QATAR UNIVERSITY

COLLEGE OF HEALTH SCIENCE

MOLECULAR ANALYSIS OF THE *BRCA1* AND *BRCA2* GENES IN HEREDITARY BREAST OR OVARIAN CANCER PATIENTS IN QATAR (2015-2016)

BY

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ABSTRACT

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Title: Molecular Analysis of the *BRCA1* and *BRCA2* Genes in Hereditary Breast or Ovarian Cancer Patients in Qatar (2015-2016)

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The frequency of *BRCA1* and *BRCA2* mutations is understudied in Arab women and there are no studies investigating *BRCA1*/*2* mutations in Qatar. In this study, we sequenced *BRCA1* and *BRCA2* genes in 183 patients suspected of hereditary breast or ovarian cancer residing in Qatar from 2015-2016 using both Next Generation Sequencing Ion torrent PGM and Sanger sequencing with genetic analyzer. Twelve *BRCA1*/*2* (8.22%) mutations were reported in 13 out of 158 patients in this study. Around 5.06% *BRCA1* and 3.16% *BRCA2* mutations were observed in this study. The mean age at diagnosis of breast cancer in *BRCA1* mutation carriers was 39 years and *BRCA2* mutation carriers was 43 years. Around 62.5% of *BRCA1* mutation carriers exhibited TNBC and no *BRCA2* mutations carriers exhibited TNBC. While Sanger sequencing is still the gold standard of sequencing *BRCA1*/*2* gene, high-throughput next-generation sequencing technologies has been an effective, cost efficient and time saving method for molecular genetic testing of *BRCA1* and *BRCA2*. Further extensive studies are required to find the frequently occurring *BRCA1/2* mutations and other hereditary breast or ovarian cancer genes that may contribute to breast cancer.

DEDICATION

I would like to dedicate this work to my kids.

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This research project was possible with the support of many people. I would like to thank my supervisor, Dr. Hatem Zayed, for the guidance support and supervision he provided during my research project. I would also like to thank Dr Fatemeh Abbaszadeh for her support throughout the project. I would also like to thank the heads of department Dr. Asma Al Thani, Dr. Nasser Rizq, Dr Luqman and Dr. Ahmed Al Malki for their assistance. Special thanks also to family and friends without whom this would not have been possible.

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ABBREVATIONS USED

ATM	Ataxia Telangiectasia mutation
BRCA1	Breast cancer gene 1
BRCA2	Breast cancer gene 2
CDH1	Cadherin 1
CHK2	Checkpoint Kinase 2
MEN	Multiple Endocrine Neoplasia
PTEN	Phosphatase and Tensin homolog
STK11	Serine-Threonine Kinase 11
TNBC	Triple negative breast cancer
SSCP	Single-stranded conformation polymorphism
HBOC	Hereditary breast and ovarian cancer
IDC	Invasive Ductal Carcinoma
DCIS	Ductal Carcinoma *In situ*

Chapter 1. Literature review

1.1. Breast Cancer

Breast cancer is the second most common cancer in the world and the most common cancer in women. There were nearly 1.67 million identified cases in 2012, which accounted for 29% of all cancers according to Global Cancer Statistics, 2012 (Torre et al., 2015). It is also the primary cause of cancer-related deaths in women with more than 458,000 estimated deaths worldwide in 2008 (Laraqui et al., 2015). Madigan et al., (1995) observed that there is a combination of risk factors for breast cancer. Lifestyle choices, hormonal aspects such as the use of oral contraceptives or postmenopausal hormone therapy, menstrual history such as early age of menarche, delayed menopause, delayed age at first birth, nulliparity, obesity, and a family history of breast cancer are a few of the risk factors of breast cancer(Dupont & Page, 1987; Madigan et al., 1995). Of all the aforementioned risk factors, a family history of cancer is one of the strongest for breast cancer (Madigan et al., 1995). In addition, the risk of breast cancer increases two-fold if there is a family history of cancer in first-degree female relatives, and the risk is even higher when more than two first-degree relatives have a history of cancer, or a relative is diagnosed at an early age (Madigan et al., 1995; Pharoah, Day, Duffy, Easton, & Ponder, 1997). In comparison to sporadic cancer, the majority of familial breast cancer cases present at an early age (Antoniou et al., 2003; Loman, Johannsson, Kristoffersson, Olsson, & Borg, 2001). The usual symptoms of breast cancer are general and include pain, changes to the skin, eczema, and discharge from the nipple. Around 70-80% breast cancer patients

discover a palpable lump on their own (Dixon & Mansel, 1994). In some cases, breast cancer can also be symptomless (Dixon & Mansel, 1994).

1.1.1. Prevalence of Breast Cancer

There are variations in breast cancer incidence between the developed and developing countries, which could be due to genetic factors as well as environmental, lifestyle, and reproductive factors (Antoniou et al., 2003; Uhrhammer et al., 2008). Migration pattern and westernization may affect the incidence of breast cancer in certain populations. For example, third-generation Asian Americans have a 50% higher risk than first-generation Asian Americans (Ziegler et al., 1993).

About 1.67 million breast cancer cases were identified in 2012 accounting for 29% of all cancers, according to Global Cancer Statistics, 2012 (Torre et al., 2015). More than 458,000 estimated deaths due to breast cancer have been reported worldwide in 2008 (Laraqui et al., 2015). Approximately 50% of the breast cancer cases and 60% of the deaths have been observed in developing countries (Torre et al., 2015). However, incidence rates are higher in North America, Western and Northern Europe, Australia/New Zealand; lower in sub-Saharan Africa and Asia; and intermediary in South America, the Caribbean, and Northern Africa (Siegel, Naishadham, & Jemal, 2013; Torre et al., 2015). Of all cancer cases, breast cancer is 19% in India, 24% in Sweden, 34% in Egypt and Gaza, and 18.8% in Saudi Arabia (El-Harith el, Abdel-Hadi, Steinmann, & Dork, 2002; Kahan et al., 1997; Loman et al., 2001; Saxena et al., 2006). Meanwhile, the Gulf Center for Cancer Registration reports that every year, for every 100,000 women there are 53·4 cases of breast cancer

in Bahrain, 22·8 cases in the United Arab Emirates, 17·5 cases in Saudi Arabia, 48·2 cases in Qatar, and 46·6 cases in Kuwait (Chouchane, Boussen, & Sastry, 2013).

It has further been noted that the age of the onset of breast cancer is earlier in Arab women than Caucasians of US and European origin by a decade, indicating hereditary predispositions (Chouchane et al., 2013).

1.1.1.1. Breast Cancer in Qatar

Age-adjusted breast cancer rate in Qatar is one of the highest in the Arab world. There was an increase in incidence rate of breast cancer in Qatar from 45 per 100,000 in 2003-2007 to 56 per 100,000 in 2008-2011 (Al-Bader, 2016). Breast cancer accounted for 39.41% of cancer cases in women in Qatar in 2015 (MOPH Cancer Registry, 2017). Around 32% of the patients diagnosed with breast cancer in Qatar were Qataris, and the rest of the 68% were non-Qataris from other Arab countries, South East Asian countries like Philippines, India, Nepal, Pakistan, Sri Lanka, and other countries. Around 36% of all affected women, both Qatari and non-Qatari, were of the age group of 40-50 years (Al-Bader, 2016).

1.1.2. Hereditary Genetic Predisposition for Breast and Ovarian Cancer

1.1.2.1. BRCA1 and BRCA2

People with hereditary mutations in *BRCA1* and *BRCA2* are predisposed to high risks of breast and ovarian cancers (Minion et al., 2015). Around 20 to 25% of inherited breast cancers, 5 to 10% of all breast cancers, and nearly 15 % of ovarian cancers can be attributed to mutations in *BRCA1* and *BRCA2* genes (Table1). These

tumor suppressor genes were reported and identified as breast cancer susceptibility genes for the first time in 1990 and 1994, and are located on chromosome 17q21 and 13q12-13, respectively (Miki et al., 1994; Wooster et al., 1995). Mutations in these genes have been found to be associated with breast cancer development (Uhrhammer et al., 2008; Wooster et al., 1995). The functions of *BRCA1* and *BRCA2* are crucial for normal cell function as they are involved in the cellular DNA repair and inhibition of uncontrolled cell growth.

Table 1

Breast cancer genes and their associated syndromes

	Gene	Hereditary Breast cancer risk (%)	Other Associated Cancer/Syndrome
High penetrance genes	*BRCA1*	50-85 (Brody & Biesecker, 1998)	Ovarian and fallopian tube cancer (Antoniou et al., 2003)
	BRCA2	50-85 (Brody & Biesecker, 1998)	Ovarian and fallopian tube cancer, Prostate cancer, Pancreatic and biliary cancer, Melanoma (Antoniou et al., 2003)
	CDH1	39 (Pharoah, Guilford, & Caldas, 2001)	Gastric and colorectal cancer (Pharoah et al., 2001)
	PTEN	85 (Tan et al., 2012)	Endometrial Cancer, Cowden Syndrome (Tan et al., 2012)
	STK11	32 (Hansford et al., 2015)	Peutz Jeghers Syndrome
	TP53	20 (Minion et al., 2015)	Li-Fraumeni Syndrome (Rapakko et al., 2001)
Moderate penetrance genes	*ATM*	2.37 (Renwick et al., 2006)	Ataxia Telangiectasia (Renwick et al., 2006)
	CHEK2	1.5-2.5 (Minion et al., 2015)	(Minion et al., 2015)
	BRIP1	2-3.5 (Seal et al., 2006)	Fanconi Anemia type1(Seal et al., 2006)
	PALB2	2.3 (Rahman et al., 2007)	Fanconi Anemia type N (Rahman et al., 2007)
Low penetrance genes	*BARD1*	(Shiovitz & Korde, 2015)	(Shiovitz & Korde, 2015)
	MRE11A	(Yuan et al., 2012)	(Yuan et al., 2012)
	NBN	(Frimer et al., 2016)	(Frimer et al., 2016)
	RAD50	(Frimer et al., 2016)	(Frimer et al., 2016)
	RAD51C	(Meindl et al., 2010)	(Meindl et al., 2010)

1.1.2. Non-*BRCA1/2* Genes

Other non-*BRCA1/2* genes include highly penetrant Cadherin 1 *(CDH1)*, Phosphatase and Tensin homolog (*PTEN*), Serine-Threonine Kinase 11 *(STK11), TP53,* moderately penetrant Checkpoint kinase 2 (*CHEK2),* Ataxia Telangiectasia mutation (*ATM), BRIP1, PALB2, BARD1, MRE11A, NBN, PALB2, RAD50, RAD51C,* Lynch syndrome genes, and DNA mismatch repair genes (*MLH1, MSH2, MSH6, PMS2, EPCAM*). *MUTYH* have been associated with hereditary breast or ovarian cancer (HBOC) (Coulet et al., 2013; Damiola et al., 2014; Dunlop et al., 1997; Kurian et al., 2014; Meindl et al., 2010; Pharoah et al., 2001; Rafnar et al., 2011; Walsh et al., 2006; Walsh et al., 2011) (Table1). If around 20 to 25% of inherited breast cancer can be attributed to mutations in *BRCA1* and *BRCA2* genes, the remaining 75-80% of inherited breast cancer could be attributed to possible mutations in the non-*BRCA1/2* genes mentioned in Table1.

1.1.3. Frequency of *BRCA1/2* Mutations Among Different Ethnic Groups

BRCA1 and *BRCA2* mutations are responsible for 5-10% of breast cancer and 15% of ovarian cancer (Laraqui et al., 2015). The frequency of *BRCA1* and *BRCA2* mutations could be higher in Arab women than in other populations (Mahfoudh et al., 2012; Tadmouri, 2004). El Harith *et al*. (2002) reported the contribution of *BRCA1* and *BRCA2* in familial breast cancer individuals in Saudi Arabia. Prevalence of *BRCA1* mutations in Tunisian women was 16% and 38% in two separate studies, and much higher rates of 9.8 % of early-onset sporadic cases (Mahfoudh et al., 2012). Around 36.4 % of familial cases have been reported in Algeria (Jalkh et al., 2012;

Mahfoudh et al., 2012). Nine of 72 (13%) unrelated Lebanese breast cancer patients were reported to have deleterious *BRCA1* and *BRCA2* germline mutations (Uhrhammer et al., 2008). It has also been reported that *BRCA1* c.66_67delAG, and c.5263insC, and *BRCA2* c.5946delT are founder mutations in 60% of ovarian cancer and 30% of early-onset breast cancer in Ashkenazi Jews with a family history (Abeliovich et al., 1997).

Many novel *BRCA1* and *BRCA2* mutations have been reported in the Arab world (Chouchane et al., 2013). A novel *BRCA1* mutation c.4236G>T (p.Glu1373X) has been reported in breast and ovarian cancers in Palestinian Arabs from East Jerusalem (Kadouri et al., 2007). A novel compound deletion c.2805delA/2924delA mutation along with five deleterious mutations in the *BRCA1* gene and three novel *BRCA2* mutations (c.3381delT/3609delT; c.7110delA/7338delA, and c.7235insG/7463insG) were encountered in families with breast/ovarian cancer in Morocco (Laraqui et al., 2015). A North African founder mutation c.798_799delTT along with a novel Tunisian-specific c.212+2insG *BRCA1* mutations and two novel *BRCA2* mutations: c.1313dupT and c.7654dupT were noted by Mahfoudh *et al.* in 2012 (Mahfoudh et al., 2012). Similarly, *BRCA1* alterations were linked to four of 11 familial cases in the neighboring Algerian women. Meanwhile c.2521C>T has been reported in Egypt and a few novel variants among 38 *BRCA1* and 40 *BRCA2* variants have been noted in high risk hereditary breast cancer in Lebanese women (Chouchane et al., 2013).

