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PRECLINICAL STUDY

Contribution of germline mutations in cancer predisposition genes to tumor etiology in young women diagnosed with invasive breast cancer

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Abstract
Purpose Although breast cancer in young women accounts for <10% of diagnoses annually, tumors in young patients exhibit more aggressive characteristics and higher mortality rates. Determination of the frequency of germline mutations in cancer predisposition genes is needed to improve the understanding of breast cancer etiology in young women.
Methods All female patients enrolled in the Clinical Breast Cancer Project between 2001 and 2015 and diagnosed with invasive breast cancer before age 40 were included in this study. Family history was classified using the NCCN Familial Risk Assessment guidelines. Targeted sequencing of 94 cancer predisposition genes was performed using peripheral blood DNA. Variants were detected using VariantStudio and classified using ClinVar.
Results Seven percent (141/1980) of patients were young women and 44 had a significant family history. Sequencing was completed for 118 women with genomic DNA. Pathogenic mutations were present in 27 patients: BRCA1 ($n = 10$), BRCA2 ($n = 12$), TP53 ($n = 1$), and CHEK2 ($n = 4$). Mutations classified as pathogenic were also detected in APC ($n = 1$) and MUTYH ($n = 2$). Variants of uncertain significance (VUS) were detected in an additional 17 patients in ten genes.
Discussion Pathogenic mutations in high- and moderate-risk breast cancer genes were detected in 23% of young women with an additional 3% having pathogenic mutations in colon cancer predisposition genes. VUS were observed in 14% of women in genes such as ATM, BRCA2, CDH1, CHEK2, and PALB2. Identification of those non-genetic factors is critical to reduce the burden of breast cancer in this population.

Keywords Breast cancer · Young women · Genetic predisposition · Pathogenic mutations

Introduction

More than 10,000 young women (<40 years of age) in the United States are diagnosed with breast cancer every year [1, 2] where the disease is associated with poor prognosis [3, 4]. Factors associated with breast cancer in young women include having children at an early age, ≥6 months oral contraceptive use, and breastfeeding [5, 6]. African ancestry has also been associated with increased risk of breast cancer, especially ER−, in young women [5]. Breast cancer in young women may have a hereditary component: for example, mutation rates for BRCA1 and BRCA2 are higher in young women (13%) compared to women aged 40–49 (2.2%) in the United Kingdom and in the United States (reviewed in [7]). The National Comprehensive Cancer Network (NCCN) guidelines include a diagnosis ≤45 years of age as a

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criterion for risk assessment, genetic counseling, and genetic testing of BRCA1 and BRCA2 [8].

Breast cancer genes other than BRCA1 and BRCA2 may account for breast cancer risk in young women. NCCN guidelines suggest that patients diagnosed before 31 years of age may be eligible for genetic testing of TP53, with up to 5% of young women with breast carcinomas harboring pathogenic mutations in TP53 [9]. The contribution to breast cancer risk in young women from other genes, including those associated with moderate risk for breast cancer and other genes associated with other types of cancer, is not well studied. Thus, to improve our understanding of the role of heritability in breast cancer in young women, panel testing of 94 cancer predisposition genes was performed in 118 young women diagnosed with breast cancer <40 years of age.

Methods

Between 2001 and 2015, 1980 female patients with invasive breast cancer enrolled in the Clinical Breast Care Project (CBCP). All subjects voluntarily agreed to participate in the CBCP and gave written informed consent. To be eligible to participate in this study, individuals were required to be (1) at least 18 years of age, (2) mentally competent and willing to sign the informed consent documents, and (3) a patient at Walter Reed National Military Medical Center, Bethesda, MD, Anne Arundel Medical Center, Annapolis, MD, or the Joyce Murtha Breast Care Center, Windber, PA, with evidence of current or past breast disease. Blood samples were collected with approval from the Walter Reed National Military Medical Center Human Use Committee and Institutional Review Board.

