were missense variants, 38% were synonymous and 7% were nonsense. A total of 59 variations were detected in *BRCA1* and they were categorized as exonic (n=10) and intronic (n=49) regions, *BRCA1* exonic variants were categorized into: missense (n=12), non-coding transcript (n=11) synonymous (n=6), splice region synonymous (n=2), stop gain (n=2) and 3-prime UTR (n=1) variants. Regarding variations detected in *BRCA2*, 55 intronic and 21 exonic variants were identified. Among these 21 variants; 13 novel mutations and 8 formally reported as follows: 7 as benign and one previously reported with contradictory pathogenicity according to the Clinvar database which is a freely available, public archive of reports of the relationships between human variations and phenotypes hosted by the National Center for Biotechnology Information (NCBI) and funded by intramural National Institutes of Health (NIH) funding. **Conclusion:** *BRCA1* and *BRCA2* germline profiling based on next-generation sequencing technology among Egyptian breast cancer female patients revealed 135 germline variations -104 intronic and 31 exonic, respectively. Among the exonic variants; 13 were newly reported mutations. Hence further study is required to enhance mutational analysis which may benefit clinical system.

Keywords: Breast Cancer- BRCA genes- next generation sequencer- germ line profiling

Asian Pac J Cancer Prev, **27 (2)**, 575-581

Introduction

Worldwide, breast cancer is the most common malignancy among women [1]. The National Malignancy Registry Program (NCRP) in Egypt's most recent data indicates that breast cancer represents major prevalent cancer in women, accounting for 32.04% of all cancers [2]. Breast cancer susceptibility genes may be present in 5%–10% of instances of breast cancer, increasing the chance of malignancy [3]. A significant portion of breast cancer is caused by inherited predisposition [4]. *BRCA1* (OMIM# 113705) and *BRCA2* (OMIM# 600185) are two key genes linked to hereditary breast cancer and having a high chance of developing the disease, according to genetic linkage experts. In 1990 and 1995, these tumor suppressor genes were reported and recognized as breast cancer vulnerability genes. *BRCA2* is found on chromosome 13q12–13, while *BRCA1* is found on chromosome 17q21 [5, 6]. The functions of *BRCA1* /2 are vital for normal cell function as they are included in the cellular DNA repair and inhibition of uncontrolled cell growth [7]. Germline mutations in these two highly

*[1]Biochemistry Department, Biotechnology Research Institute, Giza, Egypt. [2]High Throughput Molecular and Genetic laboratory, Central Laboratories Network and the Centers of Excellence, National Research Centre, Dokki, Giza, Egypt. [3]CSO at Omicsense, Cairo, Egypt. [4]Molecular pathology lab Children Cancer Hospital 57357, Cairo, Egypt. [5]Zoology Department, Faculty of Science, Kafr El-Sheikh University, Kafr El-Sheikh, Egypt. [6]Clinical Oncology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt. [7]Department of Medical Laboratory Technology, College of Nursing and Health Sciences, Jazan University, Jazan, Saudi Arabia. [8]Medical Oncology Department, National Cancer Institute, Cairo, Egypt. *For Correspondence: menhamswellam@gmail.com*

penetrant genes are inherited in an autosomal dominant pattern and can increase the risk of acquiring breast cancer by as much as 80%. Women who carry *BRCA1* /2 mutations have a significant increased risk of developing breast cancer before the age of 50 years [8]. Mutations for *BRCA1* /2 have been previously reported in databases of the Breast Cancer Information Core (BIC) [8]. The structure, integrity, and function of the genes are mostly affected by these mutations, which are broadly distributed across both genes. According to some studies, Arab women may have more *BRCA1*/2 mutations than women from other groups [9]. *BRCA1*/2 mutations have been found in family breast cancer patients from Morocco [10, 11], Algeria [12, 13], Tunisia [14, 15], the Middle East [16], Egypt [17], Lebanon [18], Saudi Arabia [19], and Qatar [20].

It has been reported previously that both *BRCA1* and *BRCA2* mutations have a high penetrance rate. Carriers of a *BRCA1*/2 pathogenic or possible pathogenic variant have an extreme risk for both breast cancer that necessitates consideration of more rigorous screening and defensive strategies [21, 22]. Additionally, for *BRCA1* and *BRCA2* mutation carriers, the chance of developing contralateral breast cancer is 40% and 26%, respectively, after 20 years following the original diagnosis [23].

Studies on the genetic propensity for Egyptian family breast cancer have been conducted, mostly focusing on *BRCA1* and *BRCA2* [17], but full genomic data from local patients are still lacking.

