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Exome sequencing reveals frequent deleterious germline variants in cancer susceptibility genes in women with invasive breast cancer undergoing neoadjuvant chemotherapy

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Abstract When sequencing blood and tumor samples to identify targetable somatic variants for cancer therapy, clinically relevant germline variants may be uncovered. We evaluated the prevalence of deleterious germline variants in cancer susceptibility genes in women with breast cancer referred for neoadjuvant chemotherapy and returned clinically actionable results to patients. Exome sequencing was performed on blood samples from women with invasive breast cancer referred for neoadjuvant chemotherapy. Germline variants within 142 hereditary cancer susceptibil-ity genes were filtered and reviewed for pathogenicity. Return of results was offered to patients with deleterious variants in actionable genes if they were not aware of their result through clinical testing. 124 patients were enrolled (median age 51) with the following subtypes: triple negative ($n = 43$, 34.7 %), HER2+ ($n = 37$, 29.8 %), luminal B ($n = 31$, 25 %), and luminal A ($n = 13$, 10.5 %). Twenty-eight deleterious variants were identified in 26/124 (21.0 %) patients in the following genes: *ATM* ($n = 3$), *BLM* ($n = 1$), *BRCA1* ($n = 4$), *BRCA2* ($n = 8$), *CHEK2* ($n = 2$), *FANCA* ($n = 1$), *FANCI* ($n = 1$), *FANCL* ($n = 1$), *FANCM* ($n = 1$), *FH* ($n = 1$), *MLH3* ($n = 1$), *MUTYH* ($n = 2$), *PALB2* ($n = 1$), and *WRN* ($n = 1$). 121/124 (97.6 %) patients

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consented to return of research results. Thirteen (10.5 %) had actionable variants, including four that were returned to patients and led to changes in medical management. Deleterious variants in cancer susceptibility genes are highly prevalent in patients with invasive breast cancer referred for neoadjuvant chemotherapy undergoing exome sequencing. Detection of these variants impacts medical management.

Keywords Breast cancer · Neoadjuvant chemotherapy · High-risk breast cancer · Return of results · Exome sequencing · Germline mutation/pathogenic germline variant
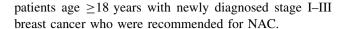
Introduction

Advances in genomic sequencing have resulted in opportunities to individualize patient care. The advent of next-generation sequencing has allowed for interrogation of the genome at a significantly reduced cost and may provide the opportunity for some cancer patients to pursue genome-guided therapy by identifying targetable somatic variants [18]. In order to determine which variants are unique to the tumor, germline sequence variants are subtracted from the tumor sequence [16]. Through this process, clinically important germline variants may be uncovered [3]. Although these variants are often labeled "incidental findings," research has demonstrated that the identification of deleterious variants causative of hereditary cancer syndromes should be anticipated in individuals undergoing next-generation sequencing tests [2, 5, 7, 10]. The identification of such variants can have a significant impact on the clinical management of a patient, including prophylactic surgeries, surveillance protocols, tailored screening, or change in therapy (e.g., chemoprevention).

The Breast Cancer Genome-Guided Therapy Study (BEAUTY) is a clinical study for patients with newly diagnosed breast cancer referred for neoadjuvant chemotherapy. The primary goal of BEAUTY is to identify novel somatic mutations associated with response to neoadjuvant chemotherapy. An additional goal of the study, which is presented here, is to determine the prevalence of deleterious germline variants in cancer susceptibility genes in these patients. Additionally, we assessed whether patients would desire to receive their germline research results and developed a procedure for return of clinically actionable results.

Methods

Participant eligibility and accrual

Patients were enrolled in BEAUTY (NCT02022202) from March 5, 2012, to May 1, 2014. Inclusion criteria were

patients age ≥18 years with newly diagnosed stage I–III breast cancer who were recommended for NAC.

Sample preparation, whole exome sequencing, and bioinformatics analyses

Methodology is described in the Online Resources.

Gene selection

A list of 142 genes associated with hereditary cancers (Online Resources, Table I) was developed by reviewing clinically available hereditary cancer gene panels, the Concise Handbook of Familial Cancer Susceptibility Syndromes, Online Mendelian Inheritance in Man (OMIM), and published literature [15, 17, 24]. Genes were divided into two tiers for analysis; tier one included genes associated with hereditary breast cancer while tier two included genes associated with other hereditary cancers.