Once informed consent was granted, nurse researchers interviewed enrollees in person to collect over 500 fields of demographic data, including family cancer histories through third-degree relatives. Presence of a family history was determined using the NCCN Familial Risk Assessment criteria [10]. Age at diagnosis was calculated by subtracting the date of birth from the date of the diagnostic biopsy. Pathologic evaluation included determination of tumor stage, size, grade, and lymph node status [11–13]. ER, progesterone receptor (PR), and HER2 status were determined using ASCO/CAP guidelines [14, 15].

Genomic DNA was isolated from blood clots using the Gentra Clotspin and Puregene DNA purification kits (Qiagen, Valencia, CA). Samples were quantitated using the Qubit™ 3.0 Fluorometer (ThermoFisher Scientific, Waltham, MA). Libraries were created from 50 ng of DNA using the TruSight Rapid Capture kit and TruSight Cancer panel and sequenced on a MiSeq (Illumina, Inc, San Diego, CA) according to manufacturer's protocols. Data were analyzed using VariantStudio version 3.0. (Illumina, Inc, San Diego, CA) and filtered to include only those variants with a read depth of ≥10 and a minor allele frequency of ≥0.25. Variants representing missense or frameshift mutations, stop gains or losses, initiator codons, in-frame insertions or deletions, and splice site alterations were included in the analysis. The predicted effect of variants was evaluated using the ClinVar database (http://www.clinvar.com/) and classified as pathogenic, likely pathogenic, uncertain significance (VUS), likely benign, or benign.

Results

Clinicopathological characteristics

One hundred forty-one (7%) patients with invasive breast cancer enrolled in the CBCP were diagnosed <40 years of age; 119 enrolled at the time of diagnosis, while the other 22 enrolled in the CBCP as post-treatment survivors. Forty-four patients (31%) had a family history of cancer. The majority of patients (90%) had unilateral disease and surgical options were divided between unilateral mastectomy (35%), breast-conserving surgery (34%), and double mastectomy (31%). Forty-two percent of young women underwent clinical testing for BRCA1 and BRCA2 mutations. Most of the patients were self-described European American (66%) followed by African American (28%; Table 1); European American women were more likely to report a family history of cancer (31%) than African American women (15%) although this difference was not significant ($P = 0.467$). The majority of patients were diagnosed more than two years after the last childbirth, although three patients (2%) had children after diagnosis.

Pathologically, the majority of the tumors were size T1 or T2 at diagnosis, poorly differentiated, lymph node negative, and ER+HER2−. Twenty-nine percent of women were diagnosed with stage III or IV tumors and 25% with TNBC. Sixteen percent of young women died of disease with a mean survival of 4.77 years (range 10 months–20.1 years) and patients alive without disease had a mean follow-up of 9.36 years. No patients died of causes other than breast cancer.

Mutation status

Of the 118 samples subjected to targeted sequencing, 117 (99%) had at least 30× coverage, with the remaining sample having 28.3× coverage. The average number of reads passing filter was 974,861. The average Q30 score was 94.3%.

Table 1 Clinical and pathological characteristics of breast tumors from 141 young women

	Frequency
Ethnicity	
African American	0.28
Asian	0.04
Hispanic	0.01
Other	0.01
Non-Hispanic white	0.66
Parity	
Non-parous	0.15
PABC[a]	0.17
Non-PABC	0.66
Pregnancy post-diagnosis	0.02
Tumor size	
T1	0.49
T2	0.42
T3	0.09
Tumor grade	
Well (grade 1)	0.10
Moderate (grade 2)	0.36
Poor (grade 3)	0.54
Tumor stage	
I	0.34
II	0.37
III	0.24
IV	0.05
Lymph node status	
Positive	0.44
Negative	0.56
ER/HER2 status	
ER+/HER2−	0.52
ER+/HER2+	0.12
ER−/HER2+	0.11
ER−/HER2−	0.25
Status[b]	
Died of disease	0.16
Alive with disease	0.01
Alive, disease-free	0.83