Finding these mutations in high-risk patients indicated by international recommendations should be a top priority due to the high penetration rate and their major effects [22]. Individuals with *BRCA1* or *BRCA2* pathogenic variant carriers are strongly advised to undergo risk-reducing procedures like bilateral mastectomy, especially among younger patients. Young women who are diagnosed with breast cancer and their families may have major consequences due to the diagnosis and any potential hereditary connections. Such complex physical and emotional issues can be overcome with the aid of doctors and genetic counsellors [24].

Locating mutation carriers may be useful therapeutically for breast cancer patients in addition to its effectiveness in preventing breast cancers. Recent research had indicated that *BRCA1* or *BRCA2* mutation-associated patients with advanced-stage breast cancer might benefit from PARP (poly ADP ribose polymerase) inhibitors like olaparib and talazoparib, both of which are now licensed for such a circumstance [25-27]. Next-generation sequencing (NGS) is a valuable tool that analyzes up to giga bases of DNA reads at a high speed and with a low cost per base. This method has also been used in worldwide collaborative projects, such as the International Genome Consortium (ICGC) [28] and The Cancer Genome Atlas (TCGA) (http://cancergenome.nih.gov). Due to the large size of *BRCA1* gene (5592 bp) and *BRCA2* gene (10257 bp) hence the need for convenient screening approaches, such as next-generation sequencing; therefore, we used Ion Torrent PGM, sequencer (Thermo Fisher Scientific, Waltham, MA, USA) to detect the variants of *BRCA1* and *BRCA2* genes.

In the current study, the authors aimed to study the prevalence and pattern of pathogenic germline *BRCA1* and *BRCA2* mutations by NGS among a group of Egyptian patients with breast cancer, benign breast lesions and control individuals using blood samples and compare the reported mutations with clinicopathological criteria for the breast cancer group.

Materials and Methods

Recruited individuals

The present prospective study was accompanied in accordance with the declaration of Helsinki and the study was approved by the Medical ethical committee – National Research Centre (ID#3-4-12), and all participants signed their informed consent. Twenty- two primary breast cancer cases were recruited from females who fulfilled the inclusion criteria as early diagnosed primary breast cancer patients and did not receive radiology, chemotherapy or hormonal therapy and confirmed with no metastasis to other organs. Also a group of patients with benign breast lesion (n=7) were enrolled. The control group (n=5) were individuals unaffected by breast cancer or other types of cancer and no family history of cancer were registered. Both benign and healthy individuals were considered as the non-cancerous group to be compared to the breast cancer group.

Breast cancer patients were diagnosed according to their staging and grading systems following the TNM classification [29] and modified Scarff-Bloom Richardson histologic grading system [30], respectively. Hormone receptors were examined using an immunohistochemical method as previously reported [31]. Both ER and PgR were considered positive if ≥10% nuclei were positively stained using ten high- power fields, and HER-2neu was considered positive if scored as +3 [32].

DNA extraction

Participants provided peripheral blood samples (3ml), then centrifuged at 10.000xg for 10 min at 4°C. After that, it was kept at -80 °C for subsequent genomic DNA isolation.

Genomic DNA was isolated by QIAamp DNA small blood kit (Cat No. 51104, Qiagen, Hilden, Germany), both purity and concentration were determined using a nano-drop spectrophotometer (Quawell, Q-500, Scribner, NE, USA), then stored at -80°C for further evaluations.

Library preparation and purification

As previously reported [33, 34], in brief: by using the Ion AmpliSeq *BRCA1* and *BRCA2* Panel (Life Technologies), the target regions were amplified. Ion AmpliSeq Library kit (Cat No. 4480441 Life Technologies) was used quantification operation, and AmpliSeqHiFi master mix, with Ion AmpliSeq primer pool (each of the three primers in a separate well for each sample ID 1-6), 10 ng of genomic DNA per reaction, and 4µl of nuclease-free water, the reaction was carried out using thermal cycler (SureCycler 8800, Agilent, Santa Clara, CA, USA). Following amplification, the primers were digested with FuPa reagent, then after ligation with

both adaptors (Cat No. A29751 Life Technologies) and barcodes using the Ion Xpress Barcode Adaptors 1-16 kit (Cat No. 4471250 Life Technologies), the primers were then sequenced. Then, as instructed in the user manual for the Ion AmpliSeq Library kit, amplification was performed using Ion Library TaqMan Quantitation kit (Cat No. 4468802 Life Technologies) (Max3005P QPCR system, Stratagene, Agilent Biotechnology, USA). The preparation of templates was done before the expanded libraries.