1.1.4. Non *BRCA1/2* Genes in Saudi Arabia

Not much is known about the role of non-*BRCA1/2* genes in breast cancer in Qatar. The 1000 genome project in Qatar is expected to provide insight into the genetic makeup of Qatari population and therefore more insight into the mutations of hereditary breast cancer causing genes in this population. A mutation in TP53 gene is known to cause breast cancer (Walsh et al., 2011). Approximately 1,400 TP53 mutations in breast cancer have been reported (Olivier et al., 2006). TP53 mutations are higher in advanced breast cancer patients of a younger age (Berns et al., 2000). Majority of breast cancer patients in Saudi Arabia are diagnosed at advanced stages and a younger age similar to African-American breast cancer patients (Al-Qasem et al., 2011). Around 40% of TP53 mutations in breast cancer patients in Saudi Arabia has been reported which is higher than the findings (34%) in African Americans (Al-Qasem et al., 2011).

1.2. Diagnosis

Current screening techniques are aimed at detecting breast cancer at early stages in asymptomatic women to reduce mortality and morbidity due to this disease. Mammography, first used in the 1980s, is one of the techniques used for early detection, and it has demonstrated a 25–30% decrease in breast cancer mortality (Schopper & de Wolf, 2009; Tabar et al., 1985; Youlden et al., 2012). Mammograms are scored using Breast Imaging-Reporting and Data System (BI- RADS) according to the American College of Radiology (Burnside et al., 2009). Other diagnostic techniques include ultrasound, MRI, fine needle or thick needle biopsy, and cytological studies (Dixon & Mansel, 1994). Furthermore, because families with a

high risk of cancer are usually predisposed to malignancy, molecular testing helps in diagnostic and precision medicine (Youlden et al., 2012). Histopathological staining of all receptors like estrogen, progesterone, and HER2neu and molecular testing assist in determining the management of breast cancer (Evans et al., 2011). Triple negative breast cancer (TNBC) is negative histopathological staining of all receptors like estrogen, progesterone, and HER2neu, and 51% *BRCA1* mutations present triple negative breast cancer (Evans et al., 2011).

Molecular genetic testing has evolved from linkage studies of *BRCA1/2* in 1994 to single-stranded conformation polymorphism (SSCP), restriction fragment length polymorphism analysis (RFLP), allele-specific oligonucleotide (ASO) for screening and detection of known common founder mutations in a population; and Sanger sequencing or next generation sequencing of hereditary predisposing genes, usually *BRCA1/2* for scanning for possible known or unknown mutations (Kurian et al., 2014; Miki et al., 1994). Other genes responsible for hereditary predisposition to breast cancer are under study for diagnostic purposes (Kurian et al., 2014).

Testing a single gene at a time for possible germline or somatic mutations is inefficient, expensive and time consuming (Walsh et al., 2010). In fact, several cancer susceptibility genes can be simultaneously sequenced using a multiplex panel of primers with next-generation sequencing (NGS) and the expenses are close to single gene testing (Walsh et al., 2010). Multigene panel with all the possible genes mentioned in Table 1 for HBOC can be run using NGS for a more efficient molecular diagnostic approach. However, unexpected data are generated from sequencing multiple genes simultaneously but with unclear clinical management protocol. Even though the Next generation sequencing of a panel of genes is commercially available,

until recently there have been very few studies assessing the frequency of genes other than *BRCA1* and *BRCA2* in breast cancer patients.

1.3. Breast Cancer Screening in Qatar

The screening program for breast cancer started in Qatar in April 2008 under Hamad Medical Corporation (HMC)(Al-Bader, 2016). The inclusion criteria for eligibility to the screening program include all asymptomatic female residents of Qatar and Qataris between 40- 69 years of age and some women less than 45 years if they have a family history of breast cancer (Al-Bader, 2016). The Primary Health Center (PHC) and private clinics refer women to the breast cancer screening clinic, and women can also self-register for a screening through the breast screening calling center (Al-Bader, 2016). Depending on breast densities, women below 50 years are screened annually and women above 50 years are screened biannually. The Risk Calculator Gail score (http://www.cancer.gov/bcrisktool/Default.aspx) is used to assess the breast cancer risk of each patient in the breast cancer screening clinic followed by breast clinical examination by a physician using a mammogram, MRI, or USS examination. A radiologist reviews the results for suspicious lesions and breast tissue densities. BI- RADS are used to score mammograms. Suspicious cases combined with other factors such as lower age of onset and family history are evaluated by an oncologist, and then according to the National Comprehensive Cancer Network (NCCN) guidelines these are sent for molecular testing of *BRCA1* and *BRCA2* to assist in treatment and management options (Figure 1). High risks cases of patients with HBOC syndrome are evaluated and referred to a genetic counsellor to guide them with risk reducing strategies if needed (Bujassoum et al., 2017).

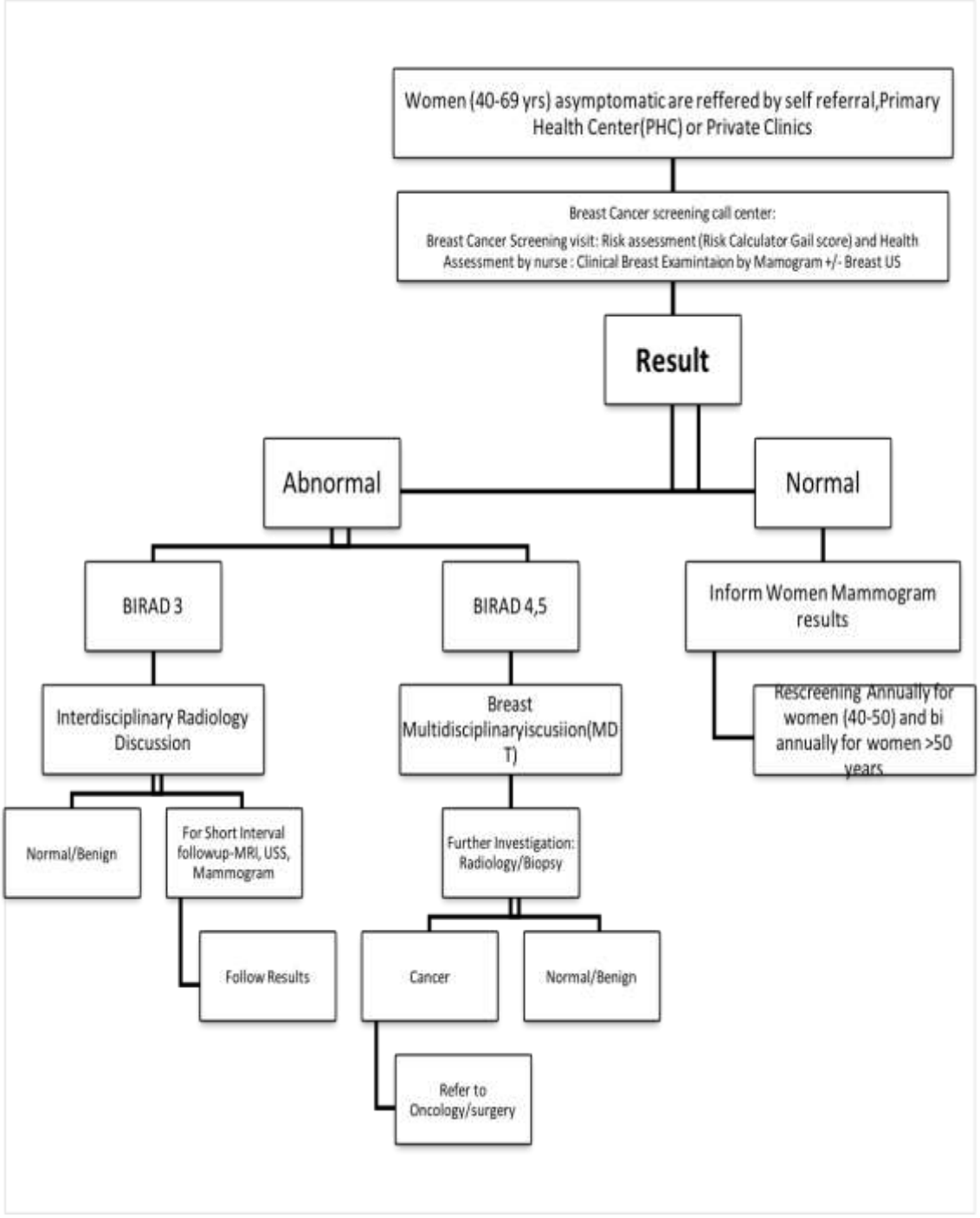

Figure 1: Pathway of Breast cancer screening in Qatar (Al-Bader, 2016).

BI-RAD: Breast Imaging-Reporting and Data System

1.4. Management and Prognosis

Treatment and management options for breast cancer depends on the stage of cancer, tissue, immunochemistry, histological reports and genetic testing of the patient (Dixon & Mansel, 1994). Currently, breast-conserving surgery as opposed to previously performed total mastectomy besides radiation, chemotherapy, or hormone treatment help in better prognosis and survival rate. Clinical trials indicate PARP (poly adenosine diphosphate [ADP] ribose) polymerase inhibitors, agents that hinder the DNA damage repair mechanism is useful *BRCA*-associated tumors (Fong et al., 2009). Moreover, Trastuzumab (Herceptin) chemotherapy has proven to be a more effective form of treatment for HER2-positive tumours than TNBC (Evans et al., 2011).

The advancement of technology in early detection and improved treatment options over the past decade have contributed to a decrease in breast cancer death rates in North America and several European countries and thereby improved prognosis for breast cancer. Five Year Survival rates are as high as 90% in developed countries such as the United States, Australia and Canada due to population screening programs using mammography and appropriate treatments but as low as 12% in parts of Africa. There is a possibility that the rates of breast cancer is higher in Africa, Asia, Arab countries and Qatar, but it may be going underdiagnosed and may be increasing due to a westernized lifestyle (Youlden et al., 2012). The role of *BRCA1* and *BRCA2* mutations are understudied in the Arab population (Kadouri et al., 2007). There is only one study reporting the contribution of *BRCA1/2* genes to familial breast cancer in Qatar (Bujassoum et al., 2017). The purpose of this thesis is to evaluate the prevalence of *BRCA1/2* mutations in suspected hereditary breast or ovarian cancer

patients in Qatar, which is expected to be lower than the prevalence in other ethnicities across the globe.

1.5. Aims and Objectives

The aim of the study is to determine the prevalence of *BRCA1* and *BRCA2* mutations in hereditary breast or ovarian cancer patients of Qatar (2015-2016).

Objectives

1. To perform molecular analysis of the identified *BRCA1* and *BRCA2* mutations in hereditary breast or ovarian cancer patients in Qatar.
2. To investigate the correlation between genotype and phenotype in *BRCA1/2*mutation carriers in Qatar.

Chapter 2. Methodology

DNA was extracted using Maxwell Promega Automated DNA extraction kit (Cat. AS1290) (Promega, Madisson, USA), from 5ml blood in EDTA tubes collected from 183 residents of Qatar between 2015 and 2016. The selection criteria for testing for *BRCA1/*2 genes was according to the NCCN guidelines which included risk assessment of factors such as family history of cancer, the age of onset of breast cancer before the age of 50, reproductive history of the early first period, late last period, late first child, breast feeding, numerous primary single or bilateral breast cancer, TNBC with breast cancer below 60, and incidence of male breast cancer in the family (Petrucelli, et al., 1993). A written consent was obtained from all patients by a certified genetic counselor at the Breast Cancer High Risk Clinic at Hamad Hospital, Doha, Qatar. The Research and Ethical Committee of Hamad Medical Corporation approved this study under protocol number #16243/16. In this study, we divided the study samples into two groups: group 1 included patients diagnosed with breast or ovarian cancer and group 2 included samples of patients under high risk surveillance.

In Group 1, 158 patients had been diagnosed with breast or ovarian cancer based on physical examination; patient complaints of symptoms; radiological studies using mammogram, ultrasound (USS) or Magnetic Resonance Imaging (MRI); fine-needle biopsy; cytological studies; and histological reports. Medical records of clinical picture, estrogen, progesterone and her2 neu receptors and a family history of cancer in first-degree or second-degree relatives were noted.

2.1. Immunohistochemistry Assay

Specimen embedded in non-decalcified paraffin and fixed in 10% neutral buffer was used for ER, PR and HER2 detection. Immunohistochemistry tests for Estrogen receptor (ER) clone: EP1, progesterone receptor (PgR) clone: 636 used Envision Flex Detection System and human epidermal growth factor receptor-2 (HER2): Hercept Test kit DAKO (Dako, Denmark).