[a] PABC = pregnancy-associated breast cancer is defined as being diagnosed with breast cancer within two years of the last childbirth

[b] None of the patients died from health conditions other than breast cancer

Pathogenic or likely pathogenic mutations or VUS were detected in 15/94 (16%) cancer predisposition genes (Supplemental Table 1). DNA variants represented (1) pathogenic mutations in known breast cancer genes, (2) pathogenic mutations in colon cancer genes, and (3) VUS in a range of cancer predisposition genes. Pathogenic or likely pathogenic genes associated with hereditary breast cancer-predisposing syndromes were detected in 27/118 (23%) patients and included mutations in BRCA1 ($n = 10$), BRCA2 ($n = 12$), CHEK2 ($n = 4$), and TP53 ($n = 1$) (Table 2). Three patients had pathogenic mutations in the hereditary colon cancer genes APC ($n = 1$) and MUTYH ($n = 2$) (Table 3). Four patients with pathogenic mutations also carried VUS in BRCA2, BRIP1, CHEK2, MSH2, and PMS2. An additional 17 patients (14%) harbored VUS in 10 genes including APC ($n = 1$), ATM ($n = 4$), BRCA2 ($n = 1$), BRIP1 ($n = 2$), CDH1 ($n = 2$), CHEK2 ($n = 1$), MLH1 ($n = 2$), MSH6 ($n = 1$), PALB2 ($n = 1$), and PMS2 ($n = 2$) (Table 4). All patients with BRCA1 mutations and ER, PR, and HER2 data available had TNBC and 80% had a family history of cancer. In patients with BRCA2 mutations and ER, PR, and HER2 available, one patient with a 5104delAA mutation had TNBC, while all others had ER+ tumors with 80% ER+/HER2− and 20% ER+/HER2+ (luminal-HER2); 50% of young women with a mutation in BRCA2 had a significant family history. Mutation carriers were more common in African American women (29%) compared to European American women (22%) and the VUS in BRCA2 (S2059N) was detected in an African American woman with a luminal-HER2 tumor but without a significant family history of cancer.

Effect of mutational status on clinicopathological features

Clinicopathological factors were compared between young women with pathogenic mutations and those with no mutations detected in any of the 94 cancer predisposition genes; given the uncertain status of women with VUS, these 17 patients were excluded from the analyses. Presence of a family history was significantly higher ($P < 0.002$) in mutation carriers (57%) compared to non-carriers (24%); no other clinicopathological factors differed significantly (Table 5).

Discussion

Mutation frequencies in BRCA1 and BRCA2 in young women from the United States, Canada, Europe, China, and Brazil range from 9 to 23% [16–24]. Panel testing increases the frequency of detection of pathogenic mutations to 18–26% [25–28]. In our study which represents, to our knowledge, the first study to utilize panel testing to identify germline mutations in cancer predisposition genes in young women unselected for family history, ethnicity, or subtype, we detected a mutation frequency of 23% in known breast cancer genes and 3% in genes associated with other familial cancers.

Table 2 Pathogenic or likely pathogenic mutations in known breast cancer genes identified in 27/118 women diagnosed with invasive breast cancer <40 years of age