Template preparation

To achieve at least 500X depth of coverage for each sample, purified and quantified libraries were combined in a pool at a molar equivalent ratio. The Ion OneTouch 2 instrument (Life Technologies) was used to clonally amplify the pooled libraries using the Ion PGM Hi-Q view OT2 kit (Cat No# A29900 Life Technologies). Then, in accordance with the manufacturer's instructions, template ion sphere particles (ISP) were enriched using ion PGM enrichment beads (Cat No# 4478525 Life Technologies) as previously reported [33, 34].

Sequencing using ion torrent PGM platform

All barcoded enriched samples were sequenced on the Ion Torrent PGM Platform (Ion Torrent PGM, Life Technologies) using the Ion 318 Chip Kit V2 BCA (Cat No# 4488150 Life Technologies) and Ion PGM Hi-Q View Sequencing kit (Cat No# A30044 Life Technologies).

Data analysis

After aligning with the hg19 reference human genome (Genome reference Consortium GRCh37) using the cloud-based Ion Reporter Suite software (version 5.4.0 Life Technologies), variants of the *BRCA1* and *BRCA2* genes were annotated using the manufacturer-recommended parameters for the *BRCA1* and *BRCA2* panels.

The reference sequences for *BRCA1* and *BRCA2* were NM 007300.3 and NM 000059.3, respectively, and analysis of variations was conducted without bias. Ensembl Variation Effect Predictor (VEP), an online tool, was employed for variant annotation and effect prediction.

Results

Individual characteristics

The current study was carried out on a total of 34 individuals, they were grouped into primary breast cancer (n=22), patients with primary breast benign lesions (n=7) and age matched healthy individuals (n=5). Breast cancer group was staged into two groups (early stage and late stage) and graded into (low grade and high grade). Their demographic and clinicalopathological features were reported in Table 1.

NGS results

In general, 135 genetic variants were found, including 59 in *BRCA1* and 76 in *BRCA2* (2 insertion-deletion; small genetic mutation where one or more nucleotides are either inserted into or deleted from the genome [indels]), and 133 single nucleotide variations; involves the substitution of one nucleotide for another in a DNA sequence [SNV]) and they were grouped as missense (55%), synonymous (38%) and nonsense (7%) variants. Only 59 of the 135 variations had been previously published and assigned a dbSNPrs ID.

BRCA1

Ten exonic and 49 intronic changes all variants were annotated using Variant Effect Predictors (VEP) for the *BRCA1* gene as shown in Supplementary Tables 1, 2, respectively. There were 12 overlapping intronic mutations, which were considered exonic in certain transcripts and introinc in others. Regarding variant significances per transcript; *BRCA1* exonic variants were categorized into: missense (n=12), non-coding transcript (n=11) synonymous (n=6), splice region synonymous

Table 1. Individual Demographic and Clinical Characteristics

Factors	Control group (n=5)	Benign breast lesion (n=7)	Breast cancer (n=22)
Age			
< 50 years	2	3	15
≥ 50 years	3	4	7
Pathology status			
Fibroadenoma	-	3	-
Inflamatory cyst	-	1	-
Fibrocystic disease	-	2	-
Lipoma	-	1	-
IDC	-	-	19
ILC	-	-	3
Site of tumor			
Right side	-	5	9
Left side	-	2	11
Bilateral	-	-	2
Clinical Stage			
I = T1-T2	-	-	11
II = T3-T4	-	-	11
Histological grading			
I = G1-G2	-	-	20
II = G3-G4	-	-	2
Number Lymph node metastasis			
< 5	-	-	12
≥ 5	-	-	10
Receptor status			
Triple -ve	-	-	11
Triple +ve	-	-	11
Metastatic to other organs			
No-metastasis	-	-	16
Metastatic	-	-	6
Recurrence			
No	-	-	18
Yes	-	-	4

(n=2), stop gain (n=2) and 3-prime UTR (n=1) variants (3′UTR variants are non-coding and do not affect the protein-coding sequence. However, they are still located within exons - specifically, un-translated exonic regions - and thus classified as non-coding exonic variants). The stop gain variants they were formally reported as pathogenic in Clinvar database where a total of 59 *BRCA1* genetic variants were processed in the analysis, with none filtered out. Among these, 31 variants (52.5%) were novel, while 28 (47.5%) were previously known. The variants overlapped with 2 genes, 32 transcripts, and 2 regulatory features. When examining coding consequences, the majority were missense variants (55%), followed by synonymous variants (33%) and stop-gained mutations (8%). Expanding to all annotated consequences, intron variants dominated the dataset (61%), with smaller proportions of missense (8%), downstream gene variants (7%), NMD transcript variants (6%), synonymous variants (6%), splice region variants (4%), 3′UTR variants (3%), upstream gene variants (1%), and non-coding transcript variants (1%), along with a few others."

