Breast Cancer: Targets and Therapy

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ORIGINAL RESEARCH

Guideline-Based, Multi-Gene Panel Germline Genetic Testing for at-Risk Patients with Breast Cancer

Hikmat Abdel-Razeq[1,2], Lama Abujamous[3], Khansa Al-Azzam[1], Hala Abu-Fares[1], Hira Bani Hani[1], Mais Alkyam[1], Baha' Sharaf[1], Shatha Elemian[1], Faris Tamimi[1], Fawzi Abuhijla[4], Sarah Edaily[1], Osama Salama[1], Hazem Abdulelah[1], Rand Daoud[1], Mohammad Abubaker[1], Areej Al-Atary[5]

[1]Department of Internal Medicine, King Hussein Cancer Center, Amman, Jordan; [2]School of Medicine, The University of Jordan, Amman, Jordan; [3]Department of Cell Therapy & Applied Genomic, King Hussein Cancer Center, Amman, Jordan; [4]Department of Radiation Oncology, King Hussein Cancer Center, Amman, Jordan; [5]Department of Nursing, King Hussein Cancer Center, Amman, Jordan

Correspondence: Hikmat Abdel-Razeq, Department of Internal Medicine, King Hussein Cancer Center, School of Medicine, University of Jordan, Queen Rania Al Abdullah Street, P.O. Box 1269, Amman, 11941, Jordan, Tel +962-6 5300460, Ext 1000, Email habdelrazeq@khcc.jo

Background: Genetic testing for at-risk patients with breast cancer should be routinely offered. Knowledge generated may influence both treatment decisions and cancer prevention strategies among the patients themselves and their relatives. In this study, we report on the prevalence and patterns of germline mutations, using commercially available next-generation sequencing (NGS)-based multi-gene panels (MGP).

Patients and Methods: Consecutive at-risk breast cancer patients, as determined by international guidelines, were offered germline genetic testing using a 20-gene NGS-based panel at a reference lab. Samples of peripheral blood were obtained for DNA extraction and genetic variants were classified as benign/likely benign (negative), pathogenic/likely pathogenic (positive) or variants of uncertain significance (VUS).

Results: A total of 1310 patients, median age (range) 43 (19–82) years, were enrolled. Age ≤45 years (n = 800, 61.1%) was the most common indication for testing. Positive family history of breast, ovarian, pancreatic or prostate cancers, and triple-negative disease were among the common indications. Among the whole group, 184 (14.0%) patients had pathogenic/likely pathogenic variants; only 90 (48.9%) were in *BRCA1* or *BRCA2*, while 94 (51.9%) others had pathogenic variants in other genes; mostly in *APC, TP53, CHEK2* and *PALB2*. Mutation rates were significantly higher among patients with positive family history (p = 0.009); especially if they were 50 years or younger at the time of breast cancer diagnosis (p < 0.001). Patients with triple-negative disease had relatively higher rate (17.5%), and mostly in *BRCA1/2* genes (71.4%). Variants of uncertain significance (VUS) were reported in 559 (42.7%) patients; majority (90.7%) were in genes other than *BRCA1* or *BRCA2*.

Conclusion: Pathogenic mutations in genes other than *BRCA1/2* are relatively common and could have been missed if genetic testing was restricted to *BRCA1/2*. The significantly high rate of VUS associated with multi-gene panel testing can be disturbing.

Keywords: breast cancer, *BRCA1*, *BRCA2*, multigene panel, hereditary breast cancer, next generation sequencing

Introduction

Breast cancer continues to be the most common cancer among women worldwide.[1] Regionally, it constitutes one-fifth of all cancer cases and almost 40% of all female cancers.[2] With a median age of 51 years, breast cancer in the Arab world is diagnosed at much younger age compared to the West. Additionally, more than 30% of patients present late with locally-advanced or metastatic disease.[3,4]

Recent data had shown that 5–10% of breast cancers are related to inherited germline mutations, mostly in *BRCA1* or *BRCA2*.[5,6] Carriers of these mutations are at higher risk for both breast and ovarian cancers. Professional societies had

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published genetic testing guidelines for patients with breast cancer based on their family or personal history of cancer or tumor molecular subtypes.[7,8]

