original reports

Evaluation of Germline Genetic Testing Criteria in a Hospital-Based Series of Women With Breast Cancer

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abstract

PURPOSE To determine the sensitivity and specificity of genetic testing criteria for the detection of germline pathogenic variants in women with breast cancer.

MATERIALS AND METHODS Women with breast cancer enrolled in a breast cancer registry at a tertiary cancer center between 2000 and 2016 were evaluated for germline pathogenic variants in 9 breast cancer predisposition genes (*ATM, BRCA1, BRCA2, CDH1, CHEK2, NF1, PALB2, PTEN*, and *TP53*). The performance of the National Comprehensive Cancer Network (NCCN) hereditary cancer testing criteria was evaluated relative to testing of all women as recommended by the American Society of Breast Surgeons.

RESULTS Of 3,907 women, 1,872 (47.9%) meeting NCCN criteria were more likely to carry a pathogenic variant in 9 predisposition genes compared with women not meeting criteria (9.0% *v* 3.5%; $P < .001$). Of those not meeting criteria (n = 2,035), 14 (0.7%) had pathogenic variants in *BRCA1* or *BRCA2*. The sensitivity of NCCN criteria was 70% for 9 predisposition genes and 87% for *BRCA1* and *BRCA2*, with a specificity of 53%. Expansion of the NCCN criteria to include all women diagnosed with breast cancer at ≤ 65 years of age achieved > 90% sensitivity for the 9 predisposition genes and > 98% sensitivity for *BRCA1* and *BRCA2*.

CONCLUSION A substantial proportion of women with breast cancer carrying germline pathogenic variants in predisposition genes do not qualify for testing by NCCN criteria. Expansion of NCCN criteria to include all women diagnosed at ≤ 65 years of age improves the sensitivity of the selection criteria without requiring testing of all women with breast cancer.

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ASSOCIATED CONTENT

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Appendix

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INTRODUCTION

The National Comprehensive Cancer Network (NCCN) hereditary cancer testing criteria is one of the current standards for identifying women at increased risk of hereditary breast, ovarian, or pancreatic cancer.[1] Although the criteria have expanded over time to be more inclusive, many women with breast cancer currently do not qualify for genetic testing according to NCCN criteria. Two recent studies noted that a significant proportion of carriers of germline pathogenic variants were not identified (up to 50%) if women underwent testing solely based on NCCN criteria (v3.2019).[2,3] On this basis, the American Society of Breast Surgeons (ASBrS) recommended that germline genetic testing should be made available to all women with a personal history of breast cancer.[4]

The implications of the ASBrS recommendations for women with breast cancer not selected for age at diagnosis or family history of cancer have not been adequately studied. Prior studies[2,3] were prone to significant ascertainment and selection biases in favor of women with high-risk breast cancer.[5] Patients included in prior studies may also have undergone genetic testing because of concern for inherited risk of cancer, despite not meeting NCCN testing criteria. More importantly, the majority of the genes included in the multigene panels in both studies have uncertain clinical relevance for breast cancer,[2,3,6,7] which makes it challenging to interpret the significance of the results. Furthermore, these studies and the ASBrS recommendations did not consider the increase in the number of tests needed to detect clinically actionable

germline pathogenic variants in all women with breast cancer. In a field with unmet needs for genetic counseling and management, understanding the impact of the ASBrS criteria on genetic testing services is critical for the responsible allocation of resources.[6]

The conflicting recommendations from NCCN and ASBrS have created a debate on whether all women with breast cancer need to undergo germline genetic testing.[8-13] To alleviate some of the confusion associated with these recommendations and to inform genetic testing practice, we evaluated the sensitivity and specificity of NCCN, ASBrS, and other genetic testing criteria for germline pathogenic variants in a large series of women with breast cancer from a breast cancer registry at a tertiary cancer center.

MATERIALS AND METHODS

Sample Selection

The study sample was derived from the Mayo Clinic Breast Cancer Study (MCBCS), a prospective registry offering participation to all women evaluated at Mayo Clinic Rochester for a diagnosis of first invasive breast cancer or ductal carcinoma in situ between May 15, 2000, and May 31, 2016. Of 7,300 women approached, 6,198 consented to the study and were asked to provide a blood sample and a baseline questionnaire on personal and family history adapted from the Breast Cancer Family Registry.[14] Only baseline questionnaire and tumor characteristics from the initial diagnosis were considered in the current study. Patient demographics, tumor characteristics, and family history were also abstracted from electronic medical records to verify existing information. Family history information was available from 4,516 women providing blood samples (Appendix Fig A1, online only). Genetic testing results were offered and/or disclosed to the study participants through pretest and post-test counseling procedures by certified genetic counselors. This study was approved by the Institutional Review Board at Mayo Clinic.

Germline Sequencing and Bioinformatics Analysis

Germline DNA extracted from peripheral blood mononuclear cells was analyzed for germline pathogenic variants in the coding regions and consensus splice sites of 37 genes (Appendix Table A1, online only) using a custom amplicon-based QIAseq panel (Qiagen, Hilden, Germany) and sequencing on a HiSeq4000 (Illumina, San Diego, CA) as described previously[15] and in the Appendix. Pathogenic and likely pathogenic variants were analyzed together as pathogenic variants. Low penetrance missense variants in *CHEK2* were excluded from analyses.

Selection of Genes Based on Clinical Actionability

The primary analysis was restricted to 9 established breast cancer predisposition genes (*ATM, BRCA1, BRCA2, CDH1, CHEK2, NF1, PALB2, PTEN,* and *TP53*)[16-18] with clear management recommendations in the NCCN guidelines.[1] Analyses were performed separately for pathogenic variants in 6 high-risk genes (*BRCA1, BRCA2, CDH1, PALB2, PTEN*, and *TP53*) or pathogenic variants in *BRCA1* or *BRCA2* only. Separate analysis including *BARD1*, *RAD51C*, and *RAD51D* was performed because pathogenic variants in these genes have been associated with triple-negative breast cancer.[1,17,19,20] These genes have recently been included in genetic testing recommendations (NCCN v1.2020),[1] although risk management guidelines are not available.

Assessment of NCCN Hereditary Cancer Testing Criteria

Women with a first- or second-degree relative with breast cancer were classified as having a family history of breast cancer. Similar definitions were used for other cancers. Of the NCCN hereditary cancer testing criteria relevant to women with a personal history of breast cancer (v1.2020),[1] 12 of 15 were fully evaluable (Appendix Table A2, online only). For the other criteria, women provided information on family history of prostate cancer rather than stage or Gleason score of prostate cancer in family members and on number of third-degree relatives with breast, ovarian, pancreatic, and prostate cancer rather than individual-level information. The influence of this information on guideline performance was assessed in a sensitivity analysis. Women meeting any of the evaluated criteria were considered qualified for genetic testing according to NCCN guidelines. Of those with *BRCA1* or *BRCA2* pathogenic variants who did not meet NCCN criteria, we used the Tyrer-Cuzick risk evaluation tool (v8.0b)[21,22] to assess whether these women had $> 5\%$ pretest probability of carrying *BRCA1* or *BRCA2* pathogenic variants.

Statistical Analysis

NCCN, ASBrS, and other age-of-diagnosis– and family-history–based criteria were evaluated for sensitivity and specificity of the testing criteria, the number of women tested, frequency of germline pathogenic variants in the criteria evaluated, and the variant of uncertain significance (VUS)-to-pathogenic-variant ratio (defined in the Appendix, online only). Fisher's exact test was performed to compare the frequency of pathogenic variants among women meeting and not meeting NCCN criteria, and results were reported as odds ratios with 95% CIs. All tests were 2 sided, and a P value $< .05$ was considered statistically significant. All analyses were performed using IBM SPSS Statistics (v25; SPSS, Chicago, IL).

RESULTS

Results of Germline Genetic Testing

A total of 3,907 women with a diagnosis of invasive breast cancer (84.0%) or ductal carcinoma in situ (16.0%) were included in the final analysis. The median age of breast cancer diagnosis was 57 years (range, 21-94 years). A family history of breast cancer was present in 46.7% of

TABLE 1. Patient and Tumor Characteristics

Characteristic	Total (N = 3,907)	Meeting NCCN Criteria (n = 1,872)	Not Meeting NCCN Criteria (n = 2,035)
Age at diagnosis of first breast cancer, years			
Median	57	48	63
≤ 45	747 (19.1)	747 (39.9)	0 (0.0)
46-50	538 (13.8)	385 (20.6)	153 (7.5)
51-55	530 (13.6)	175 (9.3)	355 (17.4)
56-60	491 (12.6)	174 (9.3)	317 (15.6)
61-65	511 (13.1)	127 (6.8)	384 (18.9)
66-70	477 (12.2)	129 (6.9)	348 (17.1)
71-75	327 (8.4)	80 (4.3)	247 (12.1)
≥ 75	286 (7.3)	55 (2.9)	231 (11.4)
Race/ethnicity			
White	3,719 (95.2)	1,730 (92.4)	1,989 (97.7)
Black	29 (0.7)	15 (0.8)	14 (0.7)
Other/unknown	159 (4.1)	127 (6.8)	32 (1.6)
Ashkenazi-Jewish ancestry	6 (0.2)	6 (0.3)	0 (0.0)
Histology			
Invasive	3,282 (84.0)	1,565 (83.6)	1,717 (84.4)
In situ	625 (16.0)	307 (16.4)	318 (15.6)
Estrogen receptor status			
Positive	3,300 (84.5)	1,476 (78.9)	1,824 (89.6)
Negative	554 (14.2)	358 (19.1)	196 (9.6)
Unknown	53 (1.3)	38 (2.0)	15 (0.8)
Progesterone receptor status			
Positive	2,928 (74.9)	1,336 (71.4)	1,592 (78.2)
Negative	920 (23.5)	494 (26.4)	426 (20.9)
Unknown	59 (1.5)	42 (2.2)	17 (0.9)
HER-2 receptor status[a]			
Positive	378 (11.5)	220 (14.1)	158 (9.2)
Negative	2,390 (72.8)	1,143 (73.0)	1,247 (72.6)
Borderline	12 (0.4)	4 (0.3)	8 (0.5)
Unknown	502 (15.3)	198 (12.6)	304 (17.7)
Personal history of other cancers			
Any cancer	492 (12.6)	226 (12.1)	266 (13.1)
Ovarian	38 (1.0)	38 (2.0)	0 (0.0)
Pancreatic	10 (0.3)	10 (0.5)	0 (0.0)
Family history of cancer[b]			
Breast	1,823 (46.7)	1,059 (56.6)	764 (37.5)
Ovarian	286 (7.3)	286 (15.3)	0 (0.0)
Pancreatic	303 (7.8)	303 (16.2)	0 (0.0)
Prostate	852 (21.8)	432 (23.1)	420 (20.6)

NOTE. Data are No. (%).