BRCA2

Regarding the *BRCA2* gene, a total of 21 exonic and 55 intronic variants were detected in BRCA2, as reported in Supplmentary Tables 3,4, respectively. Based on variant consequences per transcript the exonic variants in *BRCA2* can be grouped like the following 10 synonymous variants, one 5-prime UTR variant (5′UTR variants are non-coding and do not affect the protein-coding sequence. However, they are still located within exons- specifically, untranslated exonic regions- and thus classified as non-coding exonic variants) which was predicted to have a modifier effect and 10 missense variants. Concerning the intronic variants there were two splice site variants, where BRCA2, 76 genetic variants were processed with no filtering applied. Of these, 45 variants (59.2%) were novel, while 31 (40.8%) were previously reported. The variants overlapped with 4 genes, 16 transcripts, and 4 regulatory features. Among coding consequences, missense variants accounted for 55% and synonymous variants for 45%, indicating a predominance of protein-altering changes. When considering all annotated consequences, intron variants were most frequent (50%), followed by downstream gene variants (17%), missense (8%), synonymous (7%), NMD transcript variants (5%), upstream gene variants (5%), regulatory region variants (3%), non-coding transcript exon variants (3%), and 5′UTR variants (1%), with a small fraction falling under other categories.

From the 21 exonic mutations, 13 variants were newly reported and 8 formally reported variations among them: 7 were detected to be benign and 1 was described to be with contradictory pathogenicity on the Clinvar database. Among the enrolled individuals, mutational allele frequencies (MAF) were reported in Supplementary Table 5.

Discussion

Breast cancer is the most common cancer in Egyptian women, accounting for 22,700 cases approximately 38.8% of cancers in this population in 2020, and this number is forecasted to jump to 46,000 in 2050 [35], and several studies have been conducted on Egyptian patients among this type of cancer for its early detection and prediction its prognosis [36-39]. Among the genetic mutations being detected in breast cancer are BRCA genes variants, however studies conducted among Egyptians concerning their mutations are sparse, hence authors aimed to shed light on the genetic makeup of *BRCA1* and *BRCA2* in Egyptian women with breast cancer as compared to patients with benign breast lesions and a group of healthy controls using blood sample as minimally invasive approach to detect BRCA genes mutations using a high throughput technology as NGS.

In the current study, 135 genetic variations were reported in *BRCA1* and *BRCA2* as 59 and 76, respectively, out of these variations 59 were previously reported. It has been stated earlier that revealing of *BRCA1* and 2 genetic variations may participate to recognize the genetic pathogenesis of breast cancer [40]. Identification of common and rare genetic variations in regulatory elements may alter gene expression and be linked with disease risk [41], in our results common mutations were reported in both genes as shown in Supplementary Tables (1-5) [supplementary data], thus detecting escalating number of variants in non-coding areas of the genome by NGS may offering a considerable clinical challenge [42-44]. The present study reported the prevalence of mutational allele frequency (MAF) among enrolled groups (Supplementary Table 5, supplementary data) and the 13 mutations were reported newly, in previous studies among Egyptians and other countries from North Africa it has been reported that detection of pathogenic mutations in BRCA genes is a great approach for understanding breast cancer predisposition and early diagnosis [45-48].

Genetic screening for *BRCA1* in the current study has revealed exonic and intronic variations as 10 and 49, respectively. In an earlier study carried out by Montalban and his colleagues [49], stated the first *BRCA1* deep intronic variant related to inheritance breast and ovarian cancer amongst Spanish population by Sanger sequencing, although in an another report on 20 families from France and USA to reveal *BRCA1* variants reported pathogenic variants (PV) with decreased penetrance [50]. In another recent study among Egyptian breast cancer patients screened BRCA genes for some exons using high resolution melting analysis (HRM) then direct sequencing for the detected variants reported PV for *BRCA1* [43]. However, in the current study sequencing of the *BRCA1*gene using NGS as a sensitive and high throughput technology serves as powerful and new input data among Egyptian breast cancer patients.