Variant filtering and classification

Germline variants were filtered to identify missense, nonsense, frameshift, and splice-site variants within 142 hereditary cancer susceptibility genes. We also filtered for all intronic variants captured by exome sequencing in this list of genes that were previously reported as deleterious or pathogenic in the Human Gene Mutation Database (HGMD) or ClinVar [13, 25]. Variants were classified by a Certified Genetic Counselor according to the 2007 American College of Medical Genetics (ACMG) recommendations as either deleterious (category 1), likely deleterious (category 2), variant of uncertain significance (category 3), likely benign (category 4), or benign (category 5) [21]. Variant classification was determined based on reported minor allele frequencies from the Exome Variant Server, 1000 Genomes, and dbSNP; predicted protein impact; in silico models; review of databases such as HGMD, ClinVar, and locus specific databases; and review of published literature [1, 8, 13, 22, 25, 26].

After classifying variants from the first 91 patients, filtering strategies were designed that reduced the total number of variants for review. In the remaining patients, all tier two variants were filtered to include only those with a minor allele frequency of less than one percent that were either classified as a "disease causing mutation" in HGMD or predicted to be protein truncating [25]. Tier one variants were not filtered in this manner given our desire to maximize sensitivity and the greater likelihood of identifying clinically actionable variants in breast cancer-associated genes.

As an internal validation, we randomly selected approximately 10 % of all variants, making sure to sample all deleterious and likely deleterious variants (hereafter,

collectively termed deleterious). These variants were then independently classified by a second Certified Genetic Counselor. In scenarios where variant classifications were discrepant between the reviewers, the reviewers met to discuss the discrepancy and to reach a consensus about the final classification.

Return of results

Patients were invited to consent to the return of germline research results. Patients who consented to the return of results were informed that they would be contacted by a genetic counselor if a clinically actionable deleterious result that the patient was unaware of was identified. After identification of a clinically actionable deleterious variant, a study coordinator contacted eligible patients by telephone to offer an appointment with a genetic counselor. Risks, benefits, and limitations of receiving results were discussed during the genetic counseling appointment. If the patient was interested in receiving results, a second appointment was scheduled for results disclosure. Appointments occurred in-person or by telephone. It was highly recommended that patients proceed with confirmatory testing in a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory prior to making any medical management decisions based on the research results.

From the list of 142 cancer susceptibility genes (Online Resources, Table I), a subset of 39 genes were chosen for inclusion in the return of results protocol as they were determined to be "clinically actionable" based upon having existing medical management guidelines (National Comprehensive Cancer Network or other guidelines in published literature available on PubMed). Return of results was not performed for childhood-onset conditions, autosomal recessive conditions (as it was not possible to determine whether two variants identified within a gene were in *cis* or in *trans*), or carrier status for autosomal recessive conditions.

Ethics

This study was approved by the Mayo Clinic Institutional Review Board. All patients were required to provide written informed consent before participation, and patients were consented to the use of blood and tumor samples for whole exome sequencing as well as return of results

Results

Patient characteristics

The patient and disease characteristics are provided in Table 1. The median age was 51 (range 21–73) and most women had high-risk disease based upon the clinical T-stage [median tumor size 5 cm (1.1–9.9)], nodal status (56.4 % node positive), and clinical molecular subtype [89.5 % with luminal B, HER2+, or triple negative breast cancer (TNBC)].

Clinical genetic test results

Review of medical records showed that of the 124 patients enrolled, 81 (65.3 %) discussed the option of genetic testing with a clinical provider independent of the study, and 66/81 (81.5 %) underwent clinical genetic testing, including: single site *BRCA1* (n = 2), *BRCA1/BRCA2* sequencing (n = 7), *BRCA1/BRCA2* sequencing and deletion/duplication analysis (n = 45), *BRCA1/BRCA2* and *TP53* sequencing and deletion/duplication analysis (n = 2), a hereditary breast cancer panel (n = 9), and immunohistochemistry screening for Lynch syndrome (n = 1) (Online Resources, Table II). Nine patients tested positive for deleterious germline variants, including four in *BRCA1* and five in *BRCA2*.