Abbreviations: HER2, human epidermal growth factor receptor 2; NCCN, National Comprehensive Cancer Network.

[a]Among patients with invasive breast cancer (n = 3,282).

[b]First- or second-degree relatives.

women (Table 1). The majority (40.5%) had only 1 relative with breast cancer. Among the 3,907 women, 241 (6.2%) had germline pathogenic variants in the 9 established predisposition genes. Pathogenic variants in *CHEK2* (1.7%), *BRCA2* (1.4%), *BRCA1* (1.3%), and *ATM* (1.1%) were the most frequent. The c.1100delC allele accounted for 52 of 67 *CHEK2* pathogenic variants (Appendix Table A3, online only). Among women with 2 or more relatives with breast cancer, the frequency of germline pathogenic variants was > 5% for *BRCA1* or *BRCA2* and > 10% for 9 predisposition genes (Appendix Fig A2, online only). A total of 449 (11.5%) women had a VUS in any of the 9 genes (Appendix Fig A3, online only).

Comparison of NCCN and ASBrS Criteria

Of the 3,907 women, 1,872 (47.9%) met NCCN testing criteria, whereas 2,035 (52.1%) did not. The characteristics of women in these categories are shown in Table 1. Testing of all women as recommended by ASBrS identified pathogenic variants in 9 actionable predisposition genes in 6.2% of women, in 6 high-risk genes in 3.4% of women, and in *BRCA1* or *BRCA2* in 2.7% of women (Table 2). Those meeting NCCN criteria were more likely to carry a pathogenic variant in the 9 genes than women not meeting criteria (9.0% *v* 3.5%; $P < .001$). Similar results were observed for the 6 high-risk genes (5.7% *v* 1.4%; $P < .001$) and for *BRCA1* or *BRCA2* (5.0% *v* 0.7%; $P < .001$). However, 72 (29.9%) of the 241 women with pathogenic variants in the 9 genes, 28 (20.9%) of 134 with pathogenic variants in the 6 high-risk genes, and 14 (13.1%) of 107 with pathogenic variants in *BRCA1* or *BRCA2* did not qualify for genetic testing based on NCCN criteria (Table 2; Fig 1). The NCCN v1.2020 testing guidelines differ from the v3.2019 guidelines by recommending testing of those with > 5% pretest probability of a *BRCA1* or *BRCA2* pathogenic variant. None of the 14 women with *BRCA1* or *BRCA2* pathogenic variants who did not qualify for testing under previous NCCN v3.2019 criteria had > 5% pretest probability (all14 women had < 2.5% probability) of a *BRCA1/2* pathogenic variant, as measured using the Tyrer-Cuzick model. Thus, this additional criterion did not improve the sensitivity of the testing guidelines in this study. Additional analyses considering all women with a family history of prostate cancer (Appendix Table A4, online only) or all women with third-degree relatives with breast, ovarian, pancreatic or prostate cancer (Appendix Table A5, online only) did not substantially alter the 70.1% sensitivity from the primary analysis. The specificity of NCCN criteria was approximately 53% for pathogenic variants in 9 predisposition genes, 6 high-risk genes, and *BRCA1* or *BRCA2* (Table 3). The VUS rate for the 9 genes was only marginally higher in women meeting NCCN criteria compared with women not meeting criteria (12.7% *v* 10.4%; $P = .02$). However, this resulted in a higher VUS-to-pathogenic variant ratio for women not meeting NCCN criteria (2.9 vs. 1.4). Interestingly, only 2 of 6 carriers of *TP53* pathogenic

TABLE 2. Comparison of Frequencies of Germline Pathogenic Variants Between Patients Meeting and Not Meeting NCCN Criteria for Genetic Testing

Gene	Total Meeting ASBrS Recommendations N = 3,907 No. (%)	Meeting NCCN Guidelines n = 1,872 No. (%)	Not Meeting NCCN Guidelines n = 2,035 No. (%)	Odds Ratio (95% CI) NCCN *v* Non-NCCN[a]	*P* for NCCN *v* Non-NCCN[a]	Pathogenic Variant Carriers Missed by NCCN Criteria[b] (%)
BRCA1/2	107 (2.7)	93 (5.0)	14 (0.7)	7.5 (4.2 to 13.5)	$< 2.2 \times 10^{-16}$	13.1
6 high-risk genes[c]	134 (3.4)	106 (5.7)	28 (1.4)	4.3 (2.8 to 6.6)	8.34×10^{-14}	20.9
9 breast cancer genes[d]	241 (6.2)	169 (9.0)	72 (3.5)	2.7 (2.0 to 3.6)	7.89×10^{-13}	29.9
ATM	43 (1.1)	28 (1.5)	15 (0.7)			34.9
BRCA1	51 (1.3)	46 (2.5)	5 (0.2)			9.8
BRCA2	56 (1.4)	47 (2.5)	9 (0.4)			16.1
CDH1	6 (0.2)	2 (0.1)	4 (0.2)			66.7
CHEK2	67 (1.7)	39 (2.1)	28 (1.4)			41.8
NF1	1 (0.0)	0 (0.0)	1 (0.0)			100.0
PALB2	15 (0.4)	7 (0.4)	8 (0.4)			53.3
PTEN	1 (0.0)	1 (0.1)	0 (0.0)			0.0
TP53	6 (0.2)	3 (0.2)	3 (0.1)			50.0

Abbreviations: ASBrS, American Society of Breast Surgeons; NCCN, National Comprehensive Cancer Network.

[a]Odds ratios, 95% CIs, and *P* value for enrichment of germline pathogenic variants between patients meeting and not meeting NCCN guidelines.

[b]Denominators for percentages are the total pathogenic variant carriers in respective categories.

[c]*BRCA1, BRCA2, CDH1, PALB2, PTEN*, and *TP53*.

[d]*ATM, BRCA1, BRCA2, CDH1, CHEK2, NF1, PALB2, PTEN*, and *TP53*.