The risk of breast and ovarian cancers among individuals with pathogenic variants of *BRCA1* and *BRCA2* is well known.[9] In one prospective study that included a cohort of 978 *BRCA1* and 909 *BRCA2* carriers from the United Kingdom, the average cumulative risks by age 70 years for *BRCA1* carriers were estimated to be 60% for breast cancer and 59% for ovarian cancer. Women with *BRCA2* pathogenic variants had a corresponding risk of 55% and 16% for breast and ovarian cancers, respectively.[10] A meta-analysis of studies looked at the penetrance rates of *BRCA1* and *BRCA2* reached similar conclusions.[11] Risk-reduction interventions including bilateral mastectomies[12,13] and oophorectomies[14,15] are highly recommended in such patients.[16]

We recently reported our experience on *BRCA1* and *BRCA2* mutations among a total of 517 at-risk patients tested as per the National Comprehensive Cancer Network (NCCN) guidelines.[7] Among the whole group, 72 (13.9%) patients had pathogenic or likely pathogenic *BRCA1* or *BRCA2* mutations, while 53 (10.3%) others had VUS. Higher mutation rates were observed among patients with bilateral or second primary breast cancer, those with positive family history of breast and/or ovarian cancers, and patients with triple-negative disease (negative for estrogen receptors (ER), progesterone receptors (PR), and human epidermal growth factor (HER2) receptors).[17]

About a third of patients with strong family history of breast cancers remain negative for *BRCA1* or *BRCA2* mutation.[18] Several non-BRCA genes have been recently identified and were introduced as part of multigene breast cancer gene panels. Such genes include *ATM, CHEK2, PALB2, PTEN, TP53*, and several others.

Multi-gene panels testing, utilizing next-generation sequencing (NGS) is widely used and had become more affordable. Some of these multi-gene panels are offered directly to customers and not necessarily through a controlled health-care provider setting. As such, more genes associated with breast, ovarian and other cancers were recently identified.[19,20]

Regional data on frequency of *BRCA1* and *BRCA2* pathogenic variants are scarce and data on newly identified genes does not exist.[21–23] Knowledge about pattern and prevalence of such mutations can add to our efforts to improve preventive and treatment strategies of breast and other cancers, too.

In this study, we evaluate the contribution of germline mutations in genes other than *BRCA1* and *BRCA2* to breast cancer among our local population with selected high-risk profile as recommended by the NCCN guidelines.

Methods

Breast cancer patients with selected high-risk profile as recommended by the NCCN guidelines were invited for multi-gene panel (MGP) testing. All patients had their diagnosis, treatment and follow up at our center.

Since November 2019, we introduced and included in our clinical practice guidelines, multi-gene panel testing (20 genes) for at-risk breast cancer patients. The 20 genes are: *ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, EPCAM, MLH1, MSH2, MSH6, NBN, NF1, PALB2, PMS2, PTEN, RAD51C, RAD51D, STK11* and *TP53*. Eligible patients were identified at their first encounter by their primary medical or surgical oncologists during their routine clinic visit, or during the weekly breast multidisciplinary team (MDT) meeting. Patients who consented to MGP testing were then referred to a genetic counseling clinic where a detailed pre-testing counseling is carried out by trained counselors. Clinical and psychosocial consequences of positive test results were discussed at length with the patients, and when requested, with the spouse and/or close family members.

The study was conducted in accordance with the local and international guidelines and regulations on human research including the 1964 Helsinki declaration and its later amendments. The study was approved by Institutional Review Board (IRB) at King Hussein Cancer Center (approval number: 20-KHCC-198), and all patients signed informed consent for genetic testing. Testing was done at no-cost to all patients. Cascade family screening of positive patients was also offered, almost free of charge, to all at-risk close relatives.

Samples of peripheral blood (10 milliliters) were obtained for DNA extraction utilizing methods previously detailed,[24] and MGP testing was performed at Invitae, San Francisco, USA. Mutations in breast cancer predisposing genes were classified as pathogenic/likely pathogenic (positive), no pathogenic mutations (negative) and variant of uncertain significance (VUS). Clinical and pathological data were obtained from patients' medical records, and a detailed 3-generation family history was also obtained by a genetic counselor or one of the investigators.

Analysis was performed using an Agilent SureSelect custom design reagent to screen for germline pathogenic variants. Genomic DNA regions including coding exons and intron/exon boundaries are targeted by hybridization capture and sequenced on the Illumina platform with a sensitivity of at least 95%. The target region of selected transcripts is covered to a minimum read depth of 30x. Analysis for large deletion and duplication is performed using comparative depth of coverage of NGS data and - or MLPA analysis using P087, P045, P260.