ER or PgR was considered positive when more than $\geq$1% immunoreactive cells were present and intensity was scored 1+(weak), 2+(Intermediate) and 3+(strong) according to American Society of Clinical Oncology/College of American Pathologists Clinical guideline recommendation for IHC testing of Estrogen and Progesterone (Hammond et al., 2010). Similarly, HER2 was reported positive when complete membrane staining was observed in more than 10% of the invasive tumor according to American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline (Richards et al., 2015).

2.2. Next Generation Sequencing (Ion Torrent)

One hundred and sixty of the 183 purified DNA were sequenced for *BRCA1* and *2* using Next Generation sequencing, and positives were confirmed by Sanger sequencing using B-Pure ™ EasySeq™ PCR primers. Genomic DNA library was prepared using the Ion AmpliSeq Library kit 2.0 (Thermo Fisher Scientific). Multiplex PCR was carried out using Ion AmpliSeq™ *BRCA1* and *BRCA2* Panel which contains 167 pairs of primers: 63 *BRCA1* and 104 *BRCA2* targeting coding regions of *BRCA1* and *BRCA2* genes with additional coverage of 10–20 bases beyond the targeted exon–intron boundaries. Polymerase chain reaction (PCR) amplification

was carried out under the following conditions: DNA denaturation and the activation of enzyme at 99˚C for 2 minutes, followed by 18 cycles of denaturation at 99˚C for 15 seconds, annealing and extension at 60˚C for 4 min, and finally cooling down to 10˚C. The PCR products were partially digested, PCR primers ligated with barcode adapters and ligate adapters, and pooled. DNA libraries were clonally amplified and the templates were prepared on beads. The Ion OneTouch 2 system was used for the following PCR emulsion and the enrichment of the sequencing beads of the pooled libraries (Thermo Fisher Scientific). Sequencing of 500 flows (125 cycles) on the Ion 318 Chip Kit v2 was executed with the Ion PGM System (Thermo Fisher Scientific).

2.3. Sanger Sequencing

2.3.1. PCR Reaction protocol

Seventeen samples were sequenced using Sanger Sequencing. B-Pure ™ EasySeq™ PCR plates were used for the first PCR (Figure 2). These plates are semi-skirted PCR plates pre-spotted with dried-down primer pairs and tailed with M13 primer sequences covering both genes *BRCA1* and *BRCA2,* thereby producing 80 PCR products. DNA (25 – 50 ng/µl) was added to 9 µl of the master mix as per the following table (Table 2) and loaded with Veriti® 96-Well Thermal Cycler with the program setup in Table 3.

Table 2

Master Mix components used for First PCR

Component	Volume(µl)-Per reaction
Q-water	4
AmpliTaq Gold PCR Master Mix	5
gDNA **(**25 – 50 ng/µl**)**	1
Total Volume	10

Table 3

PCR program setup in Veriti® 96-Well Thermal Cycler

1	Initial Denaturation	95 °C	10 min
	Denaturation	95 °C	15 sec
32 cycles	Annealing	60 °C	30 sec
	Extension	72 °C	1 min
	Final Extension	72 °C	7 min
1	Final Hold	°C	∝

The PCR products obtained were visualized using the Qiaxcel with an Alignment marker (15bp -1kb) and 10ng of the 50-800bp ladder. PCR products were diluted to 1:5, usually depending on the intensity of the band. The PCR products were stored in -25^{0}C till the next step.

2.3.2. PCR Sequencing for B-Pure ™ EasySeq™ PCR Products

M13 primers and BigDye® Terminator v3.1 Cycle Sequencing Kit were used for Sanger sequencing of the PCR products, which were obtained in B-Pure ™ EasySeq™ PCR plates, with 20 µl reaction volume according to Table 4 and loaded on Veriti® 96-Well Thermal Cycler according to Table 5.

Table 4

Master Mix components used for Second PCR

Components	Forward reaction (Per reaction)	Reverse reaction (Per reaction)
Q-water	13 µl	13 µl
BigDye Terminator	1 µl	1 µl
5X Sequencing Buffer	3 µl	3 µl
M13 Primer F (3.2pmol/µl)	1 µl	-
M13 Primer R (3.2pmol/µl)	-	1 µl
PCR Product (1-100ng)	2 µl	2 µl
Total (µl)	20 µl	20 µl

Table 5

Sequencing PCR program setup in Veriti® 96-Well Thermal Cycler

Stage1	1 cycle	96^0C 1 min
Stage 2	25 cycles	96^0C 10sec
		50^0C 5sec
		60^0C 4 min
Stage 3	1 cycle	4^0C ∞

Ethanol (95%)/ EDTA precipitation was used to clean the sequencing PCR products and run on genetic analyzer**:**3130xl or 3500Dx. The raw data obtained were aligned to reference sequence from NCBI database reference (*BRCA1* Transcript NM_007294.3; *BRCA2* Transcript: NM_000059.3) using SeqScape. SeqScape and Sequencing analysis softwares were used to detect any sequence variants.

2.3.3. Bioinformatics Analysis

2.3.3.1. NGS Variant Analysis

The Ion Torrent platform-specific pipeline software Torrent Suite v4.0.2 was used to process and analyze the raw data from the PGM runs and aligned to the hg19 human reference genome to call variants. Variants with quality score (QUAL) < 30 were filtered out. Subsequently, the Ion reporter v 5.0 was used to annotate all the variants and filter poor signal reads. Pathogenic *BRCA1* and *BRCA2* mutations were located in the in the Human Gene Mutation Database (HGMD) (http://www.hgmd.cf.ac.uk/ac/index.php), Breast Cancer Information Core (BIC) (https://research.nhgri.nih.gov/bic/), the 1000 Genomes Project

(http://browser.1000genomes.org) and Database of Single Nucleotide Polymorphisms (dbSNP) https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=81002852) by the Ion Reporter.

2.3.3.2. Sanger Variant Analysis

Raw data obtained were aligned to reference sequence from NCBI database reference (*BRCA1* Transcript NM_007294.3; *BRCA2* Transcript: NM_000059.3) using SeqScape v2.7 (ApplieBiosystems). SeqScape v2.7 and Sequencing analysis v5.4 (Applied Biosystems) software were used to detect sequence variants in the samples.

2.3.3.3. Variant Interpretation

The pathogenicity of the variants, which were identified through Sanger sequencing or NGS were determined by following steps:

1. Literature search for functional studies
2. Population data; identifying the allele frequency of the variant by searching Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium
3. *In silico* analysis: The bioinformatics software that is used for assessment of pathogenicity of variants is Alamut which includes tools; AlignGVGD, SIFT, Polyphen, and splicing algorithms (SpliceSiteFinder-like, MaxEntScan, NNSplice and GeneSplicer).
4. Searching disease and locus specific databases and other databases to find out whether the variant has been seen in other affected patients with breast or

ovarian cancers. Examples of such database are Breast Cancer Information Core (BIC), ClinVar, HGMD (Human Genome Mutation Database)

2.4. Statistical Analysis

Descriptive statistics were produced to compare between different groups and evaluate their association. Using Pearson's Chi-square analysis X^2 test, Categorical variables were compared and the A P-value of, 0.05 was considered significant. The level $p < 0.05$ was considered as a cut-off value for significance. All statistical analyses were carried out in IBM SPSS Statistics 19.0.0 for Windows XP (IBM, USA).

Chapter 3. Results

3.1. Ethnicity of Patient Cohort

We investigated 183 patients that were resident of Qatar, however they include 34 different nationalities, of these patients 21 were Qataris (Figure 2), 30 were Egyptians, 26 were Filipinos, 11 were Indians, 16 were Lebanese and Jordanians, and six were Syrians. In other words, 89/183 (48.6%) patients were Arabs. Few of the patients were also from Indonesia, Pakistan, Sudan, and other countries in Asia, Europe and Africa (Figure 2).

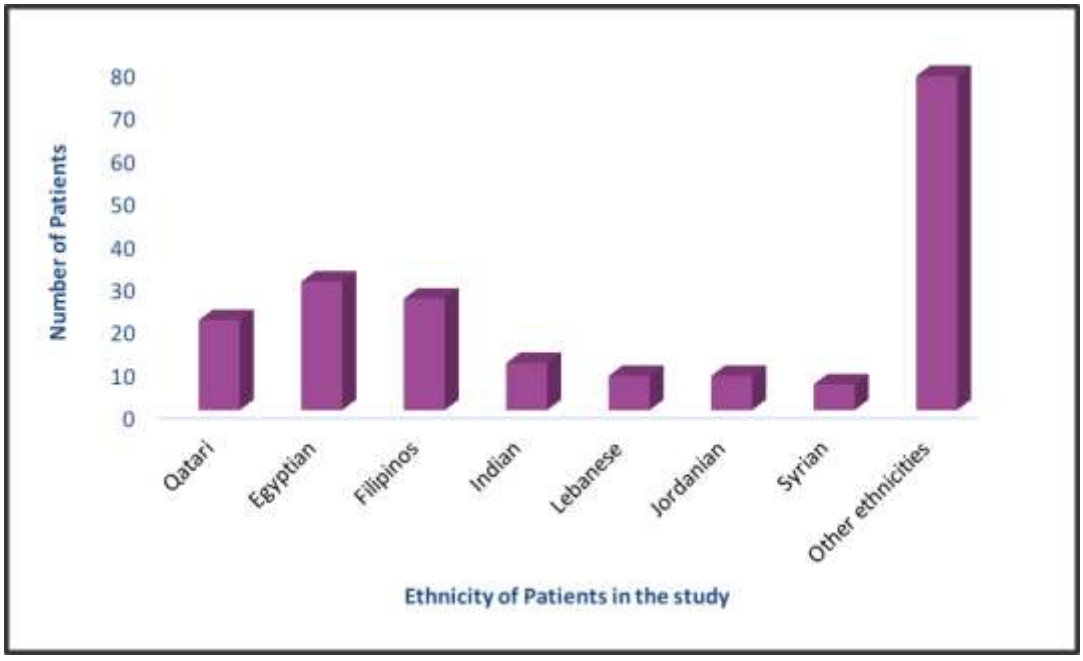

Figure 2: Ethnicities of patients diagnosed with hereditary Breast or Ovarian Cancer residing in Qatar (2015-2016). There were only 21 Qataris in this study.

3.2. Molecular Genetic Analysis for Patients with Breast and Ovarian Cancer

All patients were sequenced using Ion torrent except 17 samples that were directly sequenced by Sanger sequencing. Of the 17 samples, one was positive for *BRCA1*. All the positive mutations (12) that resulted from the Ion torrent were confirmed with Sanger sequencing.

An average output of 457,260 mapped reads were achieved, with 97.82% on target per sample. Out of 167 amplicons covered, 100% of the amplicons were covered at least once, 97.17% of the amplicons were covered at least 20 times, and 99.4% of the amplicons were covered at least 100 times. The mean uniformity of base coverage was 97.98% (Figure 3).

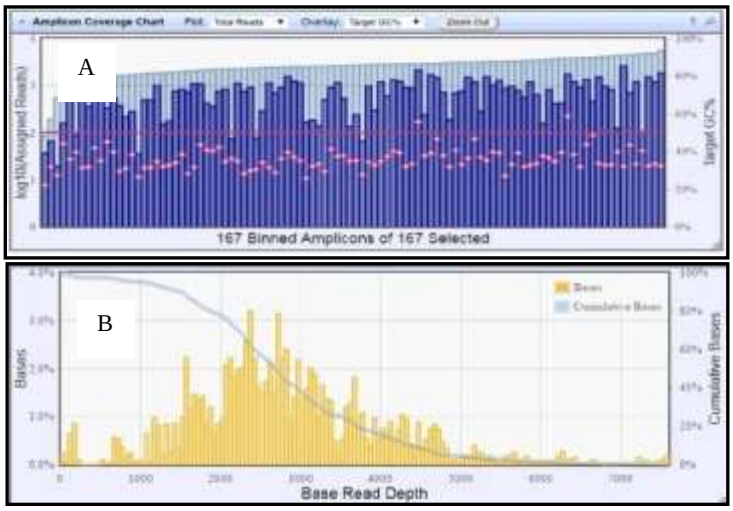

Figure 3: A. Amplicon Coverage Chart showing assigned reads in log 10 in 167 amplicons with target GC% in each 167 amplicons; B. Depth of Coverage chart showing Number of reads (%) at a particular depth with cumulative count of the number of reads at a given depth.

We divided the 183 patients into two groups, group 1 consists of 158 that were diagnosed with breast or ovarian cancer, and group 2 consist of 25 patients that were considered as under high risk surveillance as detailed in the Methods section.

3.3. Group 1

3.3.1. *BRCA1/2* Mutation Analysis in Group1

BRCA1 and *BRCA2* mutations were identified and classified from databases such as Breast Cancer Information Core (BIC), ClinVar, and Database of Single Nucleotide Polymorphisms (dbSNP) (Brookes et al., 2015) (Table 6). *In silico* analysis for splicing effects for novel mutations and for Variant of Uncertain significance (VUS) was done through software Alamut prediction analysis.