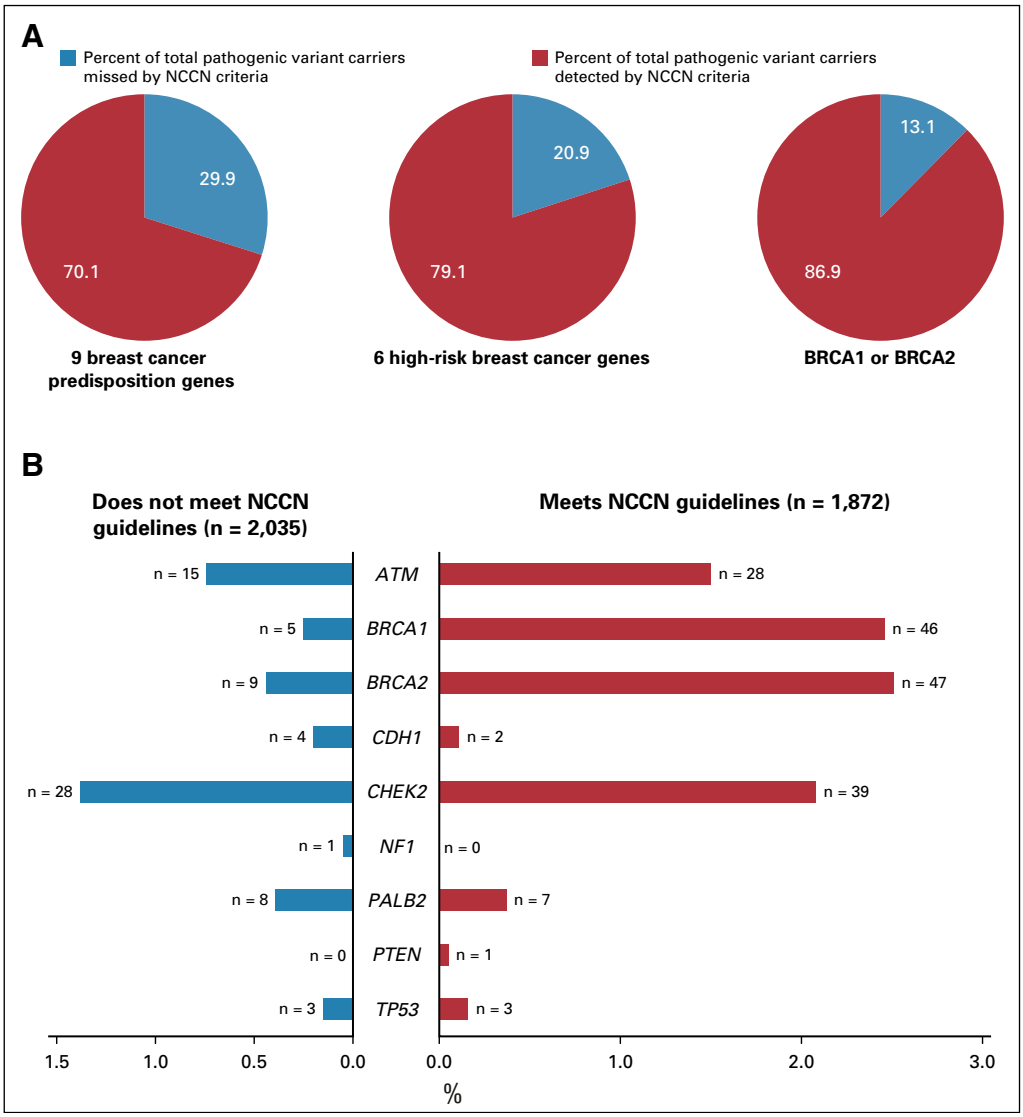

FIG 1. Evaluation of sensitivity of National Comprehensive Cancer Network (NCCN) criteria for pathogenic variant carriers in 3,907 women with breast cancer. (A) Percent of total pathogenic variant carriers missed by NCCN criteria in 9 breast cancer predisposition genes, 6 high-risk predisposition genes, and *BRCA1* or *BRCA2* (9 genes: *ATM, BRCA1, BRCA2, CDH1, CHEK2, NF1, PALB2, PTEN,* and *TP53*; 6 genes: *BRCA1, BRCA2, CDH1, PALB2, PTEN,* and *TP53*). (B) Comparison of germline pathogenic variant frequencies between women meeting and not meeting NCCN guidelines.

variants and 1 of 6 *CDH1* carriers met testing criteria for Li-Fraumeni syndrome[23] or Hereditary Diffuse Gastric Cancer criteria,[24] respectively.

Alternative Selection Criteria for Genetic Testing

Next, we explored the potential impact of combining additional age at diagnosis of breast cancer and family history of breast cancer criteria with the NCCN criteria on the sensitivity of the selection guidelines (Table 3). Expansion of NCCN criteria to include all women diagnosed with breast cancer at ≤ 65 years of age increased the sensitivity for pathogenic variants in *BRCA1* or *BRCA2* to > 98%. These criteria required testing of an additional 31% of women, leaving 21% untested, and yielding a specificity of 22%. Similar results were seen when evaluating 12 predisposition genes (Appendix Table A6, online only). Importantly, v1.2020 guidelines recommend excluding women with a breast cancer diagnosis at > 65 years of age and no family history of cancer from testing. Among 511 women in this category in this study, germline pathogenic variants were detected in only 0.2% in *BRCA1* or *BRCA2*, 0.6% in high-risk genes, and 1.4% in the 9 predisposition genes (Appendix Table A7, online only). Separately, expansion of NCCN criteria to include all women with a family history of breast cancer increased the sensitivity to 84% for pathogenic variants in the 9 predisposition genes, 91% for the 6 high-risk genes, and 94% for *BRCA1* or *BRCA2* (Appendix Table A8, online only). When combining both breast cancer family history and age at diagnosis of breast cancer with NCCN criteria, > 90% sensitivity for the 9 predisposition genes was observed for women diagnosed at ≤ 55 years of age. However, several carriers of pathogenic variants < 65 years of age did not qualify for testing (Appendix Table A8, online only). Appendix Table A9 (online only) shows a comparison of patient and tumor characteristics between women in the MCBCS and SEER Iowa Registry.

TABLE 3. Evaluation of Candidate Thresholds for Selection of Patients for Genetic Testing

Testing Criteria	Tested (%)[a]	Additional Tested (%)[b]	Not Tested (%)[c]	No. of Carriers Detected (%)	No. Not Identified (%)	Sensitivity (95% CI)[d]	Specificity (95% CI)[d]	VUS to Pathogenic Variant Ratio
9 predisposition genes[e]								
ASBrS criteria	3,907 (100)	2,035 (52.1)	0 (0)	241 (6.2)	0 (0.0)	100	0	1.9
NCCN criteria	1,872 (47.9)	0 (0)	2,035 (52.1)	169 (9.0)	72 (29.9)	70.1 (64.0 to 75.5)	53.5 (51.9 to 55.2)	1.4
NCCN criteria or age at diagnosis, years								
≤ 50	2,025 (51.8)	153 (3.9)	1,882 (48.2)	174 (8.6)	67 (27.8)	72.2 (66.2 to 77.5)	49.5 (47.9 to 51.1)	1.4
≤ 55	2,380 (60.9)	508 (13.0)	1,527 (39.1)	200 (8.4)	41 (17.0)	83.0 (77.7 to 87.2)	40.5 (38.9 to 42.1)	1.5
≤ 60	2,697 (69.0)	825 (21.1)	1,210 (31.0)	209 (7.7)	32 (13.3)	86.7 (81.8 to 90.4)	32.1 (30.6 to 33.7)	1.5
≤ 65	3,081 (78.9)	1,209 (30.9)	826 (21.1)	222 (7.2)	19 (7.9)	92.1 (88.0 to 94.9)	22.0 (20.7 to 23.4)	1.6
≤ 70	3,429 (87.8)	1,557 (39.9)	478 (12.2)	231 (6.7)	10 (4.1)	95.9 (92.5 to 97.7)	12.8 (11.7 to 13.9)	1.7
≤ 75	3,676 (94.1)	1,804 (46.2)	231 (5.9)	235 (6.4)	6 (2.5)	97.5 (94.7 to 98.8)	6.1 (5.4 to 7.0)	1.8
6-high-risk genes[f]								
ASBrS criteria	3,907 (100)	2,035 (52.1)	0 (0)	134 (3.4)	0 (0.0)	100	0	1.7
NCCN criteria	1,872 (47.9)	0 (0)	2,035 (52.1)	106 (5.7)	28 (20.9)	79.1 (71.4 to 85.1)	53.2 (51.6 to 54.8)	1.1
NCCN criteria or age at diagnosis, years								
≤ 50	2,025 (51.8)	153 (3.9)	1,882 (48.2)	107 (5.3)	27 (20.1)	79.9 (72.3 to 85.8)	49.2 (47.6 to 50.8)	1.2
≤ 55	2,380 (60.9)	508 (13.0)	1,527 (39.1)	119 (5.0)	15 (11.2)	88.8 (82.4 to 93.1)	40.1 (38.5 to 41.6)	1.3
≤ 60	2,697 (69.0)	825 (21.1)	1,210 (31.0)	122 (4.5)	12 (9.0)	91.0 (85.0 to 94.8)	31.8 (30.3 to 33.2)	1.4
≤ 65	3,081 (78.9)	1,209 (30.9)	826 (21.1)	127 (4.1)	7 (5.2)	94.8 (89.6 to 97.4)	21.7 (20.4 to 23.1)	1.4
≤ 70	3,429 (87.8)	1,557 (39.9)	478 (12.2)	129 (3.8)	5 (3.7)	96.3 (91.6 to 98.4)	12.5 (11.5 to 13.6)	1.6
≤ 75	3,676 (94.1)	1,804 (46.2)	231 (5.9)	130 (3.5)	4 (3.0)	97.0 (92.6 to 98.8)	6.0 (5.3 to 6.8)	1.6
BRCA1 or BRCA2								
ASBrS criteria	3,907 (100)	2,035 (52.1)	0 (0)	107 (2.7)	0 (0.0)	100	0	1.1
NCCN criteria	1,872 (47.9)	0 (0)	2,035 (52.1)	93 (5.0)	14 (13.1)	86.9 (79.2 to 92.0)	53.2 (51.6 to 54.8)	0.8
NCCN criteria or age at diagnosis, years								
≤ 50	2,025 (51.8)	153 (3.9)	1,882 (48.2)	94 (4.6)	13 (12.1)	87.9 (80.3 to 92.8)	49.2 (47.6 to 50.8)	0.8
≤ 55	2,380 (60.9)	508 (13.0)	1,527 (39.1)	101 (4.2)	6 (5.6)	94.4 (88.3 to 97.4)	40.0 (38.5 to 41.6)	0.9
≤ 60	2,697 (69.0)	825 (21.1)	1,210 (31.0)	102 (3.8)	5 (4.7)	95.3 (89.5 to 98.0)	31.7 (30.2 to 33.2)	1.0
≤ 65	3,081 (78.9)	1,209 (30.9)	826 (21.1)	105 (3.4)	2 (1.9)	98.1 (93.4 to 99.5)	21.7 (20.4 to 23.0)	1.0
≤ 70	3,429 (87.8)	1,557 (39.9)	478 (12.2)	105 (3.1)	2 (1.9)	98.1 (93.4 to 99.5)	12.5 (11.5 to 13.6)	1.1
≤ 75	3,676 (94.1)	1,804 (46.2)	231 (5.9)	106 (2.9)	1 (0.9)	99.1 (94.9 to 99.8)	6.1 (5.3 to 6.8)	1.1

Abbreviations: ASBrS, American Society of Breast Surgeons; NCCN, National Comprehensive Cancer Network; VUS, variant of uncertain significance.