Patient	Gene	Mutation	ER/HER2	Ethnicity	Family history	Family cancer types
19	BRCA1	NM_007294.3(BRCA1):c.68_69delAG (p.Glu23 Valfs)	TN	White	√	Mother and sister breast (premenopausal)
13	BRCA1	NM_007294.3(BRCA1):c.329dupA (p.Glu111Glyfs)	Unk	White	√	Mother breast (premenopausal)
37	BRCA1	NM_007294.3(BRCA1):c.4603G>T (p.Glu1535Ter)	TN	African American	√	Sister breast (premenopausal); Paternal aunt breast (postmenopausal)
17	BRCA1	NM_007294.3(BRCA1):c.250G>T (p.Glu84Ter)	TN	African American		Mother breast (postmenopausal) and lung
40	BRCA1	NM_007294.3(BRCA1):c.962G>A (p.Trp321Ter)	TN	White		Paternal cousin breast (premenopausal)
49	BRCA1	NM_007294.3(BRCA1):c.4986+6T>C	TN	African American	√	Mother breast (premenopausal); 2 maternal aunts breast (age unknown)
31	BRCA1	NM_007294.3(BRCA1):c.3937C>T (p.Gln1313Ter)	TN	White	√	Paternal grandmother and 2 paternal aunts breast (postmenopausal); maternal breast grandmother (premenopausal); mother ovarian
33	BRCA1	NM_007294.3(BRCA1):c.815_824dupAGCCATGTGG (p.Thr276Alafs)	TN	African American	√	Mother breast (postmenopausal); sister (premenopausal); paternal grandmother ovarian
52[a]	BRCA1	NM_007294.3(BRCA1):c.5266dupC (p.Gln1756Profs)	Unk	White	√	Paternal aunt breast (premenopausal); maternal grandfather brain
137	BRCA1	NM_007294.3(BRCA1):c.181T>G (p.Cys61Gly)	Unk	White	√	Mother and maternal aunt breast (premenopausal); father prostate and colon; paternal grandmother ovarian
138	BRCA2	NM_000059.3(BRCA2):c.4876_4877delAA (p.Asn1626Serfs)	TN	White	√	Mother breast (postmenopausal); sister (premenopausal)
118	BRCA2	NM_000059.3(BRCA2):c.4936_4939delGAAA (p.Glu1646Glnfs)	ER+HER2−	African American	√	Father and paternal uncle breast; 2 paternal aunts and 3 paternal cousins breast (premenopausal); 1 paternal aunt breast (postmenopausal)
81[b]	BRCA2	NM_000059.3(BRCA2):c.1384G>T(p.Glu462Ter)[c]	ER+HER2−	African American		Paternal aunt and maternal great-grandmother breast (postmenopausal)

Table 2 continued

Patient	Gene	Mutation	ER/HER2	Ethnicity	Family history	Family cancer types
5	BRCA2	NM_000059.3(BRCA2):c.5621_5624delTTAA (p.Ile1874Argfs)	ER+HER2−	White	√	Paternal grandmother breast (premenopausal); paternal aunt and great-grandmother (age unknown); father prostate
105	BRCA2	NM_000059.3(BRCA2):c.1813dupA (p.Ile605Asnfs)	ER+HER2−	White	√	Paternal grandmother breast (premenopausal); paternal great-grandmother and aunt (age unknown)
60	BRCA2	NM_000059.3(BRCA2):c.6331_6332delAA (p.Lys2111Glufs)	Unk	White	√	Paternal aunt breast (premenopausal)
34	BRCA2	NM_000059.3(BRCA2):c.771_775delTCAAA (p.Asn257Lysfs)	ER+HER2−	White		Mother and maternal grandmother breast (postmenopausal)
127[d]	BRCA2	NM_000059.3(BRCA2):c.7007G>A (p.Arg2336His)	Lum-HER2	White		Self cervical; father pancreatic
54[e]	BRCA2	NM_000059.3(BRCA2):c.5857G>T (p.Glu1953Ter)	ER+HER2−	White		Father skin
141	BRCA2	NM_000059.3(BRCA2):c.5857G>T (p.Glu1953Ter)	ER+HER2−	White	√	Sister breast (premenopausal); paternal aunt breast (age unknown); father prostate and skin
90	BRCA2	NM_000059.3(BRCA2):c.8821C>T (p.Gln2941Ter)	ER+HER2−	White		
29	BRCA2	NM_000059.3(BRCA2):c.9294C>G (p.Tyr3098Ter)	Lum-HER2	African American		Paternal aunt breast (postmenopausal); paternal grandfather colon
67	CHEK2	NM_007194.3(CHEK2):c.470T>C (p.Ile157Thr)	ER+HER2−	White	√	Paternal aunt breast (premenopausal)
53	CHEK2	NM_007194.3(CHEK2):c.470T>C (p.Ile157Thr)	ER+HER2−	White		Paternal great-grandmother breast (premenopausal)
79	CHEK2	NM_007194.3(CHEK2):c.1100delC (p.Thr367Metfs)	ER+HER2−	White		Paternal aunt breast (age unknown); maternal grandmother bladder
30	CHEK2	NM_007194.3(CHEK2):c.349A>G (p.Arg117Gly)	ER+HER2−	White		
39[f]	TP53	NM_000546.5(TP53):c.637C>T (p.Arg213Ter)	TN	African American		Maternal grandfather colon