Seven deleterious mutations in *BRCA1* gene were found in eight patients (Table 7). The *BRCA1* mutations were insertion, deletion, missense or nonsense mutations that lead to premature truncation of the *BRCA1* protein. Four of the seven *BRCA1* mutations (66.7%) were located in the large exon 11, and the remaining three mutations were located in exons 5,13, and 20. Patient P1 had a nonsense *BRCA1* c.2158G>T mutation (Figure 4.A). One of the variants c.3436_3439delTGTT was found in both a mother P4 and her daughter P2 who were diagnosed with breast cancer and exhibited different hormonal receptors (Figure 4.F). P3 and P7 had two global founder mutations, c.5266dupC and c.181T>G, respectively (Figure 4.B and 4.C). Another single *BRCA1* duplication, c.3627dupA was observed in P5 (Figure 4.E). P6 was found to have a nonsense *BRCA1* c.4183C>T mutation (Figure 4.D). P8 was diagnosed with ovarian cancer and found to have a missense *BRCA1* c.4327C>T mutation (Figure 4.G).

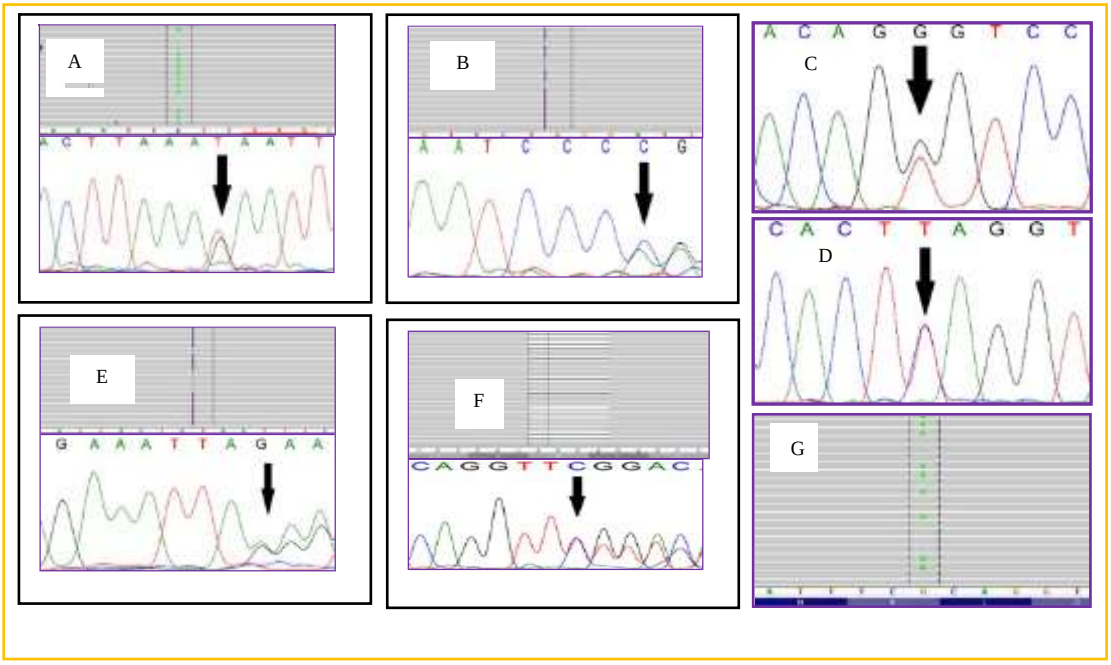

Figure 4: Electropherogram and IGV (for the ones done using NGS) showing *BRCA1* mutation A-patient P1: Heterozygous c.2158C>T; B-P-patient P3: Heterozygous insertion, c.5266dupC; C - patient P7: heterozygous mutation, c.181C>T D- patient P6: Heterozygous mutation, c.4183C>T E- patient P5: heterozygous insertion, c.3627dupA; F-patient P2 and P4: mother and daughter carrying the same heterozygous 4bp deletion c.3436_3439delTGTT; G-patient P8: Heterozygous mutation, c.4327C>T; NOTE: IGV for P1, P3 and P5 are on the reverse strand; IGV and electropherogram of P7, and P8 could not be retrieved due to technical problems.

Five *BRCA2* mutations were observed in this study (Table 6). A nonsense *BRCA2* c.4111C>T was observed in P9 (Figure 5A). P10 was found to have a single duplication c.8053dupA (Figure 5.B). We also observed a novel *BRCA2* heterozygous 13-bp insertion c.9501+7_9501+8insTAGTAAGTTAAGG (Figure 5.C) and a novel missense mutation c.353G>T (Figure 5.D). Another *BRCA2* VUS c.7435+6G>A was

observed in a Qatari patient of unknown clinical significance in various databases (Figure 5.E).

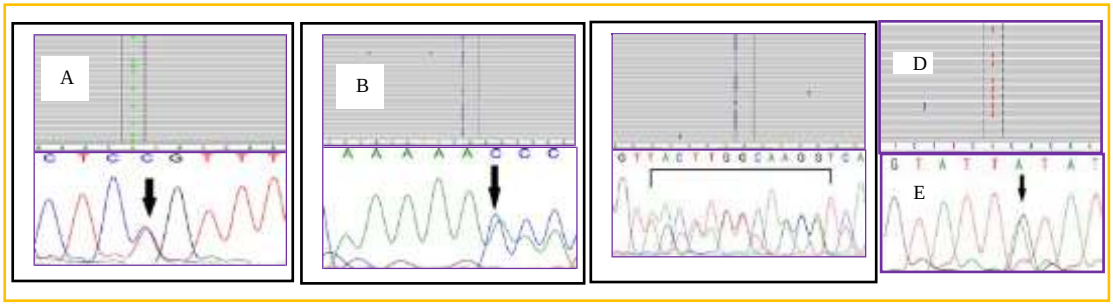

Figure 5: Electropherogram and IGV (for the ones done using NGS) showing *BRCA2* mutation A- patient 9: Heterozygous c.4111C>T; B- patient P10: heterozygous insertion, c.8053dupA; C- Patient P11: Heterozygous denevo 13bp insertion, c.9499_9500insAGGTAAGGTAGTA insertion; D- patient P12 Heterozygous c.7435+6G>A; E- patient P13: Heterozygous c.353G>T; NOTE: IGV for P8 and P9 is on the reverse strand; electropherogram of P13 couldn't be retrieved due to technical problems.

Table 6:

BRCA1/2 mutation observed in patients diagnosed with breast or ovarian cancer in Qatar.

	P_ID	dbSNP	HGVS	Exon	Mutation Type	Aminoacid change	MT SCORE	Done by Ion Torrent	Reference
BRCA1	1	rs80356875	c.2158 G>T	11	Nonsense	p.Glu720Ter	DC,Prob-0.99	Yes	(El Saghir et al., 2015; Laitman et al., 2011)
	2	rs397509067	c.3436_3439delTGTT	11	Deletion	p.Cys1146Leufs	DC,Prob-1	Yes	(Wagner et al., 1999)
	3	rs397507246	c.5265_5266insC	20	Insertion	p.Gln1756Profs	DC,Prob-1	Yes	(Hamel et al., 2011)
	4	rs397509067	c.3436_3439delTGTT	11	Deletion	p.Cys1146Leufs	DC,Prob-1	Yes	(Wagner et al., 1999)
	5	rs80357729	c.3627dupA	11	Duplication	p.Glu1210Argfs	DC,Prob-1	Yes	(Kang et al., 2015)
	6	rs80357260	c.4183C>T	11	Nonsense	p.Gln1395Ter	DC,Prob-1	No	(Langston, Malone, Thompson, Daling, & Ostrander, 1996)
	7	rs28897672	c.181T>G	5	Missense	p.Cys61Gly	DC,Prob-1	Yes	(Friedman et al., 1994)
	8	rs41293455	c.4327C>T	13	Missense	p.Arg1443Ter	DC,Prob-1	Yes	(Janavicius, 2010)
BRCA2	9	rs80358659	c.4111C>T	11	Nonsense	p.Gln1371Ter	DC,Prob-1	Yes	BIC
	10	rs397507959	c.8053dupA	18	Duplication	p.Thr2685Asnfs	DC,Prob-1	Yes	(Llort et al., 2002)
	11	rs81002852	c.7435+6G>A	Intron 14	Intronic	Intronic	Pred Plm with SS; prob-0.99	Yes	BIC
	12	N/A	c.9501+7_9501+8insTAGTAAGTTAAGG	25	Insertion	Intronic	DC,Prob-1	Yes	This study
	13	N/A	c.353G>T	4	Missense	p.Arg118Leu			This study

BIC (Breast Cancer Information Core); N/A: Not Applicable; MT: Mutation Taster score evaluation of the mutation
DC: Disease Causing; Prob: Probability; Pred: predicting; Plm: polymorphism; Mutation taste score close to 1 indicates security. *; **Reported as rs80358603 (c.353G>A) in dbSNP

3.3.2. *BRCA1/2* Mutation Frequency in Group 1

Thirteen of the 158 patients diagnosed with breast and ovarian cancer tested positive for *BRCA1/2* mutations with 8/158 (5.06%) for *BRCA1* and 5/158 (3.16) for *BRCA2* mutations (Figure 6). Around 145/158 (91.78%) tested negative for *BRCA1/2* mutations.

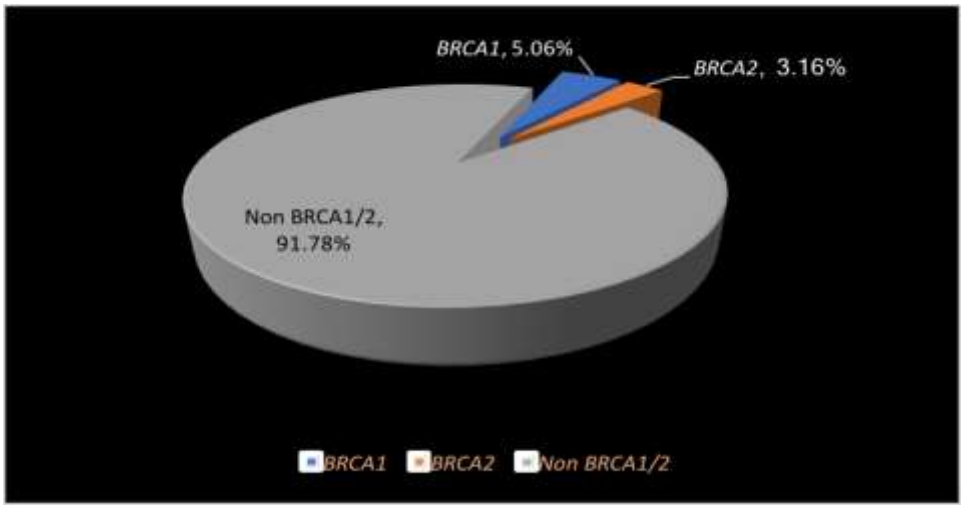

Figure 6: Frequency of *BRCA1/2* mutations in Group1 in this study. Around 91.78% of patients diagnosed with hereditary breast or ovarian cancer tested negative for *BRCA1/2* mutations.

3.3.3. Characteristics of Patient Cohort of Group1.

Samples were received from 158 patients with a mean age of 40 (Table 7). One hundred and fifty-five of them were females while three were males. One hundred and fifty-two of them were diagnosed with breast cancer, and six were diagnosed with ovarian cancer (Table 7). We observed 57/158 (36.1%) of the samples had estrogen

receptor, 56/158 (35.4%) and progesterone receptors, and 26/158 (16.4%) of the samples had Her2 receptors (Table 7).

There were no statistically significant differences in terms of the presence of mutations and age or PR, ER, HER2, TNBC status or pathological type as presented in table 7.

Table 7

Characteristics of patient cohort.

Characteristics	**n**	***BRCA1***	***BRCA2***	**Non *BRCA1/2***	**P**
		n (%)	**n (%)**	**n (%)**	**Value**
Total	**158**	**8**	**5**	**145**	
Age					
≤40	69	5 (62.5)	2 (40)	62 (42.8)	0.495
>40 years	89	3 (37.5)	3 (60)	83 (57.2)	
Female	155	8 (100)	5 (100)	142 (98)	1
Male	3	-	-	3 (2)	
Breast Cancer	152	7 (87.5)	5 (100)	139 (96.7)	
Ovarian Cancer	6	1(12.5)	-	5 (3.4)	-
Estrogen receptor					
Positive	57	2 (28.6)	3 (60)	51 (33.3)	0.38
Negative	39	4 (57.1)	-	33 (21.6)	
Unknown	62	1 (14.3)	2 (40)	69 (45.1)	
Progestogen receptor					
Positive	56	2 (28.6)	3 (60)	50 (32.7)	0.4
Negative	40	4 (57.1)	-	34 (22.2)	
Unknown	62	1 (14.3)	2 (40)	69 (45.1)	
Her2					
Positive	26		2 (33.3)	24 (15.7)	0.86
Negative	67	5 (71.4)	1 (16.7)	60 (39.2)	
Unknown	65	2 (28.6)	3 (50)	69 (45.1)	
TNBC					
Positive	31	5 (62.5)	-	26 (17)	0.47
Negative	66	3 (37.5)	2 (40)	61 (39.9)	
Unknown	70	1 (28.6)	3(60)	66 (43.1)	
Pathological Type					
IDC	60	2 (28.6)	1 (20)	57 (37.3)	
DCI	4	-	-	4 (2.6)	
Bilateral	6	1 (14.3)	-	5 (3.3)	
Unknown	72	1 (14.3)	2 (40)	69 (45.1)	

IDC: Invasive Ductal Carcinoma; DCIS: Ductal carcinoma insitu; CI

3.3.4. Age of Diagnosis of Group1

The mean age at diagnosis of the 158 cohort of patients was 41 years among the 13 patients harbored the *BRCA1/2* mutations, 39 years in *BRCA1* carriers and 43 years in *BRCA2* carriers**.** We observed 70/158 (44.3%) patients diagnosed with breast or ovarian cancer were within the age of diagnosis between 40-49 years (Figure 7). Approximately 62/158 (39.2%) of the patients were within the age of diagnosis between 30-39 years, and from this group, six patients tested positive for *BRCA1/2* mutations, which was the highest number observed in any age group. There were no *BRCA1/2* mutations for patients diagnosed with breast or ovarian cancer with age of diagnosis above 70 years.