[a]Total number of patients tested in each evaluated criterion.

[b]Additional number of patients tested compared with NCCN criteria.

[c]Number of patients in the cohort who would not undergo genetic testing based on the evaluated criteria.

[d]Sensitivity and specificity of testing criteria in percentages.

[e]*ATM, BRCA1, BRCA2, CDH1, CHEK2, NF1, PALB2, PTEN,* and *TP53.*

[f]*BRCA1, BRCA2, CDH1, PALB2, PTEN*, and *TP53.*

DISCUSSION

In a series of women with breast cancer recruited in a tertiary care center, the performance of NCCN hereditary cancer testing criteria was compared with ASBrS recommendations. The detection of pathogenic variants in 9 established predisposition genes in 9.0% of women meeting NCCN criteria was consistent with results from clinically tested cohorts.[17,18,25] However, in contrast to prior studies,[2,3,26] women meeting NCCN criteria had a higher frequency of germline pathogenic variants than women not meeting criteria (9.0% *v* 3.5%). Despite this, approximately 30% of women with pathogenic variants in the 9 predisposition genes and 13% with pathogenic variants in *BRCA1* or *BRCA2* did not qualify for testing by NCCN criteria. Thus, this study confirms that NCCN criteria are not optimal for selection of women with breast cancer for breast cancer predisposition gene testing.

Differences in results between studies may be explained by the study participants and the genes evaluated. Prior studies included women who may have undergone genetic testing despite not meeting NCCN criteria and also included several genes, such as *MUTYH*, that are not associated with increased breast cancer risk. In addition, some studies included women referred for testing over several years without taking updates in the NCCN criteria into account. The evaluation of the most recent version of the NCCN criteria (v1.2020) for pathogenic variants in established breast cancer genes using a uniform set of variables is a significant strength of this study. In addition, to fully evaluate the clinical utility of testing criteria, the sensitivity for germline pathogenic variants in *BRCA1* or *BRCA2* only, 6 high-risk genes, and 9 actionable predisposition genes were considered. Identification of a pathogenic variant in *BRCA1* or *BRCA2* can lead to significant changes in patient management through additional mammographic and magnetic resonance imaging screening, risk-reducing prophylactic surgeries,[27-29] and systemic treatment,[30-32] which may lead to improved survival. However, changes in management are limited to additional cancer screening for the moderate-risk predisposition genes.[1]

Although ASBrS criteria detect a substantially larger number of germline pathogenic variants than the NCCN criteria, there are challenges associated with testing all women with breast cancer.[6] First, the substantially higher number of women tested, estimated at another 52% in this study, will lead to increased costs. Second, the added volume may exacerbate current unmet needs for genetic services and counseling.[33,34] Third, more VUS will be detected, which may lead to anxiety[35] and unwarranted interventions.[36] Fourth, the clinical utility of testing women diagnosed with breast cancer > 65 years of age is not fully understood. Similar concerns about testing everyone with a breast cancer diagnosis have been raised in several commentaries,[6,12,37] including a recent position statement by the American College of Medical Genetics and Genomics.[5]

As an alternative to adopting testing of all women, this study demonstrates that expanding the current NCCN criteria to include all women diagnosed with breast cancer at age ≤ 65 years has the potential to achieve > 90% sensitivity for 9 predisposition genes and 6 high-risk genes, and > 98% sensitivity for *BRCA1* or *BRCA2*. These criteria reduced the proportion of women tested by 21% and decreased the VUS-to-pathogenic variant ratio compared with the ASBrS recommendations, which may translate into cost savings and lesser burden on genetic services. Importantly, this approach captured all young pathogenic variant carriers, and only older women with a low likelihood of pathogenic variants did not qualify for testing.[38] The recently updated NCCN criteria (v1.2020) recommend against genetic testing in women > 65 years of age with no family history of cancer.[1] This study found frequencies of germline pathogenic variants in the *BRCA1* or *BRCA2* and 6- or 9-gene categories of < 1.5% for women > 65 years of age without a family history and supports these recommendations.

Recently, the US Preventive Services Task Force (USPSTF) also recommended that asymptomatic women with a personal history of breast cancer should be screened by primary care clinicians for a referral to genetic counseling services.[39] Although we acknowledge the significance of the USPSTF recommendations in cancer-free women in the primary care setting, the sensitivity of the risk assessment tools recommended by USPSTF in women with breast cancer is not clearly defined. In addition, several of these tools do not take a personal history of breast cancer into account. This may result in undertesting and failure to detect those with pathogenic variants among women with breast cancer.[40-42] Probability models have also been added to the v1.2020 NCCN guidelines. Women who do not meet NCCN criteria but have a probability of > 5% of a *BRCA1* or *BRCA2* pathogenic variant based on prior-probability models now qualify for genetic testing, and those with 2.5% probability can be considered for testing. However, in this study, neither the 5% or 2.5% probability thresholds based on the Tyrer-Cuzick model changed the sensitivity of testing criteria for *BRCA1* or *BRCA2* in women with breast cancer. Thus, the utility of the probability models for selecting more women with a family history of cancer for testing is unclear. Additional studies will be needed to address this question.

The study sample was representative of women with breast cancer evaluated at a tertiary cancer center but was enriched for women diagnosed at < 46 years of age compared with patients with breast cancer reported in the SEER Iowa Registry[43] (Appendix Table A9, online only). However, the proportion of women diagnosed between the ages of 50 and 75 years, the racial composition, and the distribution of clinical tumor subtype defined by hormone

receptor status were similar to patients with breast cancer in the SEER Iowa Registry (Appendix Table A9). In addition, the proportion of women with a family history of breast cancer was similar to other studies of unselected women with breast cancer from tertiary medical centers[44,45] and a population-based study of women with breast cancer.[46] Additional studies will be needed to determine whether these findings can be applied to patients with breast cancer in the general population. Meanwhile, this study of unselected patients with breast cancer from a tertiary cancer center provides much-needed information on sensitivity and specificity of testing criteria, which will help guide personalized decision making on genetic testing.

This study has several limitations. The cost effectiveness of testing criteria was not evaluated. Although few models have suggested cost effectiveness of population-based testing,[47-49] these models have not been validated in clinical practice.[50] Another limitation of the study is that specific criteria within the NCCN guidelines were not evaluated, allowing for the potential erroneous classification of some of the women. However, sensitivity analyses evaluating the impact of the missing information yielded results similar to the primary analyses. Finally, the study sample was predominantly white, which limits the application of the findings to a racially diverse population.

Among women with breast cancer, those meeting NCCN criteria were more likely to carry a germline pathogenic variant. However, a substantial proportion of carriers do not qualify for testing by NCCN criteria. Although ASBrS recommendations are more sensitive than NCCN criteria, a substantially larger proportion of women with breast cancer must undergo testing. In a large unselected series of women with breast cancer, we demonstrate that expanding the NCCN testing criteria to include all women diagnosed with breast cancer at or before the age of 65 years has the potential to improve the sensitivity of germline genetic testing without the need for evaluation of all women with breast cancer.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Evaluation of Germline Genetic Testing Criteria in a Hospital-Based Series of Women With Breast Cancer

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APPENDIX

Information

QIAsEquation 37 gene custom amplicon panel. Germline DNA samples were analyzed for pathogenic variants in cancer predisposition genes using a custom QIAseq 37-gene amplicon panel Table A1. Genomic DNA samples were subjected to multiplex amplicon-based analysis of 746 target regions covering all coding regions and consensus splice sites from 37 cancer predisposition genes, including the 12 established breast cancer predisposition genes, including *BRCA1* and *BRCA2* (*BRCA1, BRCA2, ATM, BARD1, CDH1, CHEK2, NF1, PALB2, PTEN, RAD51C, RAD51D, TP53*). The QIAseq protocol has been optimized for high-throughput robotic processing of DNA samples (Hu C, et al: JAMA 319:2401-2409, 2018). Libraries were individually bar coded by dual indexing and sequenced in pools of 768 on a HiSeq4000. The median sequence read depth per nucleotide was 200×, with 99.7% of target regions yielding > 20× reads in all samples.

Three validation studies were conducted to assess the accuracy of the QIAseq custom panel. The first blinded study of 48 samples containing known pathogenic variants in the predisposition genes identified all 48 pathogenic variants, including 2 *BRCA1* large genomic rearrangements for the sensitivity of 100%. No false-positive variants were identified for a specificity of 100% (Hu C, et al: JAMA 319:2401-2409, 2018). The second study evaluated 48 samples from patients with pancreatic cancer selected based on results from prior genetic testing. All 32 pathogenic variants, including 2 large genomic rearrangements, were identified in a blinded analysis. The sensitivity and specificity for this study were 100% (Hu C, et al: JAMA 319:2401-2409, 2018). The third study involved QIAseq analysis of 96 samples from patients with breast cancer in a Mayo Clinic breast cancer registry that had received clinical genetic testing. All 100 pathogenic variants, including 8 large genomic rearrangements, were identified in a blinded analysis for 100% sensitivity and specificity. Overall, the QIAseq assay has greater than 99% analytical sensitivity and specificity for single nucleotide variants, insertions and deletions < 15 bp in length, and exon-level deletions and duplications.