Whole exome sequencing, variant filtering, and variant classification

After filtering for missense, nonsense, frameshift, splice-site, and previously reported deleterious/pathogenic intronic variants, 694 unique variants (observed 8214 times) were identified in 111 (78.2 %) of the 142 hereditary cancer susceptibility genes examined. Twenty-eight variants were classified as deleterious or likely deleterious, and were present in 26 of the 124 (21.0 %) patients in the following genes: *ATM* (n = 3), *BLM* (n = 1), *BRCA1* (n = 4), *BRCA2* (n = 8), *CHEK2* (n = 2), *FANCA* (n = 1), *FANCI* (n = 1), *FANCL* (n = 1), *FANCM* (n = 1), *FH* (n = 1), *MLH3* (n = 1), *MUTYH* (n = 2), *PALB2* (n = 1), and *WRN* (n = 1) (Table 2). Twenty-four of the 124 (19.4 %) patients had one deleterious variant while two patients (1.6 %) had two deleterious variants.

For the internal validation of variant classification, 81 variants were selected, independently reviewed, and classified by a second Certified Genetic Counselor. Twelve (14.8 %) were discrepant between the reviewers, including five deleterious versus likely deleterious, three likely deleterious versus uncertain significance, three uncertain significance versus likely benign, and one likely benign versus benign. The reviewers met to discuss the discrepancies and a consensus classification was reached for all variants.

All nine deleterious variants detected clinically in a CLIA-certified laboratory (four in *BRCA1* and five in *BRCA2*) were identified by our research study procedures. Classification of these nine variants was concordant in seven cases; two *BRCA2* variants that were classified as

Table 1 Patient characteristics according to the presence (yes/no) of a deleterious or likely deleterious mutation

	No (N = 98)	Yes (N = 26)	Total (N = 124)
Age group			
<30	1 (1.0 %)	1 (3.8 %)	2 (1.6 %)
30–39	17 (17.3 %)	4 (15.4 %)	21 (16.9 %)
40–49	27 (27.6 %)	7 (26.9 %)	34 (27.4 %)
50–59	27 (27.6 %)	10 (38.5 %)	37 (29.8 %)
60–69	19 (19.4 %)	3 (11.5 %)	22 (17.7 %)
70+	7 (7.1 %)	1 (3.8 %)	8 (6.5 %)
Race			
White	86 (87.8 %)	24 (92.3 %)	110 (88.7 %)
Black or African American	5 (5.1 %)	2 (7.7 %)	7 (5.6 %)
Asian	3 (3.1 %)	0 (0.0 %)	3 (2.4 %)
American Indian or Alaska Native	1 (1.0 %)	0 (0.0 %)	1 (0.8 %)
Unknown: patient unsure	3 (3.1 %)	0 (0.0 %)	3 (2.4 %)
Clinical molecular subtype			
Luminal A	9 (9.2 %)	2 (7.7 %)	11 (8.9 %)
Luminal B	23 (23.5 %)	8 (30.8 %)	31 (25.0 %)
Luminal Unknown	1 (1.0 %)	1 (3.8 %)	2 (1.6 %)
ER+/HER2+	17 (17.3 %)	0 (0.0 %)	17 (13.7 %)
ER−/HER2+	16 (16.3 %)	4 (15.4 %)	20 (16.1 %)
Triple negative	32 (32.7 %)	11 (42.3 %)	43 (34.7 %)
Clinical T-stage			
T1	10 (10.2 %)	2 (7.7 %)	12 (9.7 %)
T2	41 (41.8 %)	11 (42.3 %)	52 (41.9 %)
T3	44 (44.9 %)	12 (46.2 %)	56 (45.2 %)
T4	3 (3.1 %)	1 (3.8 %)	4 (3.2 %)
Clinical N-stage			
N0	43 (43.9 %)	11 (42.3 %)	54 (43.5 %)
N1	49 (50.0 %)	14 (53.8 %)	63 (50.8 %)
N2	3 (3.1 %)	1 (3.8 %)	4 (3.2 %)
N3	3 (3.1 %)	0 (0.0 %)	3 (2.4 %)

pathogenic/deleterious by the clinical laboratory were classified as likely deleterious in our study. Four additional deleterious variants in *BRCA2* and *FH* were detected in our study that had not been tested for clinically.

162 unique variants of uncertain significance (VUS) were found in 57/142 (40.1 %) genes examined among 103/124 (83.1 %) patients. The number of VUS per patient ranged from 0 to 6 (median 2.0). The number of unique VUS per gene (excluding genes containing no variants) ranged from 0 to 21 (median 2.0), with *ATM* containing the highest number (21 unique VUS).

No large deletions or duplications were observed.