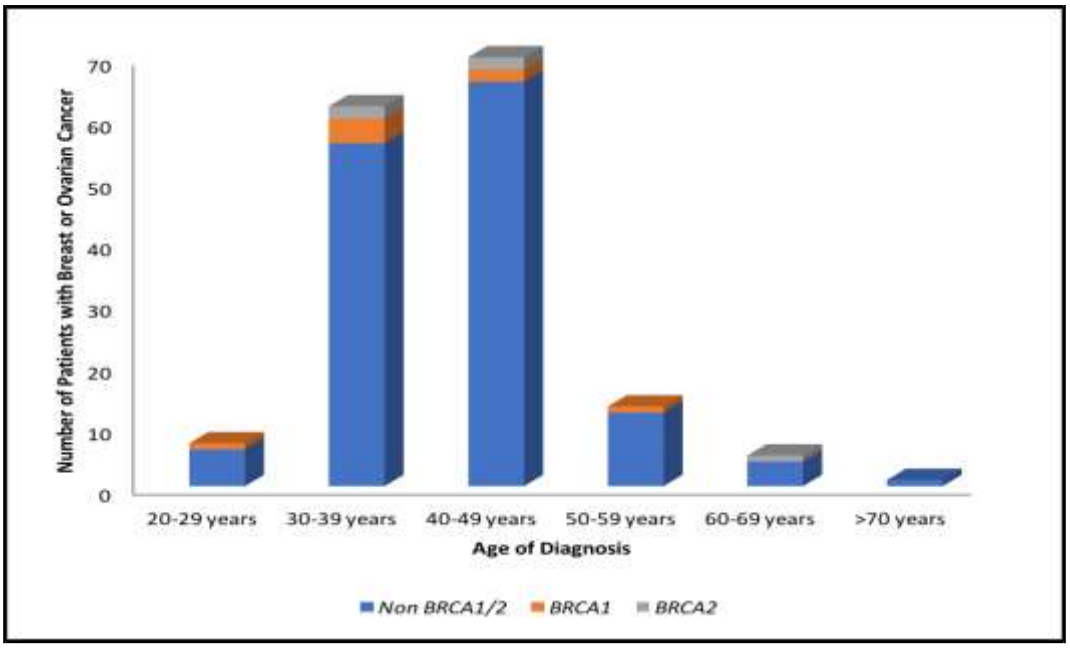

Figure 7: Number of patients diagnosed with hereditary breast or ovarian cancer in different categories of age of diagnosis with insight into number of positive *BRCA1* and *BRCA2* mutations in the respective categories. Most patients harboring *BRCA1* mutations were of 30-39 years, and those with *BRCA2* mutations were of 30-39 and 40-49 years.

3.3.5. Family History of Cancer in People with Breast Cancer with and without *BRCA1/2* Mutations

We observed that 58 of 152 patients (38.2%) with breast cancer had a family history of cancer (Figure 8). Of these, 55 had a family history of breast cancer; three had a family history of both breast and ovarian cancers, two had a family history of ovarian cancer, and two had a family history of other cancers, such as colon or pancreatic cancer (Figure 8). Of the 13 patients that were positive for *BRCA1/2*, six patients with breast cancer had a family history of breast cancer (Figure 4). Only two breast cancer patients who had no family history of cancer were tested positive for *BRCA2* (Figure 8).

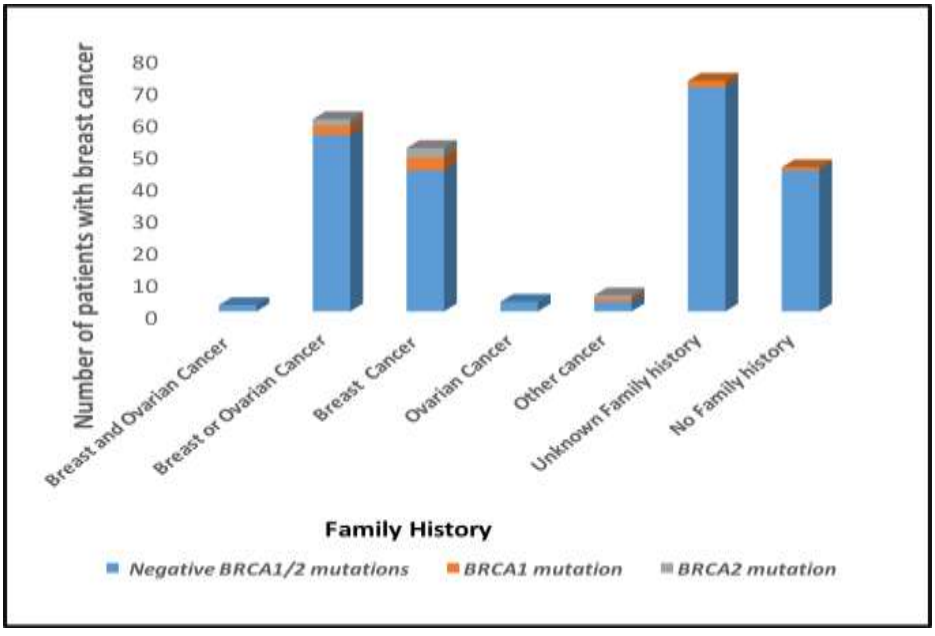

Figure 8: Family history of patients diagnosed with hereditary breast cancer with insight into number of positive *BRCA1* and *BRCA2* mutations in the respective categories. Family history was obtained from patient records provided by their physician. This summarized family history of patients does not take into account details of how many relatives were affected with cancer. Column 1 includes patients with a family history of both breast and ovarian cancers, column 2

includes patients with a family history of either breast or ovarian cancer, column 3 includes patients with a family history of breast cancer only, and column 4 includes patients with a family history of ovarian cancer only.

3.3.6. Family History of Ovarian Cancer Patients with and without *BRCA1/2* Mutations

Of the 158 patients, six patients were diagnosed with ovarian cancer, one was tested positive for *BRCA1* mutation. Three of the patients diagnosed with ovarian cancer had no family history of cancer and one had a family history of breast cancer.

3.3.7. TNBC and *BRCA1/2* Among Patients with Breast Cancer

In this study, 31/152 (20.4%) of the all breast cancer patients had TNBC while approximately 66/152 (43.4%) of these patients were non-TNBC, indicating that they were either positive for estrogen, progesterone or her2nue receptors. Seven patients with breast cancer were tested positive for the *BRCA1* mutations; of these, four were TNBC, and none of the patients who tested positive for *BRCA2* mutation had TNBC (Figure 10).

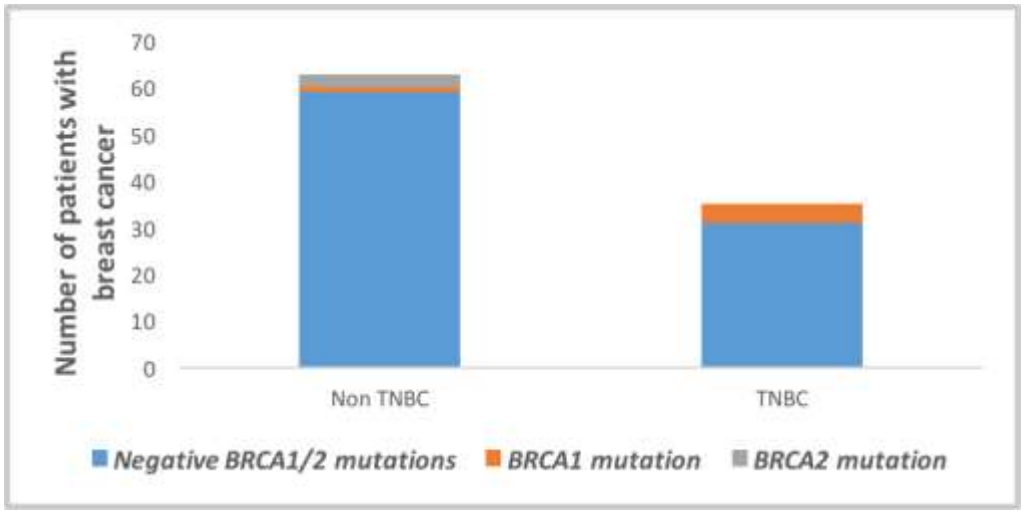

Figure 9: Number of patients with breast cancer with and without TNBC with insight into number of positive *BRCA1* and *BRCA2* mutations. No *BRCA2* mutations were found in patients with TNBC.

3.3.8. Clinical Presentation of Breast cancer in the Patients' Cohort

All the 152 patients diagnosed with breast cancer presented with different stages and types of breast cancer. 60/152 (39.5%) had Invasive ductal Carcinoma (IDC), 5/152 (3.2%) DCIS (Ductal Carcinoma *in situ*), 5/152 (3.2%) had bilateral breast cancer and pathology of 54/152 (35.5%) women diagnosed with breast cancer was unavailable. TNM classification of malignant tumor was used to describe the stages of cancer where T described the size and/or extent of the main tumor, N described the number and location of lymph nodes that contain cancer and M described metastasis. Most patients diagnosed with breast cancer and having *BRCA1/2* mutations had Invasive Ductal Carcinoma or infiltrating ductal carcinoma (IDC).

3.4. Group 2

Twenty-six patients were tested for high risk surveillance. All patients have family history of breast cancer in first degree relatives. *BRCA1/2* mutations causing breast cancer in the families of some of these patients are known.

3.4.1. *BRCA1/2* Analysis in Group2

One deleterious mutation in *BRCA1* gene and two deleterious mutations in *BRCA2* were found in three families in this study (Table 8). The *BRCA1* mutation was a single base pair duplication c.66dupA was found in family1 (Figure 10A). Two five base pair *BRCA2* deletions were found in family 2 and family 3 in this study.

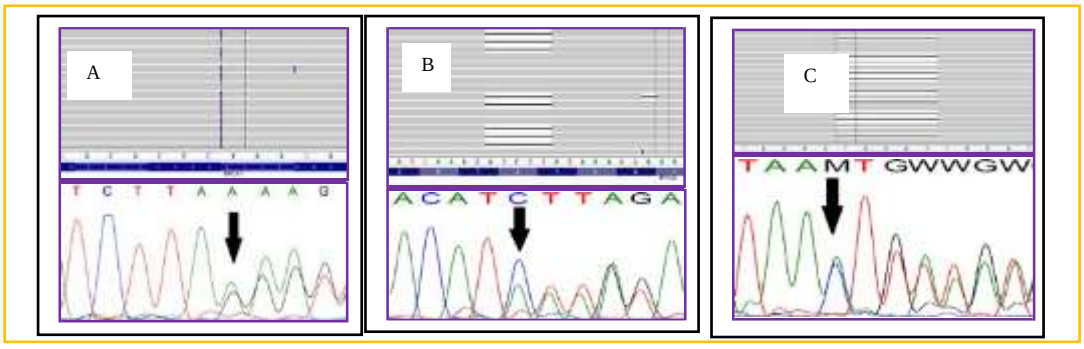

Figure 10: Electropherogram and IGV showing A-Family1: *BRCA1* Heterozygous insertion c.66dupA; B-Family2: *BRCA2* Heterozygous deletion c.1796_1800delCTTAT; C-Family3: *BRCA2* Heterozygous deletion c.6405_6409delCTTAA

Table 8

BRCA1/2 mutation observed in high risk families in Qatar.

	F_ID	dbSNP	HGVS	Exon	Mutation Type	Aminoacid change	MT SCORE	Done by Ion Torrent	Reference
BRCA1	1	rs80357783	c.66dupA	2	Duplication	p.Glu23Argfs	DC,Prob-0.99	Yes	
BRCA2	2	rs276174814	c.1796_1800delCTTAT	10		p.Ser599Terfs	DC,Prob-1	Yes	BIC
	3	rs80359585	c.6405_6409delCTTAA	11	Deletion	p.Asn2135Lysfs	DC,Prob-1	Yes	(Alexandre et al., 30 September, 2012)

Chapter 4. Discussion

We sequenced the *BRCA1* and *BRCA2* genes in the 183 patients that were suspected of hereditary breast or ovarian cancer, according to the NCCN guidelines. Some of the factors recommend by NCCN guidelines are: risk assessment of factors such as family history of cancer, age of diagnosis of breast cancer below the age of 50 years, reproductive history of early first period, late last period, late first child, breast feeding, TNBC specially with breast cancer below 60, certain histopathological reports such as estrogen, progesterone and her2 receptors, and incidence of male breast cancer in the family (Petrucelli, et al., 1993). This study was carried out in the molecular genetics laboratory at HMC. The 183 patients came from Breast Cancer High-Risk Clinic at HMC, each patient was seen by the medical oncologist, counseled by a genetic counselor and consent for *BRCA1* and *BRCA2* testing was obtained.

