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Impact of Early Testing and Analysis of Germline Genetic Mutation in Patients with Breast Cancer: A Single Institution Experience

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Breast cancer is the most common cancer among women worldwide, with germline mutations in high-penetrance genes like BRCA1 and BRCA2, and moderate-penetrance genes such as CHEK2 and ATM contributing majorly to the onset of the same. Universal germline genetic testing offers an avenue to improve early identification and develop appropriate management guidelines. Our retrospective cohort study analyzed data from 525 newly diagnosed breast cancer patients at Mercy Hospital Fort Smith from January 2020 to December 2023. Patients underwent germline genetic testing using next-generation sequencing panels irrespective of family history of cancer. Details on patient demographics, clinical characteristics, and genetic test results were collected and analyzed. The median age at diagnosis of patients was 66, with invasive ductal carcinoma (IDC) being the major subtype (66%). CHEK2 mutations were the most common pathogenic mutations (9 patients), followed by BRCA1 and MUTYH (6 each). Pathogenic mutations were more prevalent in patients over 60 years (63%). Germline mutations were identified more frequently in IDC than in ductal carcinoma in situ. Among patients with germline mutations, there was a significant drift toward mastectomy over breast-conserving surgery. Universal germline genetic testing identified pathogenic mutations in a significant proportion of breast cancer patients, especially among the older patient population. The findings further emphasize the importance of integrating universal genetic testing into routine care to guide surgical and risk-reduction management protocols effectively. Further research is needed to regularize genetic testing in similar patients.

Key Words Germline mutations, Breast neoplasms, Breast conserving surgery, Estrogen receptor, Progesterone receptor

INTRODUCTION

Breast cancer is the most common cancer in women and one of the primary causes of cancer-related deaths in women globally. According to estimates, by 2022, the annual incidence of breast cancer was 11.6% globally, with an annual mortality rate of 6.9% [1]. The 5-year survival rate of patients with breast cancer varies depending on the stage of the disease, ranging from 22% to 100%. However, this varies depending on the patient's place of origin, genetic predisposition, and other factors that play a significant role in the onset and progression of the disease [2].

Germline mutations in specific genes are known to cause familial breast cancer, accounting for approximately 5% to 10% of all breast cancers [3]. Overall, 20% to 40% of familial breast cancers are caused by BRCA1/BRCA2 mutations [4,5]. According to a recent meta-analysis, carriers of BRCA1 mutations have a 57% to 65% lifetime risk of breast cancer, and carriers of BRCA2 mutations have a 45% to 49% lifetime risk [6,7]. Although breast cancer is more prevalent in Asian populations, the majority of research to date has focused on the prevalence and range of BRCA1 and BRCA2 mutations in white populations from Europe, North America, Africa, and African American populations. Age-specific incidence rates of breast cancer vary significantly between nations and ethnic groups worldwide; therefore, to establish suitable genetic testing and management, a deeper understanding of the mutation spectrum of these high-penetrance genes and cancer risk prediction is required [8]. Germline genetic mutations are known to contribute to a proportion of breast cancer cases,

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particularly through high-risk genes such as BRCA1, BRCA2, and PALB2, which increase the risk of breast cancer [9]. Mutations in these genes are associated with hereditary breast and ovarian cancer syndromes, often leading to an earlier onset of the disease and significantly influencing treatment decisions.

In addition, moderate-penetrance genes, such as ATM and CHEK2, are increasingly recognized for their role in breast cancer susceptibility. These mutations increase breast cancer risk by approximately two to three-fold, stressing the need for broader genetic screening strategies to identify patients who might benefit from personalized treatment approaches [10]. Traditionally, genetic testing has been offered selectively to patients based on their family history, age at diagnosis, or specific clinical features. However, this approach often misses patients without a significant family history of cancer, highlighting the importance of universal genetic testing strategies. Universal germline genetic testing at diagnosis, irrespective of family history, has emerged as a promising method for the early identification of mutations impacting medical decisions. In 2020, our institution implemented universal germline genetic testing for all newly diagnosed breast cancer patients, regardless of their family history. This initiative aimed to achieve several objectives: first, to determine the prevalence of pathogenic and likely pathogenic mutations in breast cancer patients treated at our center; second, to explore the presence of germline mutations in patients diagnosed with ductal carcinoma in situ (DCIS), a non-invasive precursor to invasive breast cancer; and third, to evaluate whether the early implementation of universal germline testing influenced surgical decisions, such as the choice between bilateral mastectomy and breast-conserving surgery (BCS).