Tumor characteristics in patients with deleterious variants

The approximated clinical subtypes among the 26 patients with one or more deleterious variants included 11 (42.3 %) TNBCs, four (15.4 %) HER2+, two (7.7 %) luminal A,

eight (30.8 %) luminal B, and one (3.8 %) luminal unknown breast cancer (Table 1). In contrast, the clinical subtypes among the 98 patients without a deleterious variant included 32 (32.7 %) TNBCs, 33 (33.6 %) HER2+, nine (9.2 %) luminal A, 23 (23.5 %) luminal B, and one (1.0 %) luminal unknown breast cancer.

Return of results

Nearly all of the patients (121/124; 97.6 %) consented to the return of germline research results. Thirteen patients were found to carry a deleterious variant in a cancer susceptibility gene that met the criteria for return of test results (Table 2). Four of these 13 were not already aware of their mutation through clinical testing. Their mutations included one likely deleterious *BRCA2* variant, two deleterious *BRCA2* variants, and a likely deleterious *FH* (Fumarate hydratase, causative of Hereditary Leiomyomatosis and Renal Cell Cancer) variant. The presence of each of these

Table 2 Deleterious germline variants identified in 124 breast cancer patients

Patient characteristics			BEAUTY whole exome sequencing results					
Age at Diagnosis	Family history of BC in 1st or 2nd-degree relatives?	Clinical genetic test results	Gene (transcript)	Nucleotide change	Mutation type/predicted protein effect	Classification	Returned to patient?	Confirmed in CLIA-certified laboratory?
Eligible for return of results								
40–49	Yes	BRCA1 deleterious mutation	BRCA1 (NM_007294.3)	c.5266dupC	Frameshift p.Q1756PfsX74	Deleterious	No, patient aware	N/A
<40	No	BRCA1 deleterious mutation	BRCA1[a] (NM_007294.3)	c.4689C>G	Nonsense p.Y1563X	Deleterious	No, patient aware	N/A
<40	Yes	BRCA1 deleterious mutation	BRCA1 (NM_007294.3)	c.1504_1508delTTAAA	Frameshift p.L502AfsX2	Deleterious	No, patient aware	N/A
40–49	Yes	BRCA1 deleterious mutation	BRCA1 (NM_007294.3)	c.213-11T>G	Altered splicing	Deleterious	No, patient aware	N/A
40–49	Yes	BRCA2 deleterious mutation	BRCA2 (NM_000059.3)	c.631+2T>G	Altered splicing	Deleterious	No, patient aware	N/A
40–49	Yes	BRCA2 pathogenic mutation	BRCA2 (NM_000059.3)	c.2808delA	Frameshift p.K936NfsX24	Likely deleterious	No, patient aware	N/A
40–49	No	N/A; not pursued	BRCA2 (NM_000059.3)	c.3103G>T	Nonsense p.E1035X	Likely deleterious	Yes	Yes (deleterious)
40–49	Yes	BRCA2 deleterious mutation	BRCA2 (NM_000059.3)	c.3170_3174delAGAAA	Frameshift p.K1057TfsX8	Deleterious	No, patient aware	N/A
50–59	Yes	N/A; not pursued	BRCA2 (NM_000059.3)	c.4638delT	Frameshift p.F1546LfsX22	Deleterious	Yes	Yes (deleterious)
40–49	Yes	BRCA2 deleterious mutation	BRCA2 (NM_000059.3)	c.7069_7070delTC	Frameshift p.L2357VfsX2	Deleterious	No, patient aware	N/A
50–59	Yes	N/A; not pursued	BRCA2 (NM_000059.3)	c.7558C>T	Nonsense p.R2520X	Deleterious	Yes	Yes (deleterious)
<40	Unknown (adopted)	BRCA2 deleterious mutation and BRCA2 VUS	BRCA2 (NM_000059.3)	c.8969G>A	Nonsense p.W2990X	Likely deleterious	No, patient aware	N/A
70+	Yes	BRCA1/BRCA2 negative	FH (NM_000143.3)	c.1067T>A	Nonsense p.L356X	Likely deleterious	Yes	Yes (pathogenic)

Patient characteristics			BEAUTY whole exome sequencing results			
Age at Diagnosis	Family history of BC in 1st or 2nd-degree relatives?	Clinical genetic test results	Gene (transcript)	Nucleotide change	Mutation type/predicted protein effect	Classification
Not eligible for return of results						
50–59	Yes	N/A; not pursued	ATM (NM_000051.3)	c.4111delG	Frameshift p.D1371IfsX15	Likely deleterious
<40	Yes	BRCA1/BRCA2 negative	ATM (NM_000051.3)	c.5932G>T	Nonsense p.E1978X	Deleterious
60–69	No	N/A; not pursued	ATM[a] (NM_000051.3)	c.8264_8268delATAAG	Frameshift p.Y2755CfsX12	Deleterious