Statistical Analysis

Both clinical and pathological characteristics were tabulated and then described by percentages (%), medians and range. Analyses included all patients tested during the study period; however, close relatives diagnosed with breast cancer and tested following the identification of the index case in the family were all excluded. Proportion of patients with pathogenic/likely pathogenic variants were calculated and compared according to age at diagnosis, family history of breast, ovarian, pancreatic and prostate cancers and triple-negative status. Analysis was performed utilizing version 9.4 of SAS software (SAS Institute Inc., Cary, NC).

Results

Patients' Characteristics

Between November 2019 and October 2021, a total of 1310 patients were enrolled, median age (range) was 43 (19–82) years and 496 (37.9%) patients were 40 years or younger. All, but 24 (1.8%) were females and majority (n = 1213, 92.6%) were Jordanians. Non-Jordanians were mostly from Iraq, Libya, Palestine and Syria. Majority of the patients had hormone receptor positive disease (ER: 74.3%, PR: 73.7%), while human epidermal growth factor receptor-2 (HER2) was positive in 285 (21.8%) by immunohistochemistry (IHC) and/or Fluorescent in Situ Hybridization (FISH), and 166 (12.7%) had triple-negative disease; 160 (96.4%) of them were 60 years or younger at time of breast cancer diagnosis. Positive family history (first-, second- or third-degree) of breast, ovarian, pancreatic or high-grade prostate cancer was found in majority of the patients (n = 972, 74.2%), Table 1.

Table 1 Patients' Characteristics (n = 1310)

Characteristics		**Number**	**(%)**
Age at diagnosis (years)	Median (Range)	43 (19–82)	
	≤ 30	79	6.0
	31–40	417	31.8
	41–50	506	38.6
	51–60	189	14.4
	> 60	119	9.1
Hormonal Status	ER-Positive	973	74.3
	PR-Positive	965	73.7
HER-2 Status	HER2-Positive	285	21.8
	HER2-Negative	920	70.2
	Unknown	105	8.0
Triple Negative		166	12.7
Positive Family History* (Breast, Ovarian, Pancreas, High-grade Prostate)		972	74.2
Nationality	Jordanians	1213	92.6
	Non-Jordanians	97	7.4

Note: *First, Second or Third-degree.
Abbreviations: ER, estrogen receptors; PR, progesterone receptors; HER2, human epidermal growth factor receptor.