MATERIALS AND METHODS

Study design and setting

This small, single-centered, retrospective cohort study was conducted at Mercy Hospital Fort Smith, a tertiary care cancer center. The study period spanned from January 2020 to December 2023. This study was approved by the Institutional Review Board of the Mercy Hospital Fort Smith ([1968367-2] 23-019). Informed consent was not necessary because the study included retrospective data collection. We included all newly diagnosed breast cancer patients who presented to our institution from January 2020 onwards. Patients were identified using our institutional cancer registry. Inclusion criteria were a new diagnosis of breast cancer confirmed by histopathology, age > 18 years, and stage I through III, irrespective of family history of breast cancer. Patients with recurrent or metastatic breast cancer at initial presentation and those with insufficient data were excluded from the final analysis. Germline genetic testing was initiated as a pilot project to test all diagnosed breast cancer patients, starting in January 2020 at our institution. At the time of diagnosis, patients were offered germline genetic testing irrespective of their family history of breast cancer. Genetic testing was conducted using next-generation sequencing panels that included common breast cancer susceptibility genes, such as BRCA1, BRCA2, PALB2, CHEK2, ATM, and others. Blood samples were collected from the patients, and DNA was extracted for analysis. Testing was performed by accredited laboratories following standard protocols.

Data collection

Data on patient demographics, clinical characteristics, and treatment modalities were collected. Information on age, sex, ethnicity, tumor stage, and histopathological subtype (invasive ductal carcinoma [IDC], invasive lobular carcinoma, and DCIS) was recorded. Details of germline genetic test results, including the presence of pathogenic or likely pathogenic variants and variants of unknown significance (VUS), have been documented.

Objectives

The primary objectives of this study were as follows: 1) To determine the prevalence of germline mutations in patients with newly diagnosed breast cancer treated at our institution; 2) To evaluate the prevalence of germline mutations in patients diagnosed with DCIS; 3) To assess whether early universal germline testing influenced the choice of surgical treatment, particularly the decision between BCS and mastectomy.

Statistical analysis

Descriptive statistics were used to summarize the patient demographics, clinical characteristics, and genetic test results. The prevalence of germline mutations was calculated as the proportion of patients with pathogenic or likely pathogenic variants among all tested individuals. Subgroup analyses were performed to compare the mutation prevalence between patients with invasive breast cancer and DCIS. To evaluate the impact of germline testing on surgical decision-making, we compared the rates of BCS and mastectomy between patients who tested positive for germline mutations and those who did not.

Follow-up and data management

Patients were followed up through routine clinical visits, and data on surgical outcomes and subsequent treatments were recorded. Data were managed using a secure Health Insurance Portability and Accountability Act-compliant electronic database. Patient confidentiality was maintained throughout the study. Genetic counseling was provided to all patients undergoing germline testing to discuss the implications of the test results for themselves and their families. Patients with positive test results were offered additional support and management options, including enhanced surveillance and risk-reducing strategies.

RESULTS

From January 2020 to December 2023, 525 patients diagnosed with breast cancer were observed in the oncology clinic. The median age at diagnosis was 66 years, with ages ranging from 32 to 91 years old. IDC has emerged as the most common subtype, accounting for 66% of cases. This was followed by DCIS, which represented 18% of the patient population (Table 1). The majority of the patients (82%) had estrogen receptor-positive (ER+) cancer, while the remaining 18% had estrogen receptor-negative (ER-) cancer. Additionally, approximately 13% of the patients were diagnosed with HER2-positive breast cancer, of which 61% were classified as HER2 low (Table 2).

Prevalence of germline mutations

In terms of genetic mutations, the most common pathogenic mutation identified was CHEK2, which was found in 9 patients. This was followed by mutations in BRCA1 and MUYTH, each found in 6 patients. Predominant germline mutations were identified in patients with IDC, followed by those with DCIS. Notably, 2 patients with a pure mucinous subtype had a germline pathogenic mutation in RECQL4 (Table 3). Analysis of age-related mutation prevalence revealed a higher percentage of pathogenic breast cancer-related mutations in patients over 60 years of age (63% vs. 37%). All CHEK2 mutations were identified in patients aged > 60 years old.

Surgical outcomes

Of the 41 patients with pathogenic mutations, 17 underwent partial mastectomy, 12 underwent bilateral mastectomy, and two underwent unilateral mastectomy as evident in Table 4. All patients with BRCA1/2 mutations opted for bilateral mastectomy, and all patients with CHEK2 mutations underwent partial mastectomy. Additionally, several VUS were identified, which followed a similar pattern and were more common in patients with IDC. The most frequently reported VUS mutation involves the ATM gene (Fig. 1).