[a] Patient 52 also carries an MSH2 G683V VUS

[b] Patient 81 also carries a BRCA2 R2502C VUS

[c] BRCA2 E462X is not listed in ClinVar but classified as pathogenic by Myriad Genetics

[d] Patient 127 also carries a CHEK2 R523C VUS

[e] Patients 54 and 141 are sisters. At the time of diagnosis for patient 54 (January 2007), the only type of cancer reported in the family was skin cancer in the father. Patient 141 and another sister share the same BRCA2 mutation as patient 54. Patient 141 was diagnosed in December 2008 by which time the father had developed prostate cancer. Both women have died of disease

[f] Patient 39 also carries BRIP1 E1126D and PMS2 Y191C VUS

Clinical management will differ between young women with and without pathogenic mutations in breast cancer genes. The majority of women did not have germline mutations and should have received standard care appropriate to her tumor subtype and/or somatic mutations. In contrast, options for the 22 young women with BRCA1 and

Table 3 Pathogenic or likely pathogenic mutations in non-breast cancer predisposition genes identified in 3/118 women diagnosed with invasive breast cancer <40 years of age

Patient	Gene	Mutation	ER/HER2	Ethnicity	Family history	Family cancer types
11	APC	NM_000038.5(APC):c.449A>G (p.Lys150Arg)	Unk	White	√	Mother breast (premenopausal); Maternal grandmother colon
28	MUTYH	NM_001128425.1(MUTYH):c.536A>G (p.Tyr179Cys)	Lum-HER2	White		
102	MUTYH	NM_001128425.1(MUTYH):c.1187G>A (p.Gly396Asp)	Lum-HER2	White	√	Paternal grandmother fallopian tube; maternal grandmother breast (postmenopausal)

BRCA2 mutations include increased surveillance, prophylactic mastectomy of the unaffected breast, and oophorectomy. Of the 50 women in this study that had clinical testing for BRCA1 and BRCA2, 12 harbored pathogenic BRCA1 or BRCA2 mutations. One additional woman was tested for and found to harbor a TP53 mutation. Within these young women who underwent clinical testing, surgical treatments differed significantly ($P = 0.024$) between those who had pathogenic mutations and those with negative BRCA1 and BRCA2 results. All of the mutation carriers opted for mastectomy: seven (54%) had a unilateral mastectomy of which four developed contralateral breast cancer and one recurred; the other 46% of carriers had a double mastectomy. In those women who tested negative for BRCA1 and BRCA2 mutations, 26% opted for breast-conserving surgery, 24% for unilateral mastectomy, and 50% for double mastectomy. Rates of oophorectomy did not differ significantly between mutation carriers and non-carriers.

Utilization of gene panels, rather than single-gene tests, identified an additional four young women who carried germline pathogenic mutations in CHEK2. The CHEK2 1100delC represents a high-risk allele associated with greater than twofold increase in risk and poor prognosis; in contrast, the I157T allele is associated with moderate risk (1.4-fold) and more favorable prognosis [29]. Functional assays demonstrate that the R117G allele encodes a functionally defective protein; however, the magnitude of risk is not well characterized. Although none of the patients received clinical testing for CHEK2, both women with the I157T allele opted for double mastectomy, as did the woman with the 1100delC mutation.