Methods

Germline sequencing and bioinformatics analysis. Pooled sample libraries from 768 samples were subjected to paired-end 150 bp sequencing in each lane of a HiSeq4000. The median nucleotide coverage was 200×. Reads were trimmed with Cutadapt v1.10 (Martin M, https://doi.org/10.14806/ej.17.1.200) and aligned with bwa-mem (Li H, https://arxiv.org/abs/1303.3997). Sequence realignment, recalibration, haplotype calling, and depth of coverage were conducted using Genome Analysis Toolkit v3.4-46 (DePristo MA, et al: Nat Genet 43:491-498, 2011). Copy number variation (CNV) was detected with Pattern CNV v1.1.3 (Wang C, et al: Bioinformatics 30: 2678-2680, 2014). Annotation of variants was provided through the BioR toolkit (Kocher JP, et al: Bioinformatics 30:1920-1922, 2014) leveraging dbNSFP v3.0 (Liu X, et al: Hum Mutat 37:235-241, 2016), ClinVar (Landrum MJ, et al: Res 44:D862-D868, 2016), and CAVA (Münz M, et al: Genome Med 7:76, 2015). Population frequencies of pathogenic variants were derived from Genome Aggregation Database (gnomAD: http://gnomad.broadinstitute.org) and Exome Aggregation Consortium non-TCGA controls. Pathogenic variants were viewed with VCF-Miner (Hart SN, et al: Brief Bioinform 17:346-351, 2016). A 5-tier system was used to classify pathogenic variants based on the American College of Medical Genetics and the Association for Molecular Pathology guidelines (Richards S, et al: Genet Med 17:405-424, 2015).

Definition of terms. Sensitivity was defined as the ability of the testing criteria to correctly designate women with germline pathogenic variants as qualifying for genetic testing. Sensitivity of different testing criteria was separately evaluated for pathogenic variants in *BRCA1* or *BRCA2*, 6 high-risk genes, and 9 breast cancer predisposition genes. It was estimated as follows:

$$\text{Sensitivity of testing criteria} = \frac{\text{Number of pathogenic variant carriers designatedas qualifying for genetic testing}}{\text{Total number of pathogenic variant carriers in the study sample}}.$$

Specificity was defined as the ability of the testing criteria to correctly reject women without a germline pathogenic variant as not qualifying for genetic testing. Specificity of different testing criteria was separately evaluated for pathogenic variants in BRCA1 or BRCA2, 6 high-risk genes, and 9 breast cancer predisposition genes. It was estimated as follows:

$$\text{Specificity of testing criteria} = \frac{\text{Total number of women without germline pathogenic variant designated as not qualifying for genetic testing}}{\text{Total number of women without germline pathogenic variant in the study sample}}.$$

The variant of uncertain significance (VUS)-to-pathogenic variant ratio was defined as the ratio of the number of women with VUS results to the number of women with germline pathogenic variants identified by the testing criteria. This was estimated separately for *BRCA1* or *BRCA2*, 6 high-risk genes, and 12 breast cancer predisposition genes.

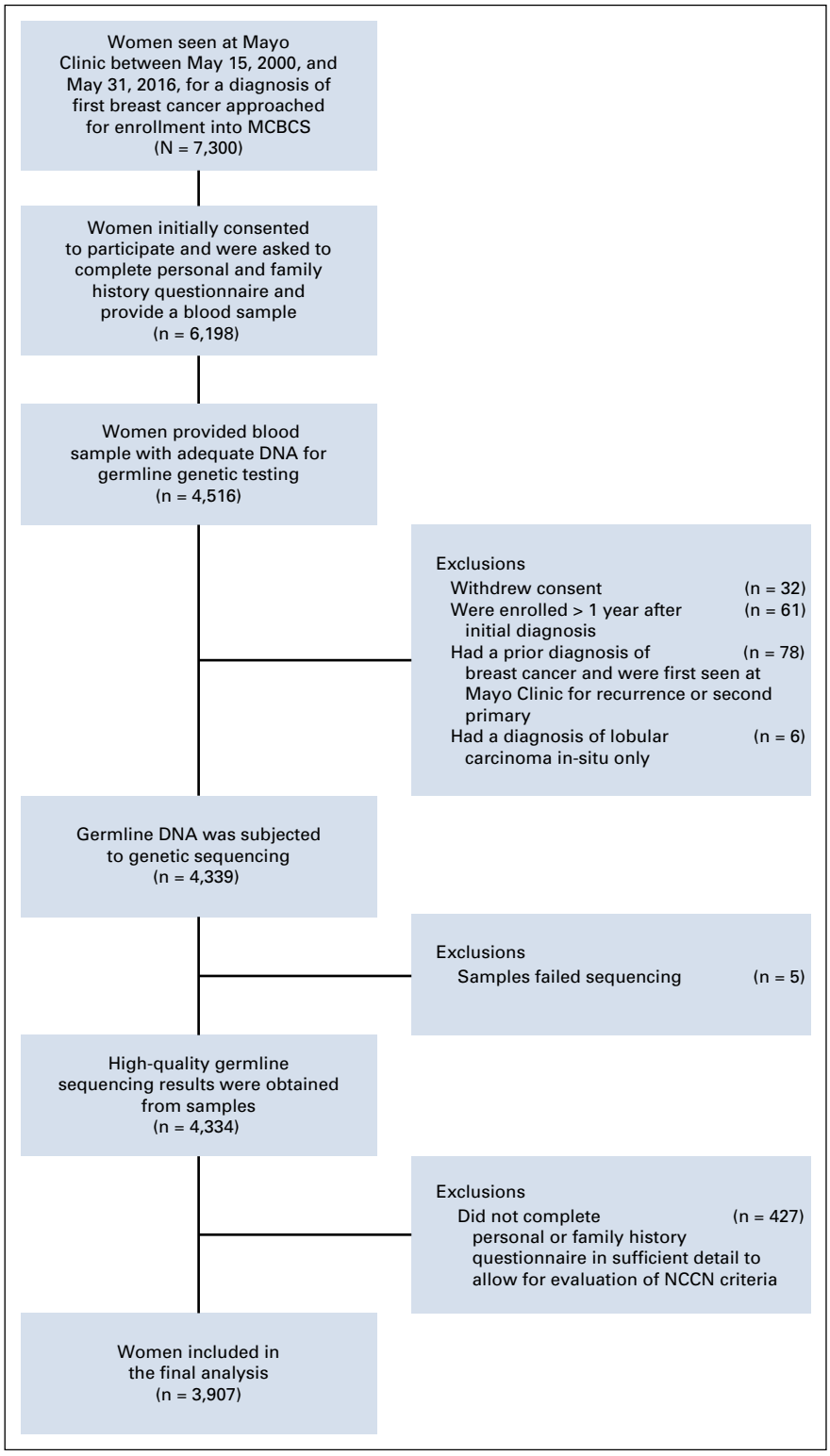

FIG A1. Sample selection. MCBCS, Mayo Clinic Breast Cancer Study; NCCN, National Comprehensive Cancer Network.

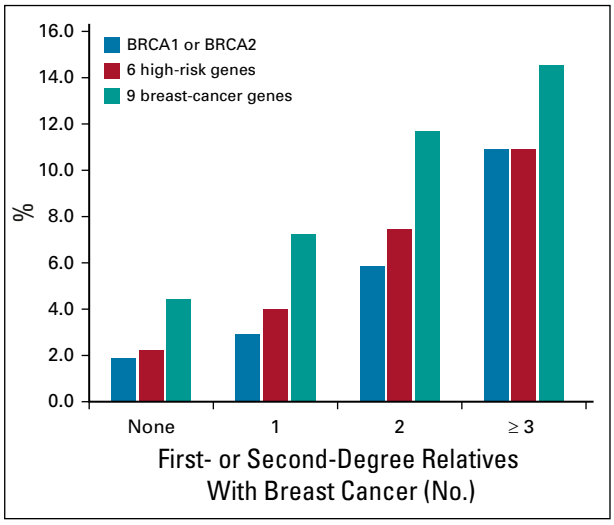

FIG A2. Frequency of germline pathogenic variants by family history of breast cancer according to the number of first- or second-degree relatives with breast cancer.

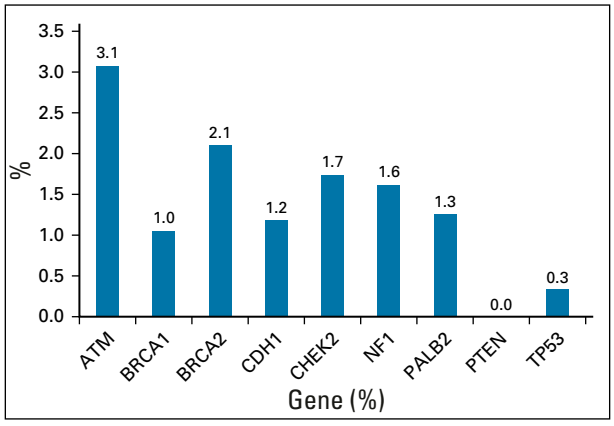

FIG A3. Frequencies of variants of uncertain significance by gene.