Table 2 continued

Patient characteristics			BEAUTY whole exome sequencing results			
Age at Diagnosis	Family history of BC in 1st or 2nd-degree relatives?	Clinical genetic test results	Gene (transcript)	Nucleotide change	Mutation type/predicted protein effect	Classification
60–69	Yes	N/A; not pursued	BLM (NM_001287246.1)	c.3014_3015ins TATCA	Frameshift p.M1006IfsX3	Likely deleterious
50–59	Yes	BRCA1/BRCA2 negative	CHEK2 (NM_007194.3)	c.470T>C	Missense p.I157T	Deleterious
50–59	Yes	N/A; not pursued	CHEK2 (NM_007194.3)	c.444+1G>A	Altered splicing	Deleterious
50–59	Yes	BRCA1/BRCA2 negative	FANCA (NM_000135.2)	c.4015delC	Frameshift p.L1339SfsX24	Likely deleterious
50–59	No	N/A; not pursued	FANCI (NM_001113378.1)	c.2422A>T	Nonsense p.K808X	Likely deleterious
50–59	Yes	BRCA1/BRCA2 negative	FANCL (NM_018062.3)	c.1096_1099dup ATTA	Frameshift p.T367NfsX13	Likely deleterious
60–69	Yes	N/A; not pursued	FANCM (NM_020937.2)	c.1491dup	Frameshift p.Q498TfsX7	Likely deleterious
<40	No	BRCA1 deleterious mutation	MLH3[a] (NM_001040108.1)	c.2116delA	Frameshift p.T706QfsX28	Likely deleterious
<40	Yes	BRCA1/BRCA2 negative	MUTYH (NM_001128425.1)	c.1187G>A	Missense p.G396D	Deleterious
60–69	No	N/A; not pursued	MUTYH[a] (NM_001128425.1)	c.536A>G	Missense p.Y179C	Deleterious
50–59	Yes	BRCA1/BRCA2 negative	PALB2 (NM_024675.3)	c.3549C>A	Nonsense p.Y1183X	Likely deleterious
50–59	No	BRCA1/BRCA2 negative	WRN (NM_000553.4)	c.487_488delAG	Frameshift p.D163CfsX4	Likely deleterious

All molecular variants are named according to GRCh37 (hg19)

BC breast cancer

[a] More than one deleterious variant identified in each of two patients

four variants was subsequently confirmed in a CLIA-certified laboratory.

Frequency of deleterious variants in databases

Of the 28 unique deleterious variants, 16 were classified as "DM" (disease causing mutation) in HGMD and as pathogenic in ClinVar. Two variants were classified as DM in HGMD but were not reported in ClinVar, and two were classified as pathogenic in ClinVar but were not reported in HGMD. One variant was classified as pathogenic in ClinVar and as a "disease-associated polymorphism with additional supporting functional evidence" in HGMD. One variant was classified as "DM?" (likely disease causing but with questionable pathogenicity) in HGMD and not reported in ClinVar. All of the remaining six deleterious variants that were not reported in either of the databases were predicted to be protein truncating (nonsense, frameshift, or splice-site variants). The deleterious variants absent from ClinVar will be submitted for inclusion.

Discussion

In a prospective study of women with invasive breast cancer recommended to undergo NAC, deleterious germline variants in cancer susceptibility genes were highly prevalent and identified in 26 of 124 (21.0 %) patients. These variants were identified in a number of breast cancer susceptibility genes, including *ATM, BRCA1*, *BRCA2*, *CHEK2,* and *PALB2*. Furthermore, we identified variants in a variety of other cancer susceptibility genes including *BLM*, *FANCA*, *FANCI*, *FANCL*, *FANCM*, *FH*, *MLH3*, *MUTYH*, and *WRN*. Although the latter variants have not been classically associated with breast cancer, evidence implicates a potential role for several of these genes in the pathogenesis of breast cancer including *BLM* [6, 19, 27], *FANCA* [23], *FANCM* [9], *FH* [14], *MLH3* [4], *MUTYH* [20], and *WRN* [28].