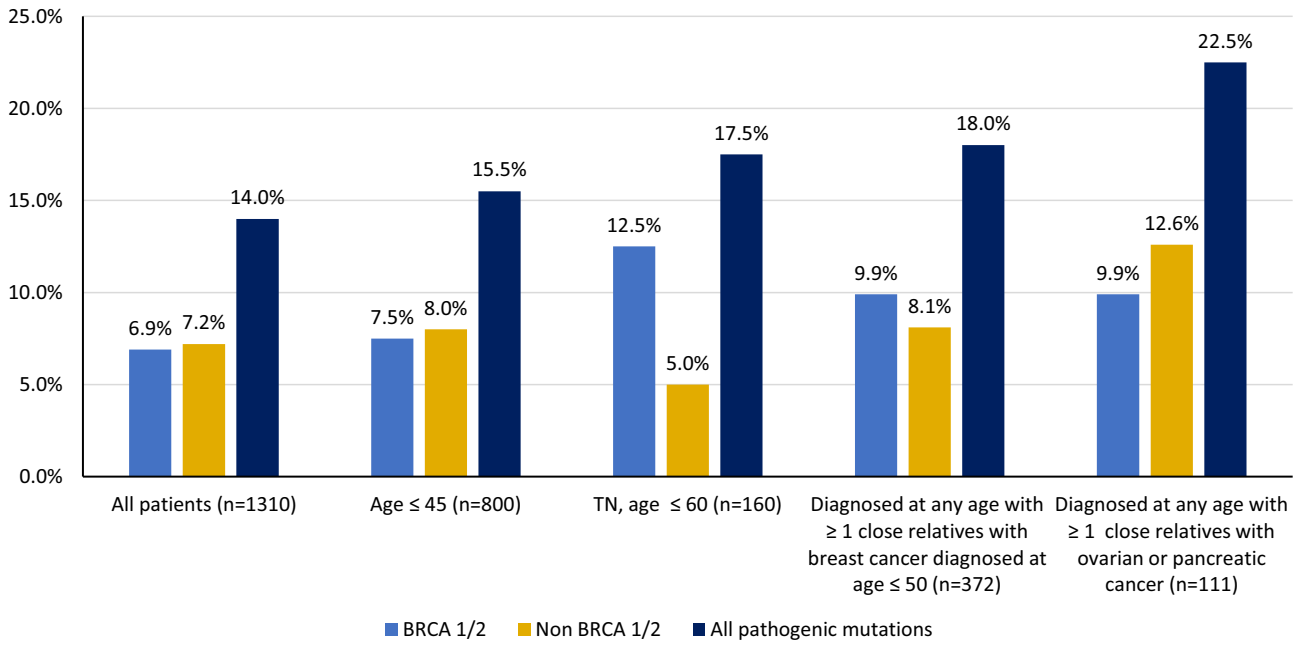

Figure 1 Rates of pathogenic/likely pathogenic variants.

Multi-Gene Panel Testing

Patients were tested according to latest NCCN guidelines. Age ≤45 years (n = 800, 61.1%) was the most common indication for genetic testing and counseling. Other common indications include family history of breast, ovarian, pancreatic or prostate cancers, and triple-negative disease.

Mutation rates were significantly higher among a group of 372 women (diagnosed at any age), with one or more close relatives with breast cancer, diagnosed at age 50 years or younger (18.0% compared to 12.5%, p = 0.009), and among 541 younger patients (diagnosed at age ≤50 years) with one or more close relatives with breast, ovarian, pancreatic, or high-grade prostate cancer (Gleason score ≥7); 18.1% vs 11.2%, p < 0.001 (Figure 1, Table 2).

Table 2 Rates of Positive Mutation Across Different Indications

Variable		Total	Pathogenic /Likely Pathogenic Mutations					
			BRCA1& BRCA2 n (%)	P-value BRCA1&2	Non-BRCA1/2 n (%)	P-value Non- BRCA1&2	All Mutations n (%)	P-value All Mutations
Age at diagnosis (years)	≤ 45	800	60 (7.5)	0.258	64 (8.0)	0.147	124 (15.5)	0.057
	> 45	510	30 (5.9)		30 (5.9)		60 (11.8)	
Age ≤ 60 with triple negative disease	Yes	160	20 (12.5)	0.003	8 (5.0)	0.254	28 (17.5)	0.180
	No	1150	70 (6.1)		86 (7.5)		156 (13.6)	
Diagnosed at any age, with ≥ close blood relative with breast cancer diagnosed at age ≤50 years	Yes	372	37 (9.9)	0.006	30 (8.1)	0.430	67 (18.0)	0.009
	No	938	53 (5.6)		64 (6.8)		117 (12.5)	
Diagnosed at any age, with ≥ 1 close blood relative with, - Epithelial ovarian cancer at any age - Exocrine pancreatic cancer at any age	Yes	111	11 (9.9)	0.187	14 (12.6)	0.020	25 (22.5)	0.007
	No	1199	79 (6.6)		80 (6.7)		159 (13.3)	

(*Continued*)

Table 2 (Continued).

Variable		Total	Pathogenic /Likely Pathogenic Mutations					
			BRCA1& BRCA2 n (%)	P-value BRCA1&2	Non-BRCA1/2 n (%)	P-value Non- BRCA1&2	All Mutations n (%)	P-value All Mutations
Diagnosed at any age with ≥2 close relatives with breast cancer diagnosed at any age	Yes	266	29 (10.9)	0.004	18 (6.8)	0.772	47 (17.7)	0.056
	No	1044	61 (5.8)		76 (7.3)		137 (13.1)	
Diagnosed at age ≤ 50 years with, - Unknown or limited family history - ≥ 1 close relatives with breast cancer at any age - ≥ 1 close relatives with pancreatic cancer - ≥ 1 close relatives with prostate cancer (Gleason score ≥7)	Yes	541	55 (10.2)	< 0.001	43 (7.9)	0.363	98 (18.1)	< 0.001
	No	769	35 (4.6)		51 (6.6)		86 (11.2)	
All patients		1310	90 (6.9)		94 (7.2)		184 (14.0)	

Triple-Negative Disease

A total of 28 (17.5%) of the 160 patients tested because of triple-negative disease had pathogenic/likely pathogenic mutations; 20 (12.5%) of them were in *BRCA1* or *BRCA2*, while only 8 (5.0%) patients had pathogenic mutations in genes other than *BRCA1* or *BRCA2* (*RAD51D, NF1 and APC* Exon 16 c.3920T>A). On the other hand, a total of 65 (40.6%) patients had VUS; 54 (33.8%) were in genes other than *BRCA1 or BRCA2*. An additional 10 (6.3%) had both VUS and pathogenic mutations.