Investigation of *BRCA2* gene sequencing disclosed the finding of 76 variants (55 intronic, and 21 exonic), eight exonic alterations of the 21 were described previously as 7 were of benign nature and only one were reported with conflicting pathogenicity as in a previous study there was no previous study (PV) was reported among 5 Egyptians families breast cancer families using whole exome sequencing (WES) [17], while in other study using

HRM reported that carriers of *BRCA2* mutations exhibit worse survival pattern which points the pathogenicity of these mutations [43]. In an anther previous study [51] the rate of *BRCA1*/ *BRCA2* PVs in triple negative breast cancer was 20.8%. By contrast to formerly reported exonic mutation records, the present study based on NGS sequencing reported 13 novel mutations which need additional assessment to their relation with medical characteristics, disease progression and survival patterns, a study in progress to report our findings.

In conclusion, to our knowledge there are few reports based on NGS technology to study germline variations of *BRCA1* and *BRCA2* genes among early diagnosed Egyptian breast cancer females as compared with benign breast lesion and healthy controls using blood samples pointing out the usefulness of this high throughput technology to explore *BRCA1* and *BRCA2* variants hence can be further applied in routine clinical use. The novelty of the current study is that among the enrolled Egyptian breast cancer patients profiling of *BRCA1* and *BRCA2* germline revealed 135 germline variations, 104 intronic and 31 exonic variants, and among the exonic variants 13 were newly reported mutations which may need future studies on large number of participants.

Study limitations

As this is a preliminary study, authors clarified the presence of some limitations as the limited number of breast cancer patients hence there was no reported significance between detected mutations and clinicopathological criteria for breast cancer, thus authors are planning to extend their work on large number of female breast cancer patients as a multicenter study in the future.

Author Contribution Statement

Study conception and design: MS, MSM, MH and MKK. Provision of samples and clinical follow-up: LEE and MES, Acquisition of data: AAIAA, AMN, MH and MKK. Analysis and interpretation of data: MS, MH, MSM, MEM, MHM and MKK. Drafting of manuscript: MS, AMN, MKK, MSM, MEM, MH and MHM. Critical revision: all authors.

Acknowledgements

The instruments listed in the current study were purchased through a grant from Science Technology Development Fund (STDF) through Capacity Building Grant Fund (CBG) [No. 4940], Egypt. The study was not part of any scientific Body or part of an approved student thesis. The study was not registered in any registration dataset.

Ethics approval and consent to participate

Ethical approval of the study was obtained from the Medical Ethical Committee of National Research Centre (ID#3-4-12), Egypt, and all participants signed their informed consent.

Availability of data and material

The datasets generated and/or analysed during the current study are not publicly available due Intellectual property/confidentiality issues, but are available from the corresponding author on reasonable request.

Ethical approval

The study was approved by the Medical ethical committee – National Research Centre (ID#3-4-12)

Competing interests: Authors report no conflicts of interest in this work

Abbreviations

BRCA, breast cancer; NGS, Next generation sequencer; indels, insertion – deletion; SNV, single neucleotide varariations; WES, whole exome sequencing; HRM, high resolution melting analysis, ICGC, International Genome Consortium; PGM, personal genome machine.

References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136(5):E359–386. https://doi.org/10.1002/ijc.29210
2. Ibrahim SS, Hafez EE, Hashishe MM. Presymptomatic breast cancer in egypt: Role of *BRCA1* and *BRCA2* tumor suppressor genes mutations detection. J Exp Clin Cancer Res. 2010;29(1):82. https://doi.org/10.1186/1756-9966-29-82.
3. Cobain EF, Milliron KJ, Merajver SD. Updates on breast cancer genetics: Clinical implications of detecting syndromes of inherited increased susceptibility to breast cancer. Semin Oncol. 2016;43(5):528–35. https://doi.org/10.1053/j.seminoncol.2016.10.001
4. Easton DF, Pharoah PD, Antoniou AC, Tischkowitz M, Tavtigian SV, et al. Gene-panel sequencing and the prediction of breast-cancer risk. N Engl J Med. 2015 Jun 4;372(23):2243-57.
5. Hall JM, Lee MK, Newman B, Morrow JE, Anderson LA, Huey B, et al. Linkage of early onset familial breast cancer to chromosome 17q21. Science. 1990;250(4988):1684–9. https://doi.org/10.1126/science.2270482
6. Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, et al. Identification of the breast cancer susceptibility gene BRCA2. Nature. 1995;378(6559):789–92. https://doi.org/10.1038/378789a0.
7. Lou DI, McBee RM, Le UQ, Stone AC, Wilkerson GK, Demogines AM, et al. Rapid evolution of *BRCA1* and *BRCA2* in humans and other primates. BMC Evol Biol. 2014;14:155. https://doi.org/10.1186/1471-2148-14-155.
8. Couch FJ, Nathanson KL, Offit K. Two decades after BRCA: Setting paradigms in personalized cancer care and prevention. Science. 2014;343(6178):1466–70. https://doi.org/10.1126/scien ce.1251827.
9. Balouch MA, Kolek MJ, Darbar D. Improved understanding of the pathophysiology of atrial fibrillation through the lens of discrete pathological pathways. Glob Cardiol Sci Pract. 2014;2014(1):24-36. https://doi.org/10.5339/gcsp.2014.5.
10. Jouali F, Laarabi FZ, Marchoudi N, Ratbi I, Elalaoui SC, Rhaissi H, et al. First application of next-generation sequencing in Moroccan breast/ovarian cancer families and report of a novel frameshift mutation of the *BRCA1* gene.