TABLE A1. List of Genes Included in the QIAseq Panel

HGNC Symbol	Reference Sequence	Ensemble Transcript ID	Chromosome	Start Position	End Position	Strand	Ensemble Gene ID
APC	NM000038.5	ENST00000257430	5	112043195	112181936	1	ENSG00000134982
ATM	NM000051.3	ENST00000278616	11	108093211	108239829	1	ENSG00000149311
BARD1	NM000465.3	ENST00000260947	2	215590370	215674428	−1	ENSG00000138376
BLM	NM000057.3	ENST00000355112	15	91260558	91358859	1	ENSG00000197299
BRCA1	NM007294.3	ENST00000357654	17	41196312	41277500	−1	ENSG00000012048
BRCA2	NM000059.3	ENST00000544455	13	32889611	32973805	1	ENSG00000139618
BRIP1	NM032043.2	ENST00000259008	17	59758627	59940882	−1	ENSG00000136492
CDH1	NM004360.4	ENST00000261769	16	68771128	68869451	1	ENSG00000039068
CDKN2A	NM000077.4	ENST00000304494	9	21967751	21995300	−1	ENSG00000147889
CHEK2	NM007194.3	ENST00000328354	22	29083731	29138410	−1	ENSG00000183765
EPCAM	NM002354.2	ENST00000263735	2	47572297	47614740	1	ENSG00000119888
ERCC2	NM000400.3	ENST00000391945	19	45853095	45874176	−1	ENSG00000104884
ERCC3	NM000122.1	ENST00000285398	2	128014866	128051752	−1	ENSG00000163161
FANCC	NM000136.2	ENST00000289081	9	97861336	98079991	−1	ENSG00000158169
FANCM	NM020937.3	ENST00000267430	14	45605143	45670093	1	ENSG00000187790
KRAS	NM004985.4	ENST00000311936	12	25357723	25403870	−1	ENSG00000133703
MEN1	NM130799.2	ENST00000312049	11	64570982	64578766	−1	ENSG00000133895
MLH1	NM000249.3	ENST00000231790	3	37034823	37107380	1	ENSG00000076242
MRE11A	NM005591.3	ENST00000323929	11	94152895	94227074	−1	ENSG00000020922
MSH2	NM000251.2	ENST00000233146	2	47630108	47789450	1	ENSG00000095002
MSH6	NM000179.2	ENST00000234420	2	47922669	48037240	1	ENSG00000116062
MUTYH	NM001128425.1	ENST00000450313	1	45794835	45806142	−1	ENSG00000132781
NBN	NM002485.4	ENST00000265433	8	90945564	91015456	−1	ENSG00000104320
NF1	NM001042492.2	ENST00000358273	17	29421945	29709134	1	ENSG00000196712
PALB2	NM024675.3	ENST00000261584	16	23614488	23652631	−1	ENSG00000083093
PMS2	NM000535.6	ENST00000265849	7	6012870	6048756	−1	ENSG00000122512
PPM1D	NM003620.3	ENST00000305921	17	58677544	58741849	1	ENSG00000170836
PRSS1	NM002769	ENST00000311737	7	142457319	142460923	1	ENSG00000204983
PTEN	NM000314.6	ENST00000371953	10	89622870	89731687	1	ENSG00000171862
RAD50	NM005732.3	ENST00000378823	5	131891711	131980313	1	ENSG00000113522
RAD51C	NM058216.2	ENST00000337432	17	56769934	56811703	1	ENSG00000108384
RAD51D	NM001142571	ENST00000345365	17	33426811	33448541	−1	ENSG00000185379
RECQL	NM002907	ENST00000444129	12	21621845	21654603	−1	ENSG00000004700
RINT1	NM021930.4	ENST00000257700	7	105172532	105208124	1	ENSG00000135249
SLX4	NM032444.2	ENST00000294008	16	3631182	3661599	−1	ENSG00000188827
TP53	NM000546.5	ENST00000269305	17	7565097	7590856	−1	ENSG00000141510
XRCC2	NM005431.1	ENST00000359321	7	152341864	152373250	−1	ENSG00000196584

Abbreviation: HGNC, HUGO Gene Nomenclature Committee.

TABLE A2. Subcategories of *BRCA1/2* Testing Criteria Evaluated in This Study Along With the Number of Patients in the Study Who Met the Criteria

Criterion	No. (%) N = 3,907
Age at diagnosis of breast cancer ≤ 45 years	747 (19.1)
Breast cancer between 46-50 years and an additional breast cancer	126 (3.2)
Breast cancer between 46-50 years and a close relative with breast cancer at any age on the same side	278 (7.1)
Triple-negative breast cancer at age < 60 years	192 (4.9)
≥ 1 first-, second-, or third-degree relative with breast cancer at age ≤ 50 years on the same side of the family	470 (12.0)
Family history of ovarian cancer	286 (7.3)
Family history of male breast cancer	31 (0.8)
Family history of pancreatic cancer	303 (7.8)
≥ 2 additional diagnoses of breast cancer in close relatives	243 (6.2)
Ashkenazi-Jewish ancestry	6 (0.2)
Personal history of ovarian cancer	38 (1.0)
Personal history of pancreatic cancer	10 (0.3)

TABLE A3. List of Pathogenic and Likely Pathogenic Variants in 9 Breast Cancer-Predisposition genes

Gene	Pathogenic Variants	Frequency
ATM	c.1339C>T_p.Arg447X	1
ATM	c.1960C>T_p.Gln654X	1
ATM	c.2251-10T>G	1
ATM	c.2849T>G_p.Leu950Arg	1
ATM	c.3085dupA	1
ATM	c.3154-2A>G	1
ATM	c.3245_3247delinsTGAT	2
ATM	c.331+5G>A	1
ATM	c.3852delA	1
ATM	c.4451delT	1
ATM	c.4632_4635delCTTA	1
ATM	c.496+5G>A	1
ATM	c.5290delC	2
ATM	c.5497-2A>C	1
ATM	c.5511_5512delTT	1
ATM	c.5712dupA	1
ATM	c.5763-2A>T	1
ATM	c.5932G>T_p.Glu1978X	1
ATM	c.6100C>T_p.Arg2034X	1
ATM	c.6154G>A_p.Glu2052Lys	1
ATM	c.717_720delCCTC	2
ATM	c.7271T>G_p.Val2424Gly	1
ATM	c.748C>T_p.Arg250X	1
ATM	c.7638_7646del9_p.Arg2547_Ser2549del	1
ATM	c.7875_7876delTGinsGC_p.Asp2625_Ala2626delinsGluPro	1
ATM	c.8010+2T>G	2
ATM	c.8098A>T_p.Lys2700X	1
ATM	c.8266A>T_p.Lys2756X	1
ATM	c.8325delC	1
ATM	c.8432delA	3
ATM	c.8655dupT	1
ATM	c.8786+1G>A	1
ATM	c.9139C>T_p.Arg3047X	1
ATM	c.943_944delTT	1
ATM	del exon 24-63	1
ATM	deletion exons 62-63	1
ATM	EX16-37del	1
BRCA1	5′UTR_EX1del	1
BRCA1	c.1016dupA	1
BRCA1	c.1127delA	1
BRCA1	c.1327A>T_p.Lys443X	1
BRCA1	c.1360_1361delAG	1
BRCA1	c.1504_1508del5	1

(continued on following page)

TABLE A3. List of Pathogenic and Likely Pathogenic Variants in 9 Breast Cancer-Predisposition genes (continued)

Gene	Pathogenic Variants	Frequency
BRCA1	c.1556delA	2
BRCA1	c.181T>G_p.Cys61Gly	2
BRCA1	c.1961delA	1
BRCA1	c.2035A>T_p.Lys679X	1
BRCA1	c.2071delA	2
BRCA1	c.213-12A>G	1
BRCA1	c.2515delC	1
BRCA1	c.2685_2686delAA	1
BRCA1	c.2709_2710delTG	1
BRCA1	c.2722G>T_p.Glu908X	2
BRCA1	c.2836_2837delAT	1
BRCA1	c.3358_3359delGT	1
BRCA1	c.3648dupA	1
BRCA1	c.3748G>T_p.Glu1250X	1
BRCA1	c.3937C>T_p.Gln1313X	2
BRCA1	c.4065_4068delTCAA	1
BRCA1	c.4146_4155dup10	1
BRCA1	c.4165_4166delAG	1
BRCA1	c.4222C>T_p.Gln1408X	1
BRCA1	c.4689C>G_p.Tyr1563X	2
BRCA1	c.5089T>C_p.Cys1697Arg	1
BRCA1	c.514C>T_p.Gln172X	1
BRCA1	c.5179A>T_p.Lys1727X	1
BRCA1	c.5251C>T_p.Arg1751X	1
BRCA1	c.5266dupC	4
BRCA1	c.5474_5481del8	1
BRCA1	c.5503C>T_p.Arg1835X	1
BRCA1	c.676delT	1
BRCA1	c.68_69delAG	2
BRCA1	c.697_698delGT	2
BRCA1	c.75_80dup6	1
BRCA1	c.923delG	1
BRCA1	del exon 1-14	1
BRCA1	dup exons1-19	1
BRCA2	c.1813dupA	1
BRCA2	c.1929delG	1
BRCA2	c.2330dupA	1
BRCA2	c.2808delA	1
BRCA2	c.3076A>T_p.Lys1026X	1
BRCA2	c.3170_3174del5	1
BRCA2	c.3744_3747delTGAG	1
BRCA2	c.3785C>G_p.Ser1262X	1
BRCA2	c.3847_3848delGT	1

(continued on following page)

TABLE A3. List of Pathogenic and Likely Pathogenic Variants in 9 Breast Cancer-Predisposition genes (continued)