Mutations in Genes Other Than BRCA1/2

Rate of mutations in non-BRCA1/2 genes is relatively common and represents 51.1% (n = 94) of all detected mutations. *APC* (n = 19, 10.3%), TP53 (n = 14, 7.6%), *CHEK2* (n = 12, 6.5%) and *PALB2* (n = 10, 5.4%) were the most encountered mutations (Figure 2). Mutation rate in non-*BRCA1/2* genes was lowest (5.0%) among patients with TN disease, and highest (12.6%) among patients diagnosed (at any age) with one or more close relatives with epithelial ovarian or pancreatic cancers (Figure 1). A list of all detected pathogenic/likely pathogenic variants is detailed in Supplementary Table.

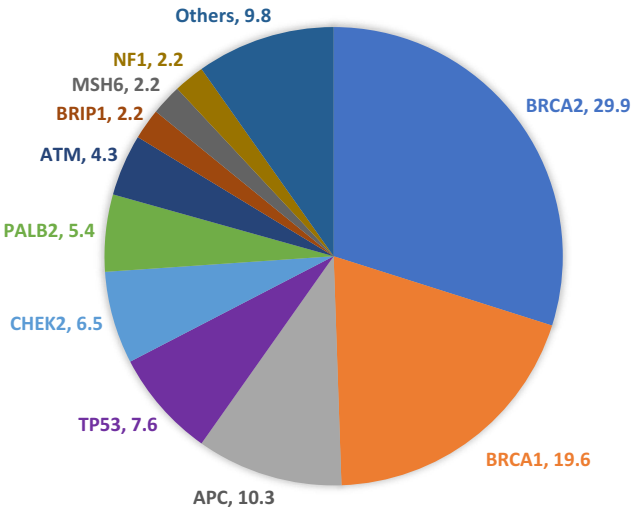

Figure 2 Positive/Likely positive variants (n=184) in percentage*. *Numbers next to gene involved represent percentage from the 184 variants detected.

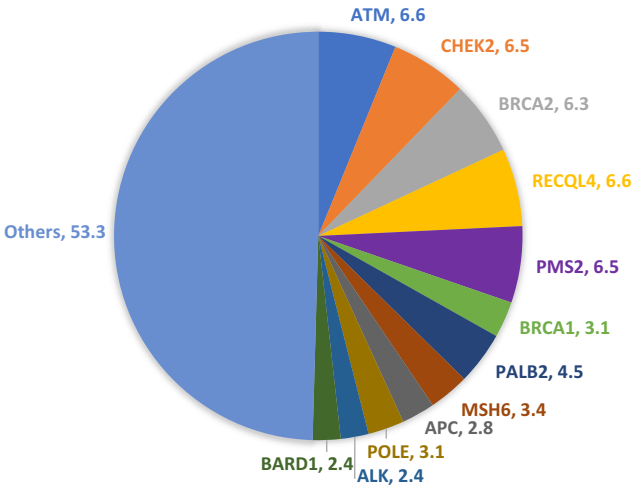

Figure 3 Rates of Variants of Uncertain Significance (VUS) in percentage*. *Numbers next to gene involved represent percentage from all VUS.