In this study, 89/183 (48.6%) of the patients were Arabs, with mostly 21 Qataris (11%) and 30 Egyptians (16.4%), and there were various other nationalities among the cohort of patients. All 183 patients were resident of Qatar during the period of the study. We divided these patients into two groups: group 1 consists of 158 patients that were diagnosed with breast or ovarian cancer, group 2 consists of 25 patients that were in high-risk surveillance. Of the 183 patients, 166 of them were tested for *BRCA1/2* using next generation sequencing. The remaining 17 were tested using Sanger sequencing. Both techniques targeted the entire coding region of *BRCA1/2* genes and the exon-intron junctions (20-30nt).

4.1. Group1

In this category, group1 consists of 158 patients that were diagnosed with breast or ovarian cancer, and sequenced for *BRCA1/2* gene. We found a total of 13/158 (8.22%) *BRCA1* or *BRCA2* mutations, with *BRCA1* mutation having a frequency rate of 5.06% and *BRCA2* mutation having a rate of 3.16%. The higher frequency of *BRCA1* relative to *BRCA2* was observed in most ethnic groups except among Koreans (Han et al., 2006) and Chinese (W. Chen et al., 2009; Li et al., 2008; Zhang et al., 2012). This low frequency of mutations among our patients' cohort could be due to lack of Multiplex ligation-dependent probe amplification (MLPA) studies and targeting sequencing of only the exons and exon-intron junctions, and the possibility of large genomic rearrangements could not be explored in this study. Other factors such as small sample size and a low percentage of Qatari population in this study might contribute to the difficulty of estimating the accurate prevalence rate of *BRCA1/2* mutation in this region.

Meta-analysis studies indicate that *BRCA1* mutation contributes to 57% and 40% risks of developing breast and ovarian cancer, respectively (Chen and Parmigiani 2007). Meanwhile, *BRCA2* mutation increases the risk of breast and ovarian cancer by 49% and 18%, respectively (Chen and Parmigiani 2007). Most of the studied cases of *BRCA1/2* mutations are Caucasian, cumulative data, indicating that around 2–8% or 23, 000–92, 000 of breast cancer cases worldwide is caused by *BRCA1/2* mutations (Kurian, 2010). The overall frequency of *BRCA1/2* mutations among our cohort of patients was 8.22% with 5.06% of *BRCA1* mutations which is much lower than other ethnicities. European studies estimated the prevalence of *BRCA1* mutation to be 25–40% in breast and ovarian cancer patients with a family history of cancer (Claes et al.,

1999; Meindl, 2002; Verhoog et al., 2001). A US study estimated *BRCA1* prevalence to be 2.2 – 2.9%, respectively, in Caucasian breast cancer patients. Similar prevalence rates were reported in Australia (Hopper et al. 1999, Malone et al. 2006). Prevalence rates of around 15.7 – 22.7% for *BRCA1* were reported in Hispanics with a strong family history of cancer (Vogel et al. 2007). *BRCA1/2* mutation studies in probands of Asian descent, such as Chinese, Japanese, Koreans, Filipinos, and Pakistanis, showed prevalence rates of 0.8 – 4.4% for *BRCA1* mutation (Fong et al. 2009, Sekine et al. 2001, Seo et al. 2004, Liede and Narod 2002). Proper prevalence rates of *BRCA1/2* mutations in Africa have not yet been reported. However, around 16.3% for *BRCA1* have been reported in African Americans with a strong family history of breast and ovarian cancer (Malone et al. 2006). We also found 3.16% of *BRCA2* mutations in this study which is also lower than other reported prevalence rates of *BRCA2* in different population. *BRCA2* mutation was reported to be 6–15% in European, 6 – 8% in Hispanic and 11.3– 14.4% in African American breast and ovarian cancer patients with a family history of cancer (Claes et al., 1999; Meindl, 2002; Verhoog et al., 2001).

Among our patient's cohort, the mean age of diagnosis of breast or ovarian cancer was 41 years in non *BRCA1/2* carriers, 39 years in *BRCA1* carriers and 43 years in *BRCA2* carriers (Table 7). The mean age of diagnosis of breast cancer in *BRCA1* carriers was less than that of *BRCA2* carriers, which are similar to the findings in a Chinese study in which the mean age at diagnosis of breast cancer in *BRCA1* carriers was 42.8 years and *BRCA2* carriers were 45.1 years (Zhang et al. 2012).

Triple-negative breast cancer (TNBC) is the negative expression of all three estrogen, progesterone and HER2 receptors in breast cancer. In this study, there were

31/152 (20.4%) TNBC cases, five (16.1%) of these had *BRCA1* mutation and none had *BRCA2* mutation (Figure 10) (Table 7). These findings are similar with most clinical studies that reported 20% of TNBC cases that showed positive *BRCA* mutations (Gonzalez-Angulo et al., 2011). About 9.6% of *BRCA1/2* mutations were reported in a group of 774 triple-negative Australian and Polish patients (Wong-Brown et al., 2015), in which the prevalence *of BRCA1/2* mutations were 9.3 % in Australia and were 9.9 % in Poland (Wong-Brown et al., 2015). Another Japanese study reported 12.4% of TNBC patients had *BRCA1/2* mutations with 10.3% *BRCA1* and 2.06% *BRCA2* (S higenaga et al., 2014). A study in the UK also reported similar findings of 12.7% of the TNBC cases <41years had *BRCA1* mutation and recommend testing for *BRCA1/2* for all TNBC cases less than 50 years of age regardless of family history (Evans et al., 2011).

In this cohort, 2/8 (25%) of the tumors with *BRCA1* mutation were ER positive while 50% of the tumors with *BRCA1* mutation in patients less than 40 years of age were ER negative (Table 7). This study concurs with the common histopathological characteristics of negative ER in *BRCA1* tumors (Honrado, Benitez and Palacios 2006). In contrast, 40% of *BRCA2* tumors in our study were ER positive which also concurs (Table 7) with other studies that *BRCA2* tumors are usually more ER positive than *BRCA1* tumors (Foulkes et al. 2004). However, HER2 expression was negative in 53.8% of tumors with *BRCA1* mutation (Table 7). This observation was similar to findings in another study in Manchester in which HER2 is negative in 97% of *BRCA1* in tissue specimen of breast cancer (Evans et al., 2016). Negative HER2 was also present in 97% of positive *BRCA1* mutation in samples of breast cancers obtained

from the International Breast Cancer Linkage Consortium (HER2 was positive 3% *BRCA1*) (Lakhani et al., 2002).

HER2-positive tumors have been known to respond more effectively than TNBC (triple negative breast cancer) to trastuzumab (herceptin) chemotherapy (Evans et al., 2011). As mentioned earlier, PARP inhibitors have been significant in the treatment of *BRCA* tumors (Park & Chen, 2012). Histopathological characteristics and genetic testing in breast cancer patients direct the strategies for treatment, management and prognosis of breast cancer, aiding in better survival rates (Honrado, Benitez, & Palacios, 2006; Park & Chen, 2012).

Seven deleterious mutations in the *BRCA1* gene were found in eight patients in this study (Table 6). The *BRCA1* mutations were an insertion, deletion, missense or nonsense mutations that lead to premature truncation of the *BRCA1* protein. Four of the *BRCA1* mutations (62.5%) were located in the large exon 11, and the remaining two mutations were located in exons 5, 13 and 20. One of the variants c.3436_3439delTGTT was found in both a mother, P4 and her daughter, P2, diagnosed with breast cancer but exhibited different hormonal receptors. Nonetheless, both mother and daughter had an early age of being diagnosed at 37 and 28 years respectively which may be associated with the location of this variant c.3436_3439delTGTT in exon 11 (Al-Mulla et al., 2009). *BRCA1* mutation located in exon 11, 13 and 20 are known to have significantly higher age of penetrance compared to when *BRCA1* mutation is located in exon 2 (Al-Mulla et al., 2009). Two other *BRCA1* mutations located in exon 11 have a similar association with lower age of diagnosis (Al-Mulla et al., 2009).

A nonsense *BRCA1* c.2158G>T was found in Patient P1. Her nationality was Sudanese and she had a strong family history of breast cancer (Figure 4A). She was diagnosed with breast cancer at the age of 29 years and was diagnosed again at 50 (Table 9). This mutation has been reported in Lebanese women (El Saghir et al., 2015). It has also been reported in non-Ashkenazi Jews and the Druze as 2277G>T (Laitman et al., 2011). Allele frequencies from Exome Aggregation Consortium (ExAC) database (http://exac.broadinstitute.org/) reports G as a major allele in high frequency and A as a minor allele in lower frequencies particularly in non-Finnish European population (Table 10).

Patients P2 and P4 were Egyptian daughter-mother pair diagnosed with breast cancer at the early ages of 28 and 37 years, respectively. We found both of them to have a deleterious *BRCA1* mutation c.3436_3439delTGTT which causes truncation of protein (Figure 4F) (Table 7). The c.3436_3439delTGTT has been reported in dbSNP as rs397509067. This mutation has also been reported as 3555del4 in BIC (https://research.nhgri.nih.gov/bic/) and in a study in hereditary breast only or hereditary breast or ovarian cancer families in1999 (Wagner et al., 1999). Histological studies for ER/PR and Her2 for the daughter P2 were negative, indicating she had TNBC. Pathological reports classified her cancer as T3NxMx, indicating stage 3 of the tumor, and status of metastasis and cancer in lymph nodes were unmeasurable. The mother P4 was in remission and diagnosed again with bilateral IDC cancer at the age of 61 years. This time, her tissue receptors tested positive for ER/PR. The pathological reports of her initial diagnosis at the age of 37 years are unavailable (Table 9). No allele frequency of this mutation has been reported in any of the known databases (Table 10).

P3 is a female French patient that was diagnosed at the age of 35 years, and was found to harbor the c.5266dupC mutation (Figure 4B). She presented with IDC and TNBC (Table 9). She had a strong family history of breast and other cancers. Initially identified as one of the founder *BRCA1* mutations in Ashkenazi Jews, c.5266dupC has been reported throughout Europe, Russia, Denmark, Czech/Slovak, Latvia, France, Poland and other Scandinavian countries (Hamel et al., 2011). c.5266dupC has been linked to a single founder individual in Scandinavia, more specifically modern day northern Russia through linkage haplotype and microsatellite studies (Hamel et al., 2011). This linkage haplotype and microsatellite studies also suggested that c.5266dupC entered Ashkenazi Jews in Poland around 400 to 500 years ago (Hamel et al., 2011). This insertion causes substitution of Glutamine with proline at amino acid 1756, resulting in a premature stop codon at amino acid 74, thereby leading to protein truncation (Table 7). c.5266dupC has been reported in other types of cancers like colon, stomach, prostate, lung, lymphoma and leukemia (Langston et al., 1996). Bilateral mastectomy has been proven to reduce risk of breast cancer in c.5266dupC carriers (Plakhins et al., 2011).

P5, a 41 year old Pakistani female diagnosed with IDC breast cancer due to *BRCA1* c.3627dupA mutation, was positive for ER/PR receptors and negative for her2 (Figure 4E). She had a strong family history of breast cancer and her mother was affected at the age of 50 (Table 9). c.3627dupA is a frameshift mutation leading to protein truncation (Table 7). The c.3627dupA mutation has been reported as c.3627_3628insA among Korean patients with a frequency of 12.3 % of *BRCA1* mutations in Korea, thereby suggesting this insertion could be a founder mutation in the breast cancer patients of Korea (Kang et al., 2015). There are reported cases of

this mutation in Poland as well (Koczkowska et al., 2016). ExAC database observed this mutation in South East Asians as well (Table 10).

We observed a nonsense *BRCA1* mutation c.4183C>T in patient P6. She was diagnosed with breast cancer at the age of 35 and is of Pakistani descent with an unknown family history of cancer (Figure 4D). Her histopathological studies indicated IDC and TNBC (Table 9). c.4183C>T was first reported in 1996 as c.4302C>T in young Caucasian women diagnosed with breast cancer before the age of 35 years. This mutation causes premature termination at amino acid 1395, therefore causing truncation of protein (Langston et al., 1996). There are reported cases of this mutation in India, Pakistan, Austria, France, and Germany (Hedau et al., 2004; Polsler et al., 2016; Rashid et al., 2006). No allele frequency of this mutation was found in ExAC or 1000 genome.