In conclusion, this comprehensive analysis of breast cancer patients over three years highlights the predominance of germline mutations in ER+ cancers, as well as the significant presence of HER2-positive cases, as explained in Table 2 and 3. This study underscores the importance of genetic testing for identifying pathogenic mutations and guiding surgical decisions, particularly in older patients.

DISCUSSION

Breast cancer is the most common malignancy worldwide and the second leading cause of cancer-related deaths in females [11]. Multiple factors influence the development of breast cancer, including age, sex, race, history of breast cancer, reproductive and hormonal factors, smoking, obesity, exposure to ionizing radiation, and genetic factors. However, BRCA1 and BRCA2 have been noted to have high penetrance susceptibility and have been the main genetic factors contributing to hereditary breast cancer in the past. Multiple genetic mutations with low and moderate penetrance, including those in PALB2, P53, PTEN, ATM, BRIP1, PALB2, and CHEK2, have been studied with advances in genomic technology and have been implicated in hereditary breast cancer [11,12]. Our understanding of the etiology of breast cancer revolves around how DNA damage responds to and

Table 1. Breast cancer subtypes among patients

Subtype	Patient
Invasive ductal carcinoma	66
Ductal carcinoma in situ	18
Other subtypes	16

Values are presented as percentage.

Table 2. Hormone receptor status and HER2 positivity

Variable	Patient
Hormone receptor status	
ER positive	82
ER negative	18
HER2 status	
HER2 positive	13
HER2 low	61

Values are presented as percentage. ER, estrogen receptor.

Table 3. Pathogenic mutations in breast cancer patients

Mutation	Total	Age above 60 yr	Age below 60 yr
CHEK2	9	9	0
BRCA1	6	3	3
MUYTH	6	4	2
BRCA2	4	2	2
RECQL4	2	0	2
PALB2	2	1	1
MSH6	2	0	2
BARD1	2	1	1
Other mutations (RAD50, MSH2, BLM, NHL1, CTNNA1, PSM2, EMN1, SDHB, VHL, FH)	10	4	6

Values are presented as number.

Table 4. Surgical interventions in patients with pathogenic mutations

Surgery type	Number of patients
Partial mastectomy	17
Bilateral mastectomy	12
Unilateral mastectomy	2

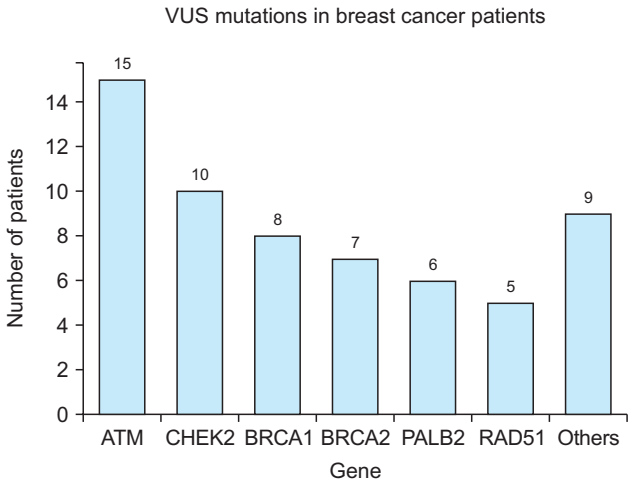

Figure 1. Distribution of VUS mutations. VSU, variants of unknown significance.

is repaired. Lifestyle choices, cellular metabolism, and environmental exposure to genotoxic factors contribute to DNA damage. Double-stranded breaks are one of the deadliest types of DNA damage. Inaccurate double-strand break repair can have catastrophic effects on cells, including aneuploidy and genetic instability. Homologous recombination or the clumsy nonhomologous end-joining method can be used to fix double-strand breaks. Since BRCA1 directly participates in the repair of double-strand breaks, coordinates proteins essential for the response to DNA damage, and influences the activation of cells, homologous recombination plays a significant role in the risk of breast cancer [13].

BRCA1/2 proteins play a unique role in DNA repair. As a result, early tumorigenesis and buildup of mutations in other genes are caused by loss-of-function mutations. While specific mutations are known, BRCA1/2 mutations are unevenly dispersed throughout the genes without hotspots. Women with BRCA1/2 germline mutations are considerably more likely to develop breast and ovarian cancers than the general population. The increased incidence of breast cancer starts at the age of 30, ovarian cancer in BRCA1 syndrome starts at the age of 40, and BRCA2 starts at the age of 50. BRCA syndrome should be suspected if breast and ovarian cancers have occurred, if male breast cancer cases are known, or if there is clustering of breast cancer in first-degree relatives, especially at a young age. Although medullary-differentiated breast carcinomas appear to be more common among BRCA disorders, other types of carcinomas can also arise. While correlation with DCIS is uncommon, BRCA germline mutations appear to be common in triple-negative breast cancers [14]. The primary cause of hereditary breast cancer, which raises a woman's lifelong risk of developing breast cancer, has been found to be BRCA1/BRCA2 mutations. Asians have an overall BRCA1/BRCA2 mutation prevalence that is similar to that of other ethnic groups. With the exception of Korea and the Philippines, the frequency of BRCA1 mutations is comparable to or marginally higher than that of BRCA2 mutations in most Asian nations. Asian parents with breast cancer are expected to benefit from better genetic counseling and cancer management given their awareness of the mutation spectrum of BRCA1/BRCA2 mutation carriers [15].