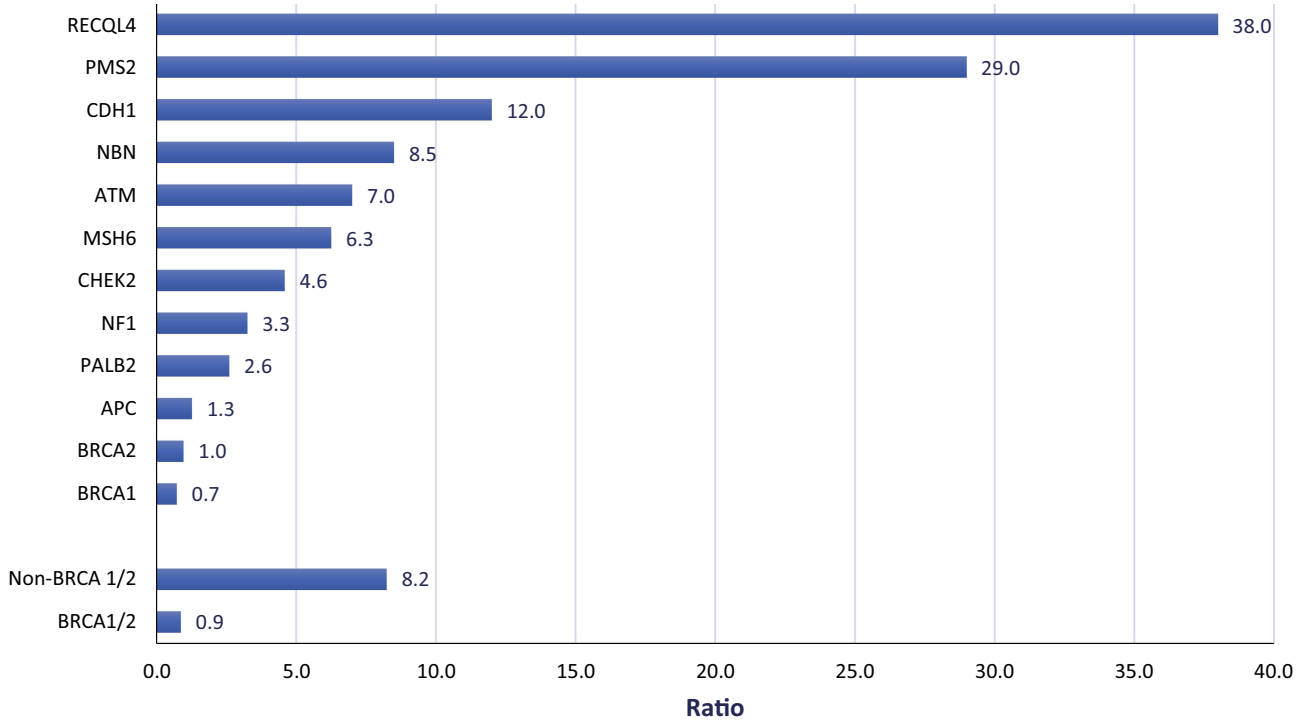

Figure 4 Ratio of variants of uncertain significance (VUS) to pathogenic/likely pathogenic variants.

VUS Rates

Variants of uncertain significance (VUS) were observed among 559 (42.7%) patients. Because many patients (n = 287, 21.9%) had more than variants, a total of 846 VUS were observed and majority (n = 767, 90.7%) were found in genes other than *BRCA1* or *BRCA2* as illustrate in Figure 3. Another 78 (6.0%) had VUS in addition to another pathogenic mutation. Ratio of VUS to positive variants was significantly higher in non-*BRCA1/2*; 8.2 versus 0.9, p =< 0 0.001. We also analyzed VUS to pathogenic/likely pathogenic ratio for the 10 most common genes as illustrated in Figure 4.

Discussion

To our knowledge, this is the first study from the region addressing the use of multi-gene panel testing in patients with breast cancer. It is clear from our study that pathogenic/likely pathogenic mutations in non-*BRCA1/2* genes are not

uncommon and represent at least 50% of all encountered positive mutations. Prior to 2020, the NCCN guidelines focused largely on testing for *BRCA1* and *BRCA2* and its associated risk management interventions. However, the latest updated version recognized several other genes and endorsed its testing.[7] Cancer risk management interventions are recommended when the absolute cancer risk for mutation carriers exceed that of average non-carrier population, which is estimated at 12–13%. The most common mutations identified in our current study are actionable and testing may be helpful. However, no clear data or recommendations exist regarding pathogenic/likely pathogenic mutations in many of such genes.[25] To complicate the issue further, variants in the same gene may be associated with different risk levels, like what we see with the *ATM* different mutation variants; some are associated with early onset and even bilateral disease while other variants are not.[26,27]

It is also clear that with the expansion of gene tested, more VUS will be encountered. Rates of VUS among non-*BRCA1/2* genes are relatively high. In our current study, at least one in three tested patients had a VUS; 90% of them were in genes other than *BRCA1* or *BRCA2*. Additionally, many of the selected genes in the panel have a high VUS to pathogenic/likely pathogenic ratio (Figure 4). Furthermore, many of the gene tested in our cohort, like *PMS2, RAD51C, MSH2, STK11* and *MLH1*, had only VUS and never pathogenic mutations. The generated anxiety for patients, families and even the treating physicians might outweigh the anticipated benefit.