Oncol Lett. 2016;12(2):1192–6. https://doi.org/10.3892/ol.2016.4739.
11. Laraqui A, Uhrhammer N, Lahlou-Amine I, Rhaffouli HEL, Baghdadi JE, Dehayni M, Bignon YJ. Mutation screening of the *BRCA1* gene in early onset and familial breast/ovarian cancer in Moroccan population. Int J Med Sci. 2013;10(1):60–7. https://doi.org/10.7150/ijms.5014.
12. Cherbal F, Bakour R, Adane S, Boualga K, Benais-Pont G, Maillet P. *BRCA1* and *BRCA2* germline mutations screening in algerian breast/ovarian cancer families. Dis Markers. 2010;28(6):377-84. https://doi.org/10.3233/dma-2010-0718
13. Henouda S, Bensalem A, Reggad R, Serrar N, Rouabah L, Pujol P. Contribution of *BRCA1* and *BRCA2* germline mutations to early algerian breast cancer. Dis Markers. 2016;2016:7869095. https://doi.org/10.1155/2016/7869095.
14. Mahfoudh W, Bouaouina N, Ahmed SB, Gabbouj S, Shan J, Mathew R, et al. Hereditary breast cancer in Middle Eastern and North African (MENA) populations: Identification of novel, recurrent and founder *BRCA1* mutations in the Tunisian population. Mol Biol Rep. 2012;39(2):1037–46. https://doi.org/10.1007/s11033-011-0829-8.
15. Riahi A, Kharrat M, Ghourabi ME, Khomsi F, Gamoudi A, Lariani I, et al. Mutation spectrum and prevalence of *BRCA1* and *BRCA2* genes in patients with familial and early-onset breast/ovarian cancer from tunisia. Clin Genet. 2015;87(2):155-60. https://doi.org/10.1111/cge.12337.
16. Bu R, Siraj AK, Al-Obaisi KA, Beg S, Al Hazmi M, Ajarim D, et al. Identification of novel brca founder mutations in middle eastern breast cancer patients using capture and sanger sequencing analysis. Int J Cancer. 2016;139(5):1091-7. https://doi.org/10.1002/ijc.30143.
17. Kim YC, Soliman AS, Cui J, Ramadan M, Hablas A, Abouelhoda M, et al. Unique features of germline variation in five Egyptian familial breast cancer families revealed by exome sequencing. PLoS ONE. 2017;12(1):e0167581. https://doi.org/10.1371/journal.pone.0167581.
18. Jalkh N, Chouery E, Haidar Z, Khater C, Atallah D, Ali H, et al. Next-generation sequencing in familial breast cancer patients from Lebanon. BMC Med Genomics. 2017;10(1):8. https://doi.org/10.1186/s12920-017-0244-7.
19. Merdad A, Gari MA, Hussein S, Al-Khayat S, Tashkandi H, Al-Maghrabi J, et al. Characterization of familial breast cancer in Saudi Arabia. BMC Genomics. 2015;16 (Suppl 1):S3.https://doi.org/10.1186/1471-2164-16-s1-s3.
20. Bujassoum SM, Bugrein HA, Al-Sulaiman R. Genotype and phenotype correlation of breast cancer in BRCA mutation carriers and non-carriers. J Cancer Sci Ther. 2017;9(2):358–62. https://doi.org/10.4172/1948-5956.1000442.
21. Chen S, Parmigiani G. Meta-analysis of *BRCA1* and *BRCA2* penetrance. J Clin Oncol. 2007;25(11):1329-33. https://doi.org/10.1200/jco.2006.09.1066
22. Daly MB, Pal T, Berry MP, Buys SS, Dickson P, Domchek SM, et al. Genetic/familial high-risk assessment: Breast, ovarian, and pancreatic, version 2.2021, nccn clinical practice guidelines in oncology. J Natl Compr Canc Netw. 2021;19(1):77-102. https://doi.org/10.6004/jnccn.2021.0001.
23. Machackova E, Claes K, Mikova M, Házová J, Sťahlová EH, Vasickova P, et al. Twenty years of *BRCA1* and *BRCA2* molecular analysis at mmci - current developments for the classification of variants. Klin Onkol. 2019;32(Supplementum2):51-71. https://doi.org/10.14735/amko2019S51
24. Abdel-Razeq H, Abujamous L, Abunasser M, Edaily S, Bater R. Prevalence and predictors of germline *BRCA1* and *BRCA2* mutations among young patients with breast cancer in jordan. Sci Rep. 2021;11(1):14906. https://doi.org/10.1038/s41598-021-94403-1