In addition to identification of women with BRCA1 and BRCA2 mutations who had not had clinical testing and women with CHEK2 mutations, we also identified three young women with breast cancer who had mutations in hereditary colon cancer genes. While these mutations may be secondary to the development of breast tumors, they are associated with increased risk for colon cancer. One woman died of breast cancer within three years of her breast cancer diagnosis, but the other two may benefit from increased colon cancer screening. Other studies have demonstrated that identification of mutations in non-breast cancer genes through panel testing may result in changes in clinical management. DNA samples from 198 women referred for BRCA1 and BRCA2 testing were subjected to panel testing of 42 cancer-associated genes; 29% of the women carried BRCA1 or BRCA2 mutations, while an additional 15 women had pathogenic mutations in other genes [30]. Six of these women had actionable mutations in CDH1, MLH1, and MUTYH and were advised to undergo frequent colonoscopy or endoscopy; one of these women was found to have a tubular adenoma, an incidental tumor that may not have been discovered had panel testing not been performed.

While the percentage of young women with invasive breast cancer enrolled in the CBCP (7%) is similar to national estimates [1], this represents only 141 patients enrolled over a 14-year period. Larger sample sizes may alter frequency estimates or patterns of germline mutations; however, our finding of a mutation frequency of 23% pathogenic mutations is similar to larger studies when subpopulations of younger women were considered. Patients in the CBCP enroll as individuals, and thus medical records and DNA samples from family members are not available to determine segregation patterns for the identified risk alleles, thereby limiting the ability to improve classification of the 17 VUS identified in this cohort. Finally, the use of a targeted sequencing approach precludes the ability to detect large rearrangements, which have been shown to account for up to 6% of BRCA1 and BRCA2 mutations in high-risk individuals from the United States [31]. Given this frequency, a small number (2–3) of young women with a family history of breast cancer may have undetected large rearrangements in BRCA1 or BRCA2, thus increasing the mutation rate in known breast cancer genes from 23 to 26%.

In conclusion, etiology of breast cancer has been associated with pathogenic mutations in 23% of known breast cancer predisposition genes, with an additional 3% of

Table 4 VUS identified in 17/118 women diagnosed with invasive breast cancer <40 years of age