P7 is a Greek female that was diagnosed with breast cancer at the age of 37 years and was found to have c.181 T>G, and was characterized with IDC and TNBC (Figure 4C and Table 9). This missense mutation was first reported in 1994 as amino acid change p.Cys61Gly in two unrelated German and Polish families with an average age of onset of 39-43 years, and found to be a global founder mutation (Friedman et al., 1994). Since then it has been reported in several studies on different families from Poland, Israel, Australia, Japan, and USA (Górski et al., 2000; Johannsson et al., 1996; Sekine et al., 2001). This mutation has been reported on other types of cancer such as skin (Górski et al., 2000). This causes substitution of cysteine with glycine at amino acid 61, leading to the loss of the zinc-binding motif of the *BRCA1* protein, finally resulting in non-functional protein in homology directed recombination. Several modified homologous recombination assays have been experimented to

analyze specific *BRCA1* missense variants in the homologous recombination process and to aid in determining the predisposition of a *BRCA1* variant to breast cancer (Ransburgh, Chiba, Ishioka, Toland, & Parvin, 2010). Allele frequencies from ExAC report T as a major allele in high frequency and G as a minor allele in lower frequencies particularly in non-Finnish European population (Table 10). This mutation was predicted as pathogenic with Alamut which combines Phylop, Align GVGD and SIFT score to classify its pathogenicity.

Patient P8, a Qatari female diagnosed with epithelial ovarian cancer at the age of 46 years old was positive for *BRCA1* missense mutation c.4327C>T (Figure 4G). She had two sisters diagnosed with breast cancer at the age of 46 and 50 years, respectively (Table 9). This mutation has been reported in ExAc database in Europeans and Latino population. Allele frequencies from ExAC report C as a major allele in high frequency and T as a minor allele in lower frequencies mostly in Africa in a sample count of 121,390 (Table 10).

Patient P9 was diagnosed with breast cancer at the age of 36 years. She was tested positive for frameshift *BRCA2* mutation c.4111C>T (Figure 5A). Pathological reports classified her cancer as T3N2M0, indicating stage 3 of the tumor, and unknown status of metastasis and cancer in 2 of the lymph nodes. Tissue receptors were positive for ER/PR and negative for HER2. The patient was from the Philippines and had no family history of cancer (Table 9). This mutation was reported in dbSNP as rs80358659, and in the BIC in an Italian patient. No allele frequency of this mutation was found in ExAC or 1000 genomes.

Patient P10, an Indian patient diagnosed with breast cancer at the age of 65 years, tested positive for *BRCA2* insertion c.8053dupA (Figure 5B). Her brother was diagnosed with breast cancer as well. She was positive for all three receptors: ER/PR/Her2 (Table 9). Interestingly, this insertion has been reported in Spain as 8281insA and associated with colorectal cancer(Llort et al., 2002). No allele frequency of this mutation was found in ExAC or 1000 genomes.

A Qatari female diagnosed with breast cancer at the age of 42 was positive for a *BRCA2* variant c.7436+6G>A (Figure 5D). Pathological reports classified her cancer as T4N2M0, indicating stage 4 of the tumor, and unknown status of metastasis and cancer in 2 of the lymph nodes (Table 9), and was reported as rs81002852 in dbSNP, the pathogenicity of this mutation is controversial. It has been reported 14 times in BIC with unknown clinical significance mostly in Africa and Europe. It is six bases from the donor splice site and expected to cause splice site changes and thereby pathogenicity. However, there are no functional studies yet to confirm this. Allele frequencies from ExAC report G as a major allele in high frequency and A as a minor allele in lower frequencies mostly in Africa in a sample count of 121,390 (Table 10).

Patient P12, an Indian patient diagnosed with breast cancer at the age of 41 years, was positive for a novel *BRCA2* insertion c.9501+7_9501+8insTAGTAAGTTAAGG (Figure 5C). She was affected with IDC, and the receptors were negative for ER/PR and positive for Her2. She had a strong family history of pancreatic cancer in an uncle who was diagnosed at the age of 48 years and breast cancer in a maternal aunt who was diagnosed at 50 years (Table 9). This mutation has not been reported in any of the databases. It has not been found in normal 200 normal chromosomes sample count (100 normal individual).

The *BRCA2* mutation c.353G>T (p.Arg118Leu) is a novel missense mutation that was observed in a 31 year old Omani patient P13 diagnosed with stage 4 cancer (Figure 5E) (Table 9). This mutation has not been reported in any known mutation databases to date, and was absent in 200 normal chromosomes. At the same nucleotide position, a G to A transition (c. 353 G>A; p.Arg118His) was reported in dbSNP as rs80358603 with inconclusive clinical significance as per ClinVar. The p.Arg118Leu was predicted as tolerated by SIFT, benign by PolyPhen, and disease causing by SNPs&GO (data not shown).

Table 9

Personal and family history of cancer, pathological characteristics, immunohistochemistry for tissue staining (ER/PR, HER2 and TNBC) for all positive BRCA1/2 mutations

	P_ ID	HGVS	Country of Origin	Age at Diagnosis	Family history (Type of Cancer Breast/ Other kinds)	Histology/Grade	Tissue staining		
							HER 2-ve	ER/ PR	TN BC
BRCA1	1	c.2158 G>T	Sudan	29	B	NA	NA	NA	NA
	2	c.3436_3439delTGTT	Egypt	28	B	-/T3NxMx	-	-	+
	3	c.5265_5266insC	France	35	B+ca*	IDC/-	-	-	+
	4	c.3436_3439delTGTT	Egypt	37	B	IDC/BL	NA	+	-
	5	c.3627dupA	Pakistan	41	B	IDC/-	-	+	-
	6	c.4183C>T	Pakistan	35	NA	IDC/T3N3M1	-	-	+
	7	c.181T>G	Greece	37	NA	IDC/T2NOMO	-	-	+
	8	c.4327C>T	Qatari	46	B	Epithelial Ovarian cancer	NA	NA	NA
BRCA2	9	c.4111C>T	Philippines	36	NO	-/T3N2M0	-	+	-
	10	c.8053dupA	India	65	B	NA	+	+	-
	11	c.7435+6G>A	Qatar	42	NO	-/T4N2M0	NA	NA	NA
	12	c.9501+7_9501+8insTAGTAAGTTAAGG	India	41	B/pan**	IDC/T3N3M2	+	-	-
	13	c.353G>T	Oman	31	NA	Stage4	NA	NA	NA

B: Breast Cancer; NA: Not available; IDC: Invasive Ductal Carcinoma; TxNxMx tumor staging as described earlier; Ca:Cancer ;**Pan: Pancreatic; BL:Bilateral

Table 10

Allele Frequencies of BRCA1/2 mutation

	ID	dbSNP	c.DNA	Sample count	ExAc Aggregrated Populations	
					Wild Type/ Mutated	**Specific Ethnicity**
BRCA1	1	rs80356875	c.2158G>T	121408	0.9999G/0.00001647A	E (NF)(66700)
	2	rs397509067	c.3436_3439delTGTT	NA	NA	NA
	3	rs397507246	c.5265_5266insC	12142	0.9998-/0.00015649C	E (NF)(0.0002 in 66740)
	4	rs397509067	c.3436_3439delTGTT	NA	NA	NA
	5	rs80357729	c.3627dupA		0.9999-/0.00000824A	E-A(0.0001166 in 8576)
	6	rs80357260	c.4183C>T	NA	NA	NA
	7	rs28897672	c.181T>G	121394	0.9999T/0.0000659G	E(NF)(0.0001224in65364)
	8	rs28897672	c.4327C>T	121390	0.99998355C/0.00001647T	NA
BRCA2	9	rs80358659	c.4111C>T	NA	NA	NA
	10	rs397507959	c.8053dupA	NA	NA	NA
	11	rs81002852	c.7435+6G>A	121390	0.999G/0.00028A	AF (0.002640in9850) L (0.00026in11416) E (NF)(0.0000625in63930) S-A(0.000062in16108)
	12	NA	c.9501+7_9501+8insTAGTAAGTTAAGG	NA	NA	NA
	13	NA	c.353G>T	NA	NA	NA

Exome Aggregation Consortium (ExAC); E: European; NF: Non Finish; E-A: East Asian; S-A: South Asian; L: Latino; Af: African; NA: Not Available

4.2. Group2

One *BRCA1* duplication c.66dupA was found in family 1 (Figure 10A). This mutation was found in three brothers that were 30, 31 and 38 years old, respectively, with family history of mother diagnosed with breast and ovarian cancer that were positive for c.66dupA (Table 8; Figure 11A). This mutation has been reported in Japan and Pakistan (Rashid et al., 2006). This mutation maybe a founder mutation in Pakistan. It has been reported as 185insA in several Pakistani families and haplotype studies may otherwise indicate the same (Rashid et al., 2006). This mutation has been reported in different ethnicities in BIC database. No allele frequency of this mutation was found in ExAC or 1000 genome.

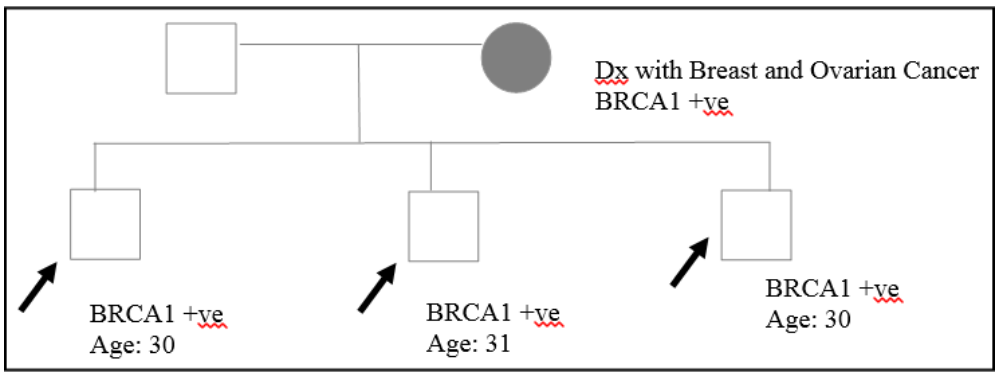

Figure 11.A. Pedigree chart depicting *BRCA1* mutation c.66dupA in Family 1. All three brothers were positive for the mutation and the mother was diagnosed with breast and ovarian cancer and positive for this mutation.

Two *BRCA2* five base pair deletion were found in two families (Table 8). The first mutations *BRCA2* c.1796_1800delCTTAT is a deleterious that was found in a Qatari Family 2 (Figure 10B; Figure 11B). This family has strong family history of breast and ovarian cancer. The patient tested was 55 years old when tested. Three of her brothers were negative for the same mutation.

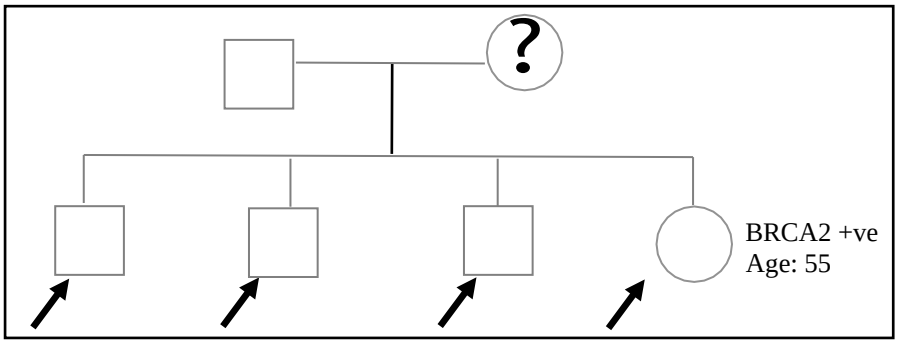

Figure 11.B. Pedigree chart depicting *BRCA2* mutation *c.1796_1800delCTTAT* in Family 2. Three of her brothers were negative for *BRCA2* mutation.

The second mutation *BRCA2* c.6405_6409delCTTAA was found to be deleterious and has been observed in a 30 year-old Sudanese female patient from family 3 (Figure 11.C). She had a strong family history of breast cancer with her mother and aunt diagnosed at 30 years and grandmother diagnosed at 50 years. This mutation, however, has been linked with cases of gastric cancer in Portugal (Alexandre et al., 30 September, 2012). ExAC database reports this deletion in Latinos.

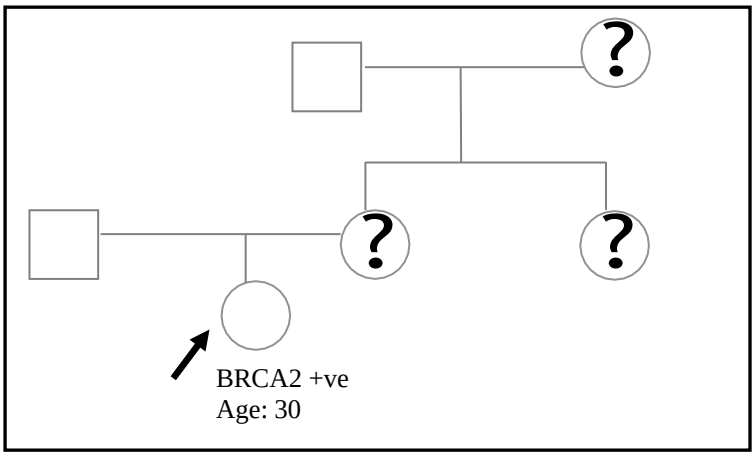

Figure 11.C. Pedigree chart depicting *BRCA2* mutation c.6405_6409delCTTAA in Family 3. She has strong family history of cancer.