Gene	Pathogenic Variants	Frequency
BRCA2	c.3975_3978dupTGCT	2
BRCA2	c.4405_4409del5	1
BRCA2	c.4472_4475delTGAA	1
BRCA2	c.4638delT	1
BRCA2	c.5073dupA	1
BRCA2	c.5217_5223del7	1
BRCA2	c.5290_5291delTC	1
BRCA2	c.5350_5351delAA	1
BRCA2	c.5351dupA	1
BRCA2	c.5682C>G_p.Tyr1894X	1
BRCA2	c.5701G>T_p.Glu1901X	1
BRCA2	c.5864C>A_p.Ser1955X	1
BRCA2	c.5946delT	1
BRCA2	c.5966C>G_p.Ser1989X	1
BRCA2	c.6037A>T_p.Lys2013X	1
BRCA2	c.6275_6276delTT	2
BRCA2	c.658_659delGT	1
BRCA2	c.6641dupC	1
BRCA2	c.6644_6647delACTC	1
BRCA2	c.6664dupT	1
BRCA2	c.7007+1G>C	1
BRCA2	c.7007G>A_p.Arg2336His	2
BRCA2	c.7025_7026delAA	1
BRCA2	c.7069_7070delCT	3
BRCA2	c.7254_7255delAG	2
BRCA2	c.7480C>T_p.Arg2494X	1
BRCA2	c.7558C>T_p.Arg2520X	1
BRCA2	c.7681C>T_p.Gln2561X	1
BRCA2	c.7913_7917del5	1
BRCA2	c.793+1G>A	1
BRCA2	c.7976+1G>A	1
BRCA2	c.7976G>A_p.Arg2659Lys	1
BRCA2	c.8537_8538delAG	1
BRCA2	c.8904delC	2
BRCA2	c.8969G>A_p.Trp2990X	1
BRCA2	c.9004G>A_p.Glu3002Lys	1
BRCA2	c.9117G>A_p.=	1
BRCA2	c.9253dupA	1
BRCA2	c.961C>T_p.Gln321X	1
BRCA2	exon 3 rearrangement	1
BRCA2	Exon25 rearrangement	1
CDH1	c.1590dupC	1
CDH1	c.1711+1dupG	1

(continued on following page)

TABLE A3. List of Pathogenic and Likely Pathogenic Variants in 9 Breast Cancer-Predisposition genes (continued)

Gene	Pathogenic Variants	Frequency
CDH1	c.1792C>T_p.Arg598X	1
CDH1	c.2064_2065delTG	1
CDH1	del exon 1-16	1
CDH1	del exon 3-10	1
CHEK2	c.1100delC	52
CHEK2	c.1425dupT	1
CHEK2	c.1555C>T_p.Arg519X	1
CHEK2	c.277delT	1
CHEK2	c.444+1G>A	6
CHEK2	c.507delT	1
CHEK2	c.555delC	1
CHEK2	del exon 9-10	4
NF1	c.7971-1G>A	1
PALB2	c.109-2A>G	1
PALB2	c.115C>T_p.Gln39X	1
PALB2	c.172_175delTTGT	1
PALB2	c.223A>T_p.Lys75X	1
PALB2	c.2257C>T_p.Arg753X	2
PALB2	c.2748+1G>T	1
PALB2	c.3048delT	1
PALB2	c.3507_3508delTC	1
PALB2	c.3549C>A_p.Tyr1183X	2
PALB2	c.509_510delGA	4
PTEN	c.875delA	1
TP53	c.267delC	1
TP53	c.375+1dupG	1
TP53	c.455C>T_p.Pro152Leu	1
TP53	c.524G>A_p.Arg175His	2
TP53	c.743G>A_p.Arg248Gln	1

TABLE A4. Sensitivity Analysis Considering All Patients With a Family History of Prostate Cancer to Have Met the NCCN Criteria

Characteristic	*BRCA1 or BRCA2*	6 High-Risk Genes[a]	9 Breast Cancer Genes[b]
Total No. of pathogenic variant carriers detected based on ASBrS guidelines	107	134	241
Total No. of pathogenic variant carriers detected based on NCCN guidelines	97 (90.7)	113 (84.3)	198 (77.6)
Total No. of pathogenic variant carriers missed by NCCN guidelines	10 (9.3)	21 (15.7)	54 (22.4)

NOTE. Data are No. (%). A total of 2,292 women met NCCN criteria, whereas 1,615 did not meet criteria.
Abbreviations: ASBrS, American Society of Breast Surgeons; NCCN, National Comprehensive Cancer Network.
[a]*BRCA1, BRCA2, CDH1, PALB2, PTEN,* and *TP53*.
[b]*ATM, BRCA1, BRCA2, CDH1, CHEK2, NF1, PALB2, PTEN,* and *TP53*.

TABLE A5. Sensitivity Analysis Considering all Patients With a Third-Degree Family Member With Breast, Ovarian, Pancreatic, or Prostate Cancer to Have Met the NCCN Criteria

Characteristic	*BRCA1* or *BRCA2*	6 High-Risk Genes[a]	9 Breast Cancer Genes[b]
Total No. of pathogenic variant carriers detected based on ASBrS guidelines	107	134	241
Total No. of pathogenic variant carriers detected based on NCCN guidelines	94 (87.9)	108 (80.6)	174 (72.2)
Total No. of pathogenic variant carriers missed by NCCN guidelines	13 (12.1)	26 (19.4)	67 (27.8)

NOTE. Data are No. (%). A total of 1,944 women met NCCN criteria, whereas 1,963 did not meet the criteria.
Abbreviations: ASBrS, American Society of Breast Surgeons; NCCN, National Comprehensive Cancer Network.
[a]*BRCA1, BRCA2, CDH1, PALB2, PTEN,* and *TP53*.
[b]*ATM, BRCA1, BRCA2, CDH1, CHEK2, NF1, PALB2, PTEN,* and *TP53*.

TABLE A6. Evaluation of Candidate Thresholds for Pathogenic Variants in 12 Breast Cancer Predisposition Genes

Testing Criteria	No. Tested (% total)[a]	No. Additional Tested (% total)[b]	No. Not Tested (% total)[c]	No. of Carriers Detected (% tested)	No. of Carriers Not Identified (% total carriers)	Sensitivity (%)	Specificity (%)	VUS to Pathogenic Variant Ratio
12 breast cancer genes[d]								
ASBrS recommendations	3,907 (100)	2,035 (52.1)	0 (0)	255 (6.5)	0 (0)	100	0	2.1
NCCN criteria	1,872 (47.9)	0 (0)	2,035 (52.1)	180 (9.6)	75 (29.4)	70.6	53.7	1.6
NCCN criteria or age at diagnosis, years								
≤ 50	2,025 (51.8)	153 (3.9)	1,882 (48.2)	185 (9.1)	70 (27.5)	72.5	49.6	1.6
≤ 55	2,380 (60.9)	508 (13.0)	1,527 (39.1)	212 (8.9)	43 (16.9)	83.1	40.6	1.6
≤ 60	2,697 (69.0)	825 (21.1)	1,210 (31.0)	221 (8.2)	34 (13.3)	86.7	32.2	1.7
≤ 65	3,081 (78.9)	1,209 (30.9)	826 (21.1)	234 (7.6)	21 (8.2)	91.8	22.0	1.8
≤ 70	3,429 (87.8)	1,557 (39.9)	478 (12.2)	245 (7.1)	10 (3.9)	96.1	12.8	2.0
≤ 75	3,676 (94.1)	1,804 (46.2)	231 (5.9)	249 (6.8)	6 (2.4)	97.6	6.2	2.0

Abbreviations: ASBrS, American Society of Breast Surgeons; NCCN, National Comprehensive Cancer Network; VUS, variant of uncertain significance.
[a]Total number of patients tested in each evaluated criterion.
[b]Additional number of patients tested compared with NCCN criteria.
[c]Number of patients in the cohort who would not undergo genetic testing based on the evaluated criteria.
[d]*ATM, BARD1, BRCA1, BRCA2, CDH1, CHEK2, NF1, PALB2, PTEN, RAD51C, RAD51D,* and *TP53*; 6 (0.2%) women had pathogenic variants in *BARD1*, 6 (0.2%) in *RAD51C*, and 4 (0.1%) in *RAD51D*.

TABLE A7. Frequency of Germline Pathogenic Variants Among Patients Not Meeting NCCN Guidelines by Age of Diagnosis and Family History of Breast Cancer

	Frequency of Germline Pathogenic Variants in Age Groups (years)								
Gene	**≤ 45**	**46-50**	**51-55**	**56-60**	**61-65**	**66-70**	**71-75**	**≥ 75**	**Total**
Overall[a]	n = 0	n = 153	n = 355	n = 317	n = 384	n = 348	n = 247	n = 231	n = 2,035
BRCA1 or *BRCA2*	NA	1 (0.6)	7 (2.0)	1 (0.3)	3 (0.8)	0 (0.0)	1 (0.4)	1 (0.4)	14 (0.7)
6 high-risk genes	NA	1 (0.6)	12 (3.4)	3 (0.9)	5 (1.3)	2 (0.6)	1 (0.4)	4 (1.7)	28 (1.4)
9 predisposition genes	NA	5 (3.3)	26 (7.3)	6 (1.9)	13 (3.4)	9 (2.6)	4 (1.6)	6 (2.6)	72 (3.5)
Women with family history of breast cancer[b]	n = 0	n = 0	n = 158	n = 141	n = 150	n = 142	n = 92	n = 81	n = 764
BRCA1 or *BRCA2*	NA	NA	5 (3.2)	1 (0.7)	1 (0.7)	0 (0.0)	0 (0.0)	1 (1.2)	8 (1.0)
6 high-risk genes	NA	NA	7 (4.4)	3 (2.1)	2 (1.3)	1 (0.7)	0 (0.0)	3 (3.7)	16 (2.1)
9 predisposition genes	NA	NA	12 (7.6)	4 (2.8)	5 (3.3)	6 (4.2)	1 (1.1)	5 (6.2)	33 (4.3)
Women without a family history of breast cancer	n = 0	n = 153	n = 197	n = 176	n = 234	n = 206	n = 155	n = 150	n = 1,271
BRCA1 or *BRCA2*	NA	1 (0.7)	2 (1.0)	0 (0.0)	2 (0.9)	0 (0.0)	1 (0.6)	0 (0.0)	6 (0.5)
6 high-risk genes	NA	1 (0.7)	5 (2.5)	0 (0.0)	3 (1.3)	1 (0.5)	1 (0.6)	1 (0.7)	12 (0.9)
9 breast cancer genes	NA	5 (3.3)	14 (7.1)	5 (2.8)	8 (3.4)	3 (1.5)	3 (1.9)	1 (0.7)	39 (3.1)

NOTE. Data are No. (%).