Patient	Gene	Mutation	ER/HER2	Ethnicity	SIFT	PolyPhen	Family history	Family cancer types
132	APC	NM_000038.5(APC):c.7471A>G (p.Met2491Val)	TN	African American		Benign		Mother lung, kidney
126	ATM	NM_000051.3(ATM):c.7778A>G (p.Gln2593Arg)	ER+HER2−	White	Tolerated	Benign		
94	ATM	NM_000051.3(ATM):c.4148C>T (p.Ser1383Leu)	ER+HER2−	White	Deleterious	Probably damaging		Mother lung
83	ATM	NM_000051.3(ATM):c.2932T>C (p.Ser978Pro)	ER+HER2−	White	Deleterious	Probably damaging		Maternal aunt breast (postmenopausal), father lung
8	ATM	NM_000051.3(ATM):c.2927T>C (p.Val976Ala)	Unk	African American	Deleterious	Benign		
111	BRCA2	NM_000059.3(BRCA2):c.6176G>A (p.Ser2059Asn)	Lum-HER2	African American	Tolerated	Benign		Maternal aunt breast (postmenopausal); maternal grandmother lung; maternal grandfather pancreatic
32	BRIP1	NM_032043.2(BRIP1):c.550G>T (p.Asp184Tyr)	TN	White	Deleterious	Probably damaging		
68	BRIP1	NM_032043.2(BRIP1):c.1774T>G (p.Trp592Gly)	TN	White	Deleterious	Probably damaging		Mother breast (postmenopausal); maternal grandfather leukemia
14	CDH1	NM_004360.4(CDH1):c.1018A>G (p.Thr340Ala)	ER+HER2−	Asian	Tolerated	Benign		Self ovarian
10	CDH1	NM_004360.4(CDH1):c.2494G>A (p.Val832Met)	ER+HER2−	Asian	Deleterious	Probably damaging		Paternal grandfather gastric cancer
44	CHEK2	NM_007194.3(CHEK2):c.7C>T (p.Arg3Trp)	Lum-HER2	White	Deleterious	Possibly damaging		Maternal grandmother throat
57	MLH1	NM_000249.3(MLH1):c.955G>A (p.Glu319Lys)	TN	White	Deleterious	Probably damaging		Maternal grandmother breast (postmenopausal)
51	MLH1	NM_000249.3(MLH1):c.887T>C (p.Leu296Ser)	Unk	White	Deleterious	Probably damaging	√	3 maternal aunts (postmenopausal); maternal grandfather colon
99	MSH6	NM_000179.2(MSH6):c.2667G>T (p.Gln889His)	ER+HER2−	African American	Tolerated	Probably damaging		Paternal grandfather pancreatic
55	PALB2	NM_024675.3(PALB2):c.2674G>A (p.Glu892Lys)	ER+HER2−	White	Deleterious	Possibly damaging		Maternal grandmother bladder, paternal grandfather brain
23	PMS2	NM_000535.6(PMS2):c.857A>G (p.Asp286Gly)	Unk	White	Deleterious	Benign		Paternal grandmother breast (postmenopausal)
61	PMS2	NM_000535.6(PMS2):c.1004A>G (p.Asn335Ser)	Lum-HER2	White	Deleterious	Probably damaging		

Table 5 Comparison of clinical and pathological characteristics in YW with and without pathogenic mutations

	Pathogenic mutations ($n = 30$)	No detectable mutations ($n = 71$)	P value
Family history			0.002
Yes	0.50	0.50	
No	0.20	0.80	
Ethnicity			0.764
African American	0.33	0.67	
Asian	0.00	1.00	
Hispanic	0.00	1.00	
Other	0.00	1.00	
Non-Hispanic White	0.30	0.70	
Parity			0.139
Non-parous	0.54	0.46	
PABC	0.20	0.80	
Non-PABC	0.28	0.72	
Pregnancy post-diagnosis	0.00	1.00	
Tumor size			0.772
T1	0.28	0.72	
T2	0.30	0.70	
T3	0.33	0.67	
Tumor grade			0.867
Well (grade 1)	0.29	0.71	
Moderate (grade 2)	0.26	0.74	
Poor (grade 3)	0.32	0.68	
Tumor stage			0.355
I	0.28	0.72	
II	0.23	0.77	
III	0.45	0.55	
IV	0.40	0.60	
Lymph node status			0.816
Positive	0.28	0.72	
Negative	0.26	0.74	
ER/HER2 status			0.111
ER+/HER2−	0.24	0.76	
ER+/HER2+	0.27	0.73	
ER−/HER2+	0.00	1.00	
ER−/HER2−	0.45	0.55	
Status			0.399
Died of disease	0.43	0.57	
Alive with disease	0.50	0.50	
Alive, disease-free	0.27	0.73	

young women harboring mutations in hereditary colon cancer genes and 14% having VUS in cancer predisposition genes. While 43% of young women with family histories harbored pathogenic mutations, 15% of young women without a significant family history of cancer also harbored pathogenic mutations. The majority of mutations were detected in BRCA1 and BRCA2, associated with TNBC and ER+ tumors, respectively, and no other cancer predisposition genes accounted for a significant proportion of tumors in young women. Together, these data suggest that the majority of breast tumors in young women are not linked to germline mutations but may be attributable to non-genetic factors.

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Compliance with ethical standards

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

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