Although all the three patients of group 2 does not present with breast cancer but they harbor the *BRCA1/2* mutations, they might develop it at a later time in their life. For example, in family 1 that was positive for *BRCA1:* c.66dupA, all the brothers were less than 40 years of age and the mean age of diagnosis of breast cancer in *BRCA1* carriers was found to be 42.8 years among 409 Chinese patients (Zhang et al., 2012). Furthermore, breast cancer penetrance for *BRCA1* was 57% by 70 years (S. Chen & Parmigiani, 2007). Another study estimated Breast cancer penetrance to be 48% by 80 years for *BRCA1* mutation carriers. Family 2 and family 3 are positive for *BRCA2* deletions: c.1796_1800delCTTAT and c.6405_6409delCTTAA. One of the reasons of absence of onset could be that *BRCA2* mutation increases risk of penetrance of breast and ovarian cancer by 49% and 18% respectively by age 70; and 74% and 22% by age 80, respectively (Chen and Parmigiani 2007; Anglian Breast Cancer Study Group, 2000). Other factors contributing to the absence of onset of disease could be lifestyle choices and environmental impact (Dupont & Page, 1987;

Madigan et al., 1995). All these three families identified with *BRCA1/2* mutations will be counselled by a genetic counsellor with cancer risk reducing strategies and advised for regular follow ups.

4.3. Limitations

A number of limitations in this study may impact the findings and general characterization of familial breast cancer patients in Qatar. Only 12.1% of the patients were Qatari and 3.7% were from neighboring GCC countries. Two Qatari patients were found to be *BRCA1/2* mutation positive among the 21 Qatari individuals that were screened. This leads to the conclusion that the frequency of *BRCA1/2* mutation in familial breast and ovarian cancer among Qataris is lower, and therefore other HBOC genes need to be tested. There is a need for finding a HBOC gene panel that meets the needs of this population as the genetic makeup of this population is different from the Western population (Zayed & Ouhtit, 2016). Furthermore, this study did not take into account the contribution of large genomic rearrangements of *BRCA1/2* genes. Around 8-27% of *BRCA1* mutations in different populations are due to large genomic rearrangements (Armaou et al., 2007). However, no MLPA was performed to identify any large genomic rearrangements in this study.

4.4. Future prospects

Pathogenicity of the two *BRCA2* VUS observed in this study need to be clinically studied. The role of other non *BRCA1* and *BRCA2* genes need to be tested, and their role in tumorigenesis of breast cancer in this population need to be evaluated to help with better diagnostic and management strategies for better survival rates and better prognosis.

Many *BRCA1* and *BRCA2* VUS were observed in all 183 patients in this cohort (Appendix A and B). However, further haplotype studies in familial breast cancer cases will help understand the association between *BRCA1* and *BRCA2* SNP and *BRCA1* and *BRCA2* mutations (Pilato et al., 2011).

Conclusion

In summary, our study reported 12 *BRCA1/2* (8.22%) mutations in 13 residents of Qatar who had been diagnosed with familial breast or ovarian cancer from 2015-2016. The frequency of *BRCA1/2* mutations in this study is much lower than the usual frequency of germline *BRCA1/2* mutations worldwide. Around 91.78% of the patients diagnosed with HBOC were negative for *BRCA1/2* mutations, and therefore other HBOC genes should be studied to understand the cause of familial breast or ovarian cancer in Qatar. Next generation sequencing techniques have made multigene studies efficient and cost effective, and NGS on HBOC gene panel can help design better algorithms for the HBOC diagnostic clinic in Qatar. Further studies are required to find recurrent or founder *BRCA1/2* mutation or other hereditary breast or ovarian cancer genes in this population. Identification of these recurrent or founder mutations in this population will help in genetic counselling, risk management and disease management.

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APPENDICES

1. Appendix A: Frequently Observed *BRCA1* Variants

***BRCA1*- FREQUENTLY OBSERVED VARIANTS**

No.	c. DNA	Amino acid change	dbSNP	Clinical Significance	1000 genome allele count
1	c.-19-115T>C	5'UTR	rs3765640	Benign	35%
2	c.302-41T>C	Intron6	rs8176135	NA	0.30%
3	c.441+51delT	Intron7	NA	NA	NA
4	c.441+36_441+49del	Intron7	rs373413425	NA	NA
5	c.442-34C>T	Intron7	rs799923	Untested	10%
6	c.548-17G>T	Intron8	rs8176135	Benign	<0.02%
7	c.548-58delT	Intron8	rs8176144	Benign	33%
8	c.1067A>G	p.Gln356Arg	rs1799950	Bening /conflicting interpretations of pathogenecity	2%
9	c.2077G>A	p.Asp693Asn	rs4986850	Benign	3%
10	c.2082C>T	p.Ser694=	rs1799949	Benign	34%
11	c.2311T>C	p.Leu771=	rs16940	Benign	34%
12	c.2612C>T	p.Pro871Leu	rs799917	Benign	46%
13	c.3113A>G	p.Glu1038Gly	rs16941	Benign	34%
14	c.3548A>G	p.Lys1183Arg;	rs16942	Benign	35%
15	c.3625T>G	p.Leu1209Val	rs273900711	Uncertain singnificance	NA
16	c.4185+9C>T	None	rs80358034	Likely benign/uncertain significance	< 0.01%
17	c.4308T>C	p.Ser1436=	rs1060915	Benign	34%
18	c.4357+117G>A	Intron13	rs3737559	Bengin	6%
19	c.4485-63C>G	Intron14a	rs273900734	Benign	35%
20	c.4837A>G	p.Ser1613Gly	rs1799966	Benign	36%
	c.4900A>G	p.Ser1634Gly	rs1799966	Benign	
21	c.4987-68A>G	Intron16	rs8176234	Benign	35%
22	c.4987-92A>G	Intron16	rs81762133	Benign	35%

23	c.5074+65 G>A	Intron17	rs8176235	Benign	30%
24	c.5152+66 G>A	Intron18	rs3092994	Untested	34%

NA: Not Available

2. Appendix B: Frequently Observed *BRCA2* Variants

***BRCA2*- FREQUENTLY OBSERVED VARIANTS**

No.	c. DNA	Amino acid change	dbSNP	Clinical Significance	1000 genome allele count
1	c.-52A>G	5'UTR	rs206118	Benign	14%
2		5'UTR	rs1799943	Benign	21%
3	c.425+67A>C	Intron4	rs11571610	Benign	7%
4	c.681+56C>T	Intron8	rs2126042	With benign/ Uncertain significance allele.	18%
5	c.708T>C	p.His236=	rs185506536	Likely benign/ Benign	>0.1%
6	c.793+52_795+53insT	Intron9	rs760080447	Not available	NA
7	c.865A>C	p.Asn289His	rs766173	With benign/ Uncertain significance allele.	7%
8	c.1114A>C	p.Asn372His	rs144848	Benign/ conflicting intrests of pathogenecity	25%
9	c.1365A>G	p.Ser455=	rs1801439	Benign	7%
10	c.1550A>G	p.Asn517Ser	rs80358439	Uncertain significance	NA
11	c.1909+12delT	Intron10	rs276174816	Uncertain significance	NA
12	c.1910-51G>T	Intron10	rs11571651	Benign	7%
13	c.1910-74T>C	Intron10	rs2320236	Benign	17%
14	c.2229T>C	p.His743=	rs1801499	Benign on dbSNP and ClinVar	NA
15	c.2971A>G	p.Asn991Asp	rs1799944	Benign	8%
16	c.3807T>C	p.Val1269=	rs543304	Benign	16%
17	c.4563A>G	p.Leu1521=	rs206075	Benign	3%
18	c.6513G>C	p.Val2171=	rs206076	Benign	3%
19	c.6841+78_6841+81del	Inron12	rs11571661	Uncertain significance allele	29.40%
20	c.7007+115_7007+116insTTTATAAAA	Intron13	rs151155337	Benign	7%
21	c.7435+53C>T	Intron14	rs11147489	Benign	7%
22	c.7806-14T>C	Intron16	rs9534262	Benign	46%
23	c.8755-66T>C	Intron21	rs4942486	Benign	49%

24	c.9257-16T>C	Intron24	rs11571818	Not available	0.40%
25	c.10234A>G	p.Ile3412Val	rs1801426	Benign	4%
26	c.*105 A>C	3'UTR	rs.15869	Benign	16%
27	c.3396A>G	p.lys1132=	rs1801406	Benign	26.80%
28	c.7242A>G	p.ser2414=	rs1799955	Benign	21%

3. Appendix C: Supplementary Documents

مؤسسة حمد الطبية
Hamad Medical Corporation

مركز البحوث الطبية
Medical Research Center

Ref. No: MRC/0012/2017
Date: 5th January 2017

Fatemeh Abbaszadeh
Clinical Scientist
Laboratory Molecular Genetics
NCCCR

Dear Dr. Fatemeh,

Subject: Research Proposal 16243/16 "Molecular analysis of the BRCA1 and BRCA2 genes in breast cancer patients in Qatar"

The above titled Research Proposal submitted to the Medical Research Center has been reviewed and classified as **'Exempt'** under SCH guidelines **"*Category 3:* Research involving the collection or study of existing** Data, documents, records and the information is recorded by the investigator in such a manner that subjects cannot be identified, directly or through identifiers linked to the subjects" and approval is granted from 5th January 2017.

This research study should be conducted in full accordance with all the applicable sections of the rules and regulations for research at HMC and you should notify the Medical Research Center immediately of any proposed protocol changes that may affect the 'exempt' status of your research proposal. It is the Principal Investigator's responsibility to obtain review and continued approval of the proposal if there is any modification to the approved protocol.

Documents reviewed by the Research Center:

- Initial Application form submitted on 3rd January 2017
- Research Protocol- submitted on 7th December 2016
- Data collection sheet dated 27th November 2016
- Data Collection Period : 1st January 2015 to till 26th June 2016

A study progress report should be submitted annually and a final report upon study's completion.

We wish you all success and await the results in due course.

Yours sincerely,

Prof. Ibrahim Janahi
Executive Director of Research
Medical Research Center

Cc:

1. Ms. Sumaya Rahman, Laboratory Technologist, Molecular Genetics Laboratory
2. Dr. Abdulla Al Naimi ,Chairman, HGH Research Committee

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مؤسسة حمد الطبية
Hamad Medical Corporation

مركز البحوث الطبية
Medical Research Center

Ref. No: MRC/1772/2017
Date: 23rd November 2017

Fatemeh Abbaszadeh
Clinical Scientist
Laboratory Molecular Genetics
NCCCR

Dear Dr. Fatemeh,

Subject: Research Proposal 16243/16 **"Molecular analysis of the BRCA1 and BRCA2 genes in breast cancer patients in Qatar"**

Sub: Additional Investigator

The Research Center reviewed request for the addition of Co-Investigator and hereby approves your request to include Dr. Hatem Zayed from Biomedical Sciences department, Qatar University to assist Ms.Sumaya in thesis manuscript and data analysis for the above mentioned study.

We wish you all the success in your research endeavor.

Yours sincerely,

Prof. Ibrahim Janahi
Executive Director of Research
Medical Research Center

Cc:
1. Ms. Sumaya Rahman, Laboratory Technologist, Molecular Genetics Laboratory

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نبذة مختصرة

الرحمن، سمية ماجستير العلوم: يناير: 2018، العلوم الطبية الحيوية
العنوان: التحليل الجزيئي للجينات BRCA1 و BRCA2 في مرضى وراثي الثدي أو سرطان المبيض في قطر (2015-2016)

المشرف على الرسالة: حاتم زايد، دكتوراه
إن تكرر الطفرات BRCA1 و BRCA2 غير مفهومة لدى النساء العربيات، ولا توجد دراسات تجري الطفرات BRCA1 / 2 في قطر. في هذه الدراسة، قمنا بتسلسل الجينات BRCA1 و BRCA2 في 183 مريضا يشتبه في سرطان الثدي الوراثي أو سرطان المبيض المقيمين في قطر من 2015-2016 باستخدام NGS و Sanger Sequencing في الGenetic Analyzer. تم الإبلاغ عن اثني عشر (8.22% BRCA1 / 2) الطفرات في 13 من أصل 158 مريضا في هذه الدراسة. وقد لوحظ في هذه الدراسة حوالي 5. 6% من BRCA1 و 3.16٪ من الطفرات BRCA2. كان متوسط العمر عند تشخيص سرطان الثدي في .BRCA1 39 Carrier عاما و BRCA2 Carriers 43 عاما. حوالي 62.5٪ من ناقلات طفرة BRCA1 عرض تنبك ولم تظهر ناقلات BRCA2 الطفرات تنبك. في حين Sanger Sequencingلا يزال المعيار الذهبي من التسلسل BRCA1 / 2 الجينات، وكانت الإنتاجية العالية تقنيات الجيل القادم الجيل فعالة وفعالة من حيث التكلفة وتوفير الوقت طريقة لاختبار الجيني الجزيئي BRCA1 و BRCA2. مطلوب المزيد من الدراسات المكثفة للعثور على الطفرات BRCA1 / 2 التي تحدث في كثير من الأحيان وغيرها من الجينات وراثية أو سرطان المبيض الوراثية التي قد تسهم في سرطان