25. Turner NC, Telli ML, Rugo HS, Mailliez A, Ettl J, Grischke EM, et al. A phase ii study of talazoparib after platinum or cytotoxic nonplatinum regimens in patients with advanced breast cancer and germline *BRCA1*/2 mutations (abrazo). Clin Cancer Res. 2019;25(9):2717-24. https://doi.org/10.1158/1078-0432.Ccr-18-1891
26. Litton JK, Rugo HS, Ettl J, Hurvitz SA, Gonçalves A, Lee KH, et al. Talazoparib in patients with advanced breast cancer and a germline brca mutation. N Engl J Med. 2018;379(8):753-63. https://doi.org/10.1056/NEJMoa1802905.
27. Turk AA, Wisinski KB. Parp inhibitors in breast cancer: Bringing synthetic lethality to the bedside. Cancer. 2018;124(12):2498-506. https://doi.org/10.1002/cncr.31307.
28. Hudson TJ, Anderson W, Artez A, Barker AD, Bell C, Bernabé RR, et al. International network of cancer genome projects. Nature. 2010;464(7291):993-8. https://doi.org/10.1038/nature08987
29. Greene FL, Sobin LH. The staging of cancer: A retrospective and prospective appraisal. CA Cancer J Clin. 2008;58(3):180-90. https://doi.org/10.3322/ca.2008.0001.
30. Robbins P, Pinder S, de Klerk N, Dawkins H, Harvey J, Sterrett G, et al. Histological grading of breast carcinomas: A study of interobserver agreement. Hum Pathol. 1995;26(8):873-9. https://doi.org/10.1016/0046-8177(95)90010-1.
31. Umemura S, Itoh H, Ohta M, Suzuki Y, Kubota M, Tokuda Y, et al. Immunohistochemical evaluation of hormone receptor for routine practice of breast cancer: Highly sensitive procedures significantly contribute to the correlation with biochemical assays. Appl Immunohistochem Mol Morphol. 2003;11(1):62-72. https://doi.org/10.1097/00129039-200303000-00011
32. Williams SL, Birdsong GG, Cohen C, Siddiqui MT. Immunohistochemical Detection of Estrogen and Progesterone Receptor and HER2 Expression in Breast Carcinomas: Comparison of Cell Block and Tissue Block Preparations. Int J Clin Exp Pathol. 2009;2(5):476–80.
33. Khalifa MK, Bakr NM, Ramadan A, Abd Elwahab KM, Desoky E, Nageeb AM, et al. Implications of targeted next-generation sequencing for bladder cancer: Report of four cases. J Genet Eng Biotechnol. 2021;19(1):91. https://doi.org/10.1186/s43141-021-00182-7
34. Nageeb AM, Mohamed MM, Ezz El Arab LR, Khalifa MK, Swellam M. Next generation sequencing of brca genes in glioblastoma multiform egyptian patients: A pilot study. Arch Physiol Biochem. 2022;128(3):809-17. https://doi.org/10.1080/13813455.2020.1729814
35. El Orabi M and El- Ghazawy H. Cancer control in Egypt. Investing in health; 2021. Available from: https://ascopost.com/issues/march-25-2021/cancer-control-in-egypt/
36. Swellam M, Ismail M, Eissa S, Hamdy M, Mokhtar N. Emerging role of p53, bcl-2 and telomerase activity in egyptian breast cancer patients. IUBMB Life. 2004;56(8):483-90. https://doi.org/10.1080/15216540400010834
37. Swellam M, Arab LR, Bushnak HA. Clinical implications of her-2/neu overexpression and proteolytic activity imbalance in breast cancer. IUBMB Life. 2007;59(6):394-401. https://doi.org/10.1080/15216540701395074
38. Bakr NM, Mahmoud MS, Nabil R, Boushnak H, Swellam M. Impact of circulating mirna-373 on breast cancer diagnosis through targeting vegf and cyclin d1 genes. J Genet Eng Biotechnol. 2021;19(1):84. https://doi.org/10.1186/s43141-021-00174-7
39. Swellam M, El Magdoub HM, Shawki MA, Adel M, Hefny MM, El-Shazly SS. Clinical impact of lncrna xist and lncrna neat1 for diagnosis of high-risk group breast cancer