PALB2 (BRCA2 localizer and partner) is a moderate-risk gene associated with familial breast cancer. Biallelic mutations cause Fanconi anemia complementation group N, and monoallelic mutations can cause malignancies. PALB2 encodes a protein that binds and colocalizes with BRCA2 in the nuclear foci to act as a tumor suppressor. PALB2 provides a molecular scaffold for the BRCA1-PALB2-BRCA2 complex and allows BRCA2 to localize to the nucleus. PALB2 interacts with BRCA2 to swap out replication protein A for RAD51 on the processed single-stranded DNA end, in addition to working with BRCA2 to prevent cells from accumulating DNA damage. As the last stage of the interstrand cross-link repair mechanism, homologous recombination is also crucial and lacking in Fanconi anemia [13]. Further research is needed to offer a more comprehensive analysis of the involvement of PALB2 in breast cancer in different populations due to racial homogeneity, as Black American women are less likely than white American women to develop breast cancer. However, the disease tends to affect black females earlier, with more negative prognostic indicators and a higher death rate. A slightly elevated risk but distinct mutational spectrum has been found in small cohort studies of PALB2 mutation status with African ancestry [16-18].

The cell cycle checkpoint kinase 2 gene (CHEK2 or CHK2) is a tumor suppressor gene that contributes to breast cancer risk through its involvement in DNA repair and replication checkpoints. The three well-acknowledged germline variants in CHEK2 are 1100delC, R145W, and I157T. The most well-studied mutation, 1100delC, is linked to a reduced protein, CHEK2, which is defective and lacks the kinase activity [19]. Missense mutations R145W and I157T have much lower penetrance than the 1100delC mutation, which results in unstable mutant proteins and harmful binding [20]. Some studies have shown that truncating and missense CHEK2 mutations are linked to an increased risk of ovarian and breast cancer. Furthermore, carriers of CHEK2 mutations with a family history of cancer were more likely to acquire second primary tumors, and CHEK2 mutations predisposed women to particular forms of breast cancer [21]. The findings of our study suggest that pathogenic mutations in CHEK2 are particularly prevalent in the demographic population served by our institution. This observation aligns with previous studies indicating regional and ethnic variations in the prevalence of germline mutations, including CHEK2, as described by Kaufman et al. [22] and others. Additionally, our data reveals that the CHEK2 mutation was exclusively identified in patients aged over 60, consistent with findings from Laitman et al. [23].

Traditionally, genetic testing has been more readily offered to younger patients or those with significant family histories of cancer; however, our results challenge this approach and suggest that age is not a limiting factor in genetic evaluation. Moreover, approximately 9% of IDC patients in our cohort had germline mutations, which was significantly higher than the 4.2% prevalence observed in patients with DCIS. This disparity underscores the biological differences between invasive and noninvasive diseases and highlights the potential value of genetic testing across the spectrum of breast cancer diagnoses. For patients with DCIS, germline mutations may help identify those at a higher risk of progression to invasive disease, thereby aiding personalized treatment and surveillance strategies.

Our review also reveals a distinct trend among patients having BRCA1/2 mutations, where a majority opted for prophylactic mastectomy as their treatment [24]. This finding suggests that early genetic testing not only provides critical information about hereditary cancer risks but also plays a significant role in influencing patient decisions regarding surgical management. The availability of germline genetic information at the time of diagnosis enables patients and clinicians to make informed decisions regarding risk-reducing surgeries, potentially improving long-term outcomes and quality of life.

Based on our study, we firmly believe that universal germline genetic testing should be offered to all newly diagnosed breast cancer patients, regardless of age, family history, or disease stage. Incorporating genetic testing as a standard of care can lead to the earlier identification of high-risk mutations, allowing for specific treatment and preventive modalities. Furthermore, such an approach can significantly contribute to a wider and better understanding of the genetic variations in breast cancer and their use in patient care. Future studies should aim to further evaluate the cost-effectiveness of universal testing, assess patient outcomes over extended follow-ups, and explore how genetic information influences long-term decision-making in diverse populations.

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CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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