Thirteen patients were found to carry deleterious variants in actionable genes (Table 2). While the majority of patients with actionable variants were already aware of their result through clinical testing, we identified four patients with deleterious/likely deleterious variants who were not aware of their results, including three in *BRCA2* and one in *FH*. All four variants were confirmed by CLIA-certified laboratories. For these patients, the medical management plans changed significantly including plans for a prophylactic bilateral salpingo-oophorectomy (BSO) in two *BRCA2*-positive patients, a prophylactic contralateral mastectomy and BSO in one *BRCA2*-positive patient, and renal cell carcinoma surveillance and enrollment in a national Hereditary Leiomyomatosis and Renal Cell

Cancer (HLRCC) study in the patient with an *FH* variant. While guidelines do not currently recommend a specific type of chemotherapy based on the presence or absence of deleterious germline variants, evidence suggests that tumors that arise in patients with deleterious germline *BRCA1/BRCA2* variants may exhibit increased sensitivity to anthracyclines, platinum, and PARP inhibitors, and resistance to taxanes [11].

Our definition of actionable results was limited to deleterious and likely deleterious variants in genes with existing medical management guidelines. Several genes excluded from our return of results procedure may become actionable in the future as new guidelines are developed. If we used the definition of "potentially actionable" adopted by Kurian et al., which includes genes with a published association of two-fold or greater relative risk of breast cancer, the number of patients with variants in genes meeting criteria for return of results would increase from 13 (9.7 %) to 21 (16.9 %) [12]. The cancer risks associated with deleterious variants in these low–moderate penetrance genes are poorly defined, and it remains unclear whether additional surveillance or surgical management is warranted as the risk of contralateral breast cancer and other cancers is not well established. Thus, the clinical utility of returning such results to patients is uncertain.

The prevalence of deleterious germline variants identified and their potential clinical importance suggests that similar studies seeking to identify somatic variants for targeted cancer treatments should also prioritize the analysis of germline data. Classification of germline variants undoubtedly takes significant time and effort. When we tested filtering methods for tier one variants after the enrollment of 91 patients, filtering variants to include only those with a minor allele frequency of less than 1 % that were either categorized as DM in HGMD or predicted to be protein truncating reduced the number of unique variants for review from 198 to 30, while capturing all deleterious variants. If the proposed filtering strategy had been used for all tier one and tier two variants for all 124 patients, the number of unique variants for review would have been reduced from 694 to 90, while capturing 27 of 28 deleterious variants—*CHEK2* c.470T>C (p.I157T) would have been missed as it is a missense variant that is listed as a "disease-associated polymorphism with additional supporting functional evidence," not DM, in HGMD. Dewey et al. recently reported that manual variant classification required a median of 54 min (range 5–223) per genetic variant [5]. Thus, we estimate that implementing these filters would have resulted in a time savings of approximately 544 h (almost 14 full work weeks), although sensitivity would have been reduced by excluding missense mutations that are not reported as DM in HGMD. ClinVar classification was not tested in our filtering techniques as

the first full public release was not available at the time of study initiation, but was reviewed retrospectively. HGMD and ClinVar had nearly equal sensitivity, as 18/28 deleterious variants were classified as DM in HGMD and 19/28 deleterious variants were classified as pathogenic in ClinVar. Our data suggest that future studies could consider implementing germline analysis and return of results procedures using minor allele frequency, predicted protein impact, and inclusion in either HGMD or ClinVar as filters.

Limitations

This study has several limitations. CLIA-certified laboratory confirmation was not performed for all deleterious variants identified, and thus, for the non-clinically actionable variants, false positives may exist. It is possible that deleterious variants could have been missed after filtering strategies were implemented for tier two variants for the final 33 patients. Intronic, promoter, or other rare variants may have been missed given the limitations of whole exome sequencing. A large number of variants were classified as VUS, highlighting the current state of genomics knowledge and the difficulty of determining pathogenicity of variants. As demonstrated in previous studies, the burden of VUS generated by next-generation sequencing is a significant issue [2, 10, 12]. Because the median age of patients with breast cancer enrolled in our study was younger than the general population (51 vs. 61 years), the prevalence of deleterious variants is likely not generalizable to older breast cancer cohorts with low-risk tumor biology.

Conclusion

Deleterious germline variants in a variety of cancer susceptibility genes are frequent in breast cancer patients with high-risk tumor biology who were referred for neoadjuvant chemotherapy and their detection impacts medical management. Studies that seek to identify somatic variants using genomic sequencing technologies should also seek to identify actionable deleterious germline variants and return results to patients given the potential clinical implications.

Declaration The experiments performed comply with the current laws of the United States of America.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human rights All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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