The added value of extended testing might be limited to special group of at-risk breast cancer patients. Our data clearly illustrated that testing patients with TN disease beyond the usual *BRCA1/2* is associated with little added value; only 8 (5.0%) cases of non-*BRCA1/2* mutations were identified in 160 patients with TN-disease.

Family involvement in preventive decisions is inheritably limited.[28,29] Most breast cancer predisposing genes are inherited in an autosomal dominant fashion, thus the risk of carrying the same mutation is 50% among first-degree relatives. But such numbers might not necessarily be taken seriously by family members. Add to this, the fact that patients themselves are occasionally not willing to share such information with their relatives. We are in the process of collecting data on these issues which is somewhat more important in smaller communities and cultures, like ours. Our findings might add to the national efforts exercised to prevent cancer in general, and breast in particular. Identifying inherited cancer predisposing genes in a patient should reflect positively in preventing the occurrence of cancer in close relatives.

In addition to breast and ovarian cancer prevention, the utilization of our knowledge about mutations in *BRCA1* and *BRCA2*, and even in non-*BRCA* mutations, in treatment decisions is increasing.[30] Patients with TN-breast cancer and deleterious *BRCA1/2* mutations have better response rate and progression-free survival (PFS) when treated with carboplatin compared to docetaxel.[31] PARP (poly (ADP)-ribose polymerase) inhibitors were also tried in patients with pathogenic *BRCA1/2* advanced-stage breast cancer. The randomized phase-3 trial (OlympiAD) showed that olaparib, when compared to palliative chemotherapy, in a cohort of 302 HER2-negative metastatic breast cancer patients with pathogenic germline *BRCA1/2* mutation, was associated with better PFS.[32] Talazoparib, another PARP inhibitor, had shown similar results in another phase-3 randomized trial (EMBRACA).[33] More recently, PARP inhibitors were also tried in the setting of high-risk early-stage breast cancer with germline pathogenic *BRCA1/2* mutations. Following the completion neoadjuvant or adjuvant therapy and standard local treatment, adjuvant olaparib for one year was associated with significant improvement in invasive (iDFS), distant (dDFS) disease-free survivals and possibly overall survival (OS), when compared to placebo, in a randomized phase-3 trial (OlympiA).[34,35] In addition to *BRCA1* and *BRCA2*, meaningful responses were seen in patients with germline *PALB2*, but not those with *ATM* or *CHEK2* mutations alone.[36]

Given the increasing percentage of women with VUS and pathogenic/likely pathogenic variants in genes with no much data on its associated risk, pre- and post-test genetic counseling are highly needed. Studies had shown that satisfaction is significantly higher among women who had undergone genetic counseling.[37] It is also important that preventive and therapeutic decisions in relation to genetic testing made in a multidisciplinary setting with active participation of oncologists, surgeons and geneticists with high level of psychosocial support.

Conclusions

Pathogenic mutations in genes other than *BRCA1* or *BRCA2* are relatively common and could have been missed if genetic testing was restricted to *BRCA1* and *BRCA2*. MGP testing results in a significantly higher rate of VUS, a finding

that may increase the anxiety of patients and physicians, alike. Germline genetic testing had gone beyond cancer prevention and currently is incorporated in treatment decisions of both early and advanced stage breast cancer.

Abbreviations

CI, Confidence Intervals; ER, Estrogen Receptors; FISH, Fluorescent in Situ Hybridization; HER2, Human Epidermal Growth Factor Receptor; IHC, Immunohistochemistry; IRB, Institutional Review Board; MDT, Multidisciplinary Team; MGP, Multi-gene Panel; NCCN, National Comprehensive Cancer Network; NGS, Next-Generation Sequencing; PFS, Progression-Free Survival; PR, Progesterone Receptors; VUS, Variant of Uncertain Significance.

Data Sharing Statement

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

Ethics Approval and Consent to Participate

The study was approved by Institutional Review Board (IRB) at King Hussein Cancer Center (approval number, 20-KHCC-198), and all patients signed informed consent for genetic testing.

Consent for Publication

Data submitted are entirely unidentifiable, and there are no details on individuals reported within the manuscript.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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