Abbreviations: NA, not applicable because all patients in the category met NCCN criteria; NCCN, National Comprehensive Cancer Network.

[a]With or without family history of breast cancer.

[b]Family history of breast cancer in first- or second-degree relatives.

TABLE A8. Evaluation of Candidate Thresholds for Selection of Patients for Genetic Testing With Inclusion of Family History of Breast Cancer

Testing Criteria	No. Tested (% total)[a]	No. of Additional Tested (% total)[b]	No. Not Tested (% total)[c]	No. Carriers Detected (% of tested)	No. Carriers Not Identified (% of total carriers)	Sensitivity (%)	Specificity (%)	Mean No. Patients Tested per Carrier	VUS to Pathogenic Variant Ratio
9 breast cancer genes[d]									
ASBrS recommendations	3,907 (100)	2,035 (52.1)	0 (0)	241 (6.2)	0 (0)	100	0	16.2	1.9
NCCN criteria	1,872 (47.9)	0 (0)	2,035 (52.1)	169 (9.0)	72 (29.8)	70.1	53.5	11.1	1.4
NCCN criteria or family history of breast cancer	2,636 (67.5)	764 (19.6)	1,271 (32.5)	202 (7.7)	39 (16.2)	83.8	33.6	13.0	1.5
NCCN criteria or family history of breast cancer or age at diagnosis, years									
≤ 50	2,789 (71.4)	917 (23.5)	1,118 (28.6)	207 (7.4)	34 (14.1)	85.9	29.6	13.4	1.6
≤ 55	2,986 (76.4)	1,114 (28.5)	921 (23.6)	221 (7.4)	20 (8.3)	91.7	24.6	13.5	1.6
≤ 60	3,162 (80.9)	1,290 (33.0)	745 (19.1)	226 (7.1)	15 (6.2)	93.8	19.9	14.0	1.6
≤ 65	3,396 (86.9)	1,524 (39.0)	511 (13.1)	234 (6.9)	7 (2.9)	97.1	13.7	14.5	1.7
≤ 70	3,602 (92.2)	1,730 (44.3)	305 (7.8)	237 (6.6)	4 (1.7)	98.3	8.2	15.2	1.8
≤ 75	3,757 (96.2)	1,885 (48.2)	150 (3.8)	240 (6.4)	1 (0.4)	99.6	4.1	15.6	1.8
6 high-risk genes[e]									
ASBrS recommendations	3,907 (100)	2,035 (52.1)	0 (0)	134 (3.4)	0 (0.0)	100	0	29.2	1.7
NCCN criteria	1,872 (47.9)	0 (0)	2,035 (52.1)	106 (5.7)	28 (20.9)	79.1	53.2	17.7	1.1
NCCN criteria or family history of breast cancer	2,636 (67.5)	764 (19.6)	1,271 (32.5)	122 (4.6)	12 (9.0)	91.0	33.4	21.6	1.2
NCCN criteria or family history of breast cancer or age at diagnosis, years									
≤ 50	2,789 (71.4)	917 (23.5)	1,118 (28.6)	123 (4.4)	11 (8.2)	91.8	29.3	22.7	1.3
≤ 55	2,986 (76.4)	1,114 (28.5)	921 (23.6)	128 (4.3)	6 (4.5)	95.5	24.3	23.3	1.4
≤ 60	3,162 (80.9)	1,290 (33.0)	745 (19.1)	128 (4.0)	6 (4.5)	95.5	19.6	24.7	1.4
≤ 65	3,396 (86.9)	1,524 (39.0)	511 (13.1)	131 (3.9)	3 (2.2)	97.8	13.5	25.9	1.5
≤ 70	3,602 (92.2)	1,730 (44.3)	305 (7.8)	132 (3.7)	2 (1.5)	98.5	8.0	27.3	1.6
≤ 75	3,757 (96.2)	1,885 (48.2)	150 (3.8)	133 (3.5)	1 (0.7)	99.3	3.9	28.2	1.6
BRCA1 or *BRCA2*									
ASBrS recommendations	3,907 (100)	2,035 (52.1)	0 (0)	107 (2.7)	0 (0.0)	100	0	36.5	1.1
NCCN criteria	1,872 (47.9)	0 (0)	2,035 (52.1)	93 (5.0)	14 (13.1)	86.9	53.2	20.1	0.8
NCCN criteria or family history of breast cancer	2,636 (67.5)	764 (19.6)	1,271 (32.5)	101 (3.8)	6 (5.6)	94.4	33.3	26.1	0.9
NCCN criteria or family history of breast cancer or age at diagnosis, years									
≤ 50	2,789 (71.4)	917 (23.5)	1,118 (28.6)	102 (3.7)	5 (4.7)	95.3	29.3	27.3	0.9

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TABLE A8. Evaluation of Candidate Thresholds for Selection of Patients for Genetic Testing With Inclusion of Family History of Breast Cancer (continued)

Testing Criteria	No. Tested (% total)[a]	No. of Additional Tested (% total)[b]	No. Not Tested (% total)[c]	No. Carriers Detected (% of tested)	No. Carriers Not Identified (% of total carriers)	Sensitivity (%)	Specificity (%)	Mean No. Patients Tested per Carrier	VUS to Pathogenic Variant Ratio
≤ 55	2,986 (76.4)	1,114 (28.5)	921 (23.6)	104 (3.5)	3 (2.8)	97.2	24.2	28.7	1.0
≤ 60	3,162 (80.9)	1,290 (33.0)	745 (19.1)	104 (3.3)	3 (2.8)	97.2	19.5	30.4	1.0
≤ 65	3,396 (86.9)	1,524 (39.0)	511 (13.1)	106 (3.1)	1 (0.9)	99.1	13.4	32.0	1.0
≤ 70	3,602 (92.2)	1,730 (44.3)	305 (7.8)	106 (2.9)	1 (0.9)	99.1	8.0	34.0	1.1
≤ 75	3,757 (96.2)	1,885 (48.2)	150 (3.8)	107 (2.8)	0 (0.0)	100	3.4	35.1	1.1

Abbreviations: ASBrS, American Society of Breast Surgeons; NCCN, National Comprehensive Cancer Network; VUS, variant of uncertain significance.
[a]Total number of patients tested in each evaluated criterion.
[b]Additional number of patients tested compared with NCCN criteria.
[c]Number of patients in the cohort who would not undergo genetic testing based on the evaluated criteria.
[d]*ATM, BRCA1, BRCA2, CDH1, CHEK2, NF1, PALB2, PTEN,* and *TP53.*
[e]*BRCA1, BRCA2, CDH1, PALB2, PTEN,* and *TP53.*

TABLE A9. Comparison of Patient and Tumor Characteristics Between MCBCS and SEER Iowa Registry

Characteristic	MCBCS N = 3,907	SEER Iowa N= 15,679
Age at diagnosis of first breast cancer, years		
Median	57	63
≤ 45	747 (19.1)	1,563 (10.0)
46-50	538 (13.8)	1,389 (8.8)
51-55	530 (13.6)	1,800 (11.5)
56-60	491 (12.6)	1,999 (12.7)
61-65	511 (13.1)	2,166 (13.8)
66-70	477 (12.2)	1,963 (12.5)
71-75	327 (8.4)	1,627 (10.4)
≥ 75	286 (7.3)	3,172 (20.2)
Race/ethnicity		
White	3,719 (95.2)	15,249 (97.3)
Black	29 (0.7)	244 (1.6)
Other/unknown	159 (4.1)	186 (1.2)
Histology		
Invasive	3,282 (84.0)	13,179 (84.1)
In situ	625 (16.0)	2,500 (15.9)
HR status[a]		
HR+/HER2−	1,861 (76.9)	9,762 (73.8)
HR+/HER2+	240 (9.9)	1,396 (10.5)
HR−/HER2+	105 (4.3)	653 (4.9)
Triple-negative breast cancer	215 (8.9)	1,415 (10.7)

NOTE. SEER Iowa registry includes women with breast cancer diagnosed between 2010 and 2015.

Abbreviations: HER2, human epidermal growth factor receptor 2; HR, hormone receptor (HR+ tumors are positive for estrogen or progesterone receptor); MCBCS, Mayo Clinic Breast Cancer Study.

[a]Among patients with known estrogen receptor, progesterone receptor, and HER2 status.