patients. Curr Probl Cancer. 2021;45(5):100709. https://doi.org/10.1016/j.currproblcancer.2021.100709
40. Al Hannan F, Keogh MB, Taha S, Al Buainain L. Characterization of *BRCA1* and *BRCA2* genetic variants in a cohort of bahraini breast cancer patients using next-generation sequencing. Mol Genet Genomic Med. 2019;7(7):e00771. https://doi.org/10.1002/mgg3.771
41. Diederichs S, Bartsch L, Berkmann JC, Fröse K, Heitmann J, Hoppe C, et al. The dark matter of the cancer genome: Aberrations in regulatory elements, untranslated regions, splice sites, non-coding rna and synonymous mutations. EMBO Mol Med. 2016;8(5):442-57. https://doi.org/10.15252/emmm.201506055.
42. Burke LJ, Sevcik J, Gambino G, Tudini E, Mucaki EJ, Shirley BC, et al. *BRCA1* and *BRCA2* 5' noncoding region variants identified in breast cancer patients alter promoter activity and protein binding. Hum Mutat. 2018;39(12):2025-39. https://doi.org/10.1002/humu.23652
43. AbdelHamid SG, Zekri AN, AbdelAziz HM, El-Mesallamy HO. *BRCA1* and *BRCA2* truncating mutations and variants of unknown significance in egyptian female breast cancer patients. Clin Chim Acta. 2021;512:66-73. https://doi.org/10.1016/j.cca.2020.11.023
44. AbdelHamid S, El-Mesallamy H, Aziz HA, Zekri AR. Prognostic impact of *BRCA1* and *BRCA2* mutations on long-term survival outcomes in egyptian female breast cancer patients. Biology (Basel). 2021;10(7):566. https://doi.org/10.3390/biology10070566
45. Tazzite A, Jouhadi H, Nadifi S, Aretini P, Falaschi E, Collavoli A, et al. *BRCA1* and *BRCA2* germline mutations in moroccan breast/ovarian cancer families: Novel mutations and unclassified variants. Gynecol Oncol. 2012;125(3):687-92. https://doi.org/10.1016/j.ygyno.2012.03.007
46. Fourati A, Louchez MM, Fournier J, Gamoudi A, Rahal K, El May MV, et al. Screening for common mutations in *BRCA1* and *BRCA2* genes: Interest in genetic testing of tunisian families with breast and/or ovarian cancer. Bull Cancer. 2014;101(11):E36-40. https://doi.org/10.1684/bdc.2014.2049
47. ElBiad O, Laraqui A, El Boukhrissi F, Mounjid C, Lamsisi M, Bajjou T, et al. Prevalence of specific and recurrent/founder pathogenic variants in brca genes in breast and ovarian cancer in north africa. BMC Cancer. 2022;22(1):208. https://doi.org/10.1186/s12885-022-09181-4
48. Azim HA, Loutfy SA, Azim HA, Jr., Kamal NS, Abdel Fattah NF, Elberry MH, et al. The landscape of brca mutations among egyptian women with breast cancer. Oncol Ther. 2023;11(4):445-59. https://doi.org/10.1007/s40487-023-00240-9.
49. Montalban G, Bonache S, Moles-Fernández A, Gisbert-Beamud A, Tenés A, Bach V, et al. Screening of *BRCA1*/2 deep intronic regions by targeted gene sequencing identifies the first germline *BRCA1* variant causing pseudoexon activation in a patient with breast/ovarian cancer. J Med Genet. 2019;56(2):63-74. https://doi.org/10.1136/jmedgenet-2018-105606
50. Høberg-Vetti H, Ognedal E, Buisson A, Vamre TBA, Ariansen S, Hoover JM, et al. The intronic *BRCA1* c.5407-25t>a variant causing partly skipping of exon 23-a likely pathogenic variant with reduced penetrance? Eur J Hum Genet. 2020;28(8):1078-86. https://doi.org/10.1038/s41431-020-0612-1
51. Shohdy KS, Kassem L, Elnahas T, Barsoum EM, Gabriel B, Elghazawy H, et al. Genetic testing for *BRCA1*/2 variants in northern african women with ovarian and breast cancers: A multicentre study of an under-represented ancestry. ESMO Open. 2025;10(8):105510. https://doi.org/10.1016/j.esmoop.2025.105510.