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

Siddhartha Yadav, MD1; Chunling Hu, PhD2; Steven N. Hart, PhD3; Nicholas Boddicker, PhD2; Eric C. Polley, PhD1; Jie Na, MS2; Rohan Gnanaiyuvu, MS3; Kun Y. Lee, PhD2; Tricia Lindstrom, BS1; Sebastian Armasu, MS3; Patrick Fitz-Gibbon, MS3; Karthik Ghosh, MD4; Daniela L. Stan, MD3; Sandhya Pruthi, MD4; Lonzetta Neal, MD4; Nicole Sandhu, MD, PhD4; Deborah J. Rhodes, MD5; Christine Klassen, MD1; Prema P. Pethambaram, MD3; Tufia C. Haddad, MD4; Janet E. Olson, PhD3; Tanya L. Hoskin, MS3; Matthew P. Goetz, MD1; Susan M. Domchek, MD5; Judy C. Boughey, MD6; Kathryn J. Ruddy, MD, MPH1; and Fergus J. Couch, PhD2

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% vs 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 genetic testing according to 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.

J Clin Oncol 38:1409-1418. © 2020 by American Society of Clinical Oncology.
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.

The conflicting recommendations from NCCN and ASBrS have created a debate on whether all women with breast cancer need to undergo germline genetic testing. 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. 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 QiAsseq panel (Qiagen, Hilden, Germany) and sequencing on a HiSeq4000 (Illumina, San Diego, CA) as described previously 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) with clear management recommendations in the NCCN guidelines. 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. These genes have recently been included in genetic testing recommendations (NCCN v1.2020), 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), 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 Tyer-Cuzick risk evaluation tool (v8.0b) 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 statusa                 |                   |                                   |                                       |
| 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 (14.1)                        | 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 cancerb              |                   |                                   |                                       |
| 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.
aAmong patients with invasive breast cancer (n = 3,282).
bFirst- or second-degree relatives.
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% vs 3.5%; P < .001). Similar results were observed for the 6 high-risk genes (5.7% vs 1.4%; P < .001) and for BRCA1 or BRCA2 (5.0% vs 0.7%; P < .001). However, 72 (29.9%) of the 241 women with pathogenic variants had no relatives 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).

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* | P for NCCN v Non-NCCN* (%) | Pathogenic Variant Carriers Missed by NCCN Criteria a (%) |
|------|--------------------------------------------------|---------------------------------------|--------------------------------|--------------------------------------|--------------------------|---------------------------------|
| BRCA1/2 | 107 (2.7)                                      | 93 (5.0)                               | 14 (0.7)                          | 7.5 (4.2 to 13.5)                     | < 2.2 × 10^-16         | 13.1                             |
| 6 high-risk genes b | 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 b | 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.

aBRCA1, BRCA2, CDH1, PALB2, PTEN, and TP53.

bATM, BRCA1, BRCA2, CDH1, CHEK2, NF1, PALB2, PTEN, and TP53.
variants and 1 of 6 CDH1 carriers met testing criteria for Li-Fraumeni syndrome or Hereditary Diffuse Gastric Cancer criteria, 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 \( \leq \) 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 \( \geq \) 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 \( \leq \) 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.
| Testing Criteria | Tested (%) | Additional Tested (%) | Not Tested (%) | No. of Carriers Detected (%) | No. Not Identified (%) | Sensitivity (95% CI) | Specificity (95% CI) | VUS to Pathogenic Variant Ratio |
|------------------|------------|-----------------------|---------------|----------------------------|-----------------------|---------------------|----------------------|---------------------------|
| **9 predisposition genes** |            |                       |               |                            |                       |                     |                      |                           |
| 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 (86.6)                 | 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 (84.4)                 | 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 (77.7)                 | 32 (13.8)            | 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 (72.2)                 | 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** |            |                       |               |                            |                       |                     |                      |                           |
| 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 (10.9)            | 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 (10.1)            | 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 (45.5)                 | 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 (41.1)                 | 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 (38.9)                 | 5 (13.7)             | 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 (35.5)                 | 4 (13.0)             | 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 (46.6)                  | 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 (42.1)                 | 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 (38.3)                 | 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 (34.8)                 | 2 (19)               | 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 (31.1)                 | 2 (19)               | 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 (29.9)                 | 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.

*Total number of patients tested in each evaluated criterion.

*Additional number of patients tested compared with NCCN criteria.

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

*Sensitivity and specificity of testing criteria in percentages.

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

*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. However, in contrast to prior studies, women meeting NCCN criteria had a higher frequency of germline pathogenic variants than women not meeting criteria (9.0% vs 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, and systemic treatment, which may lead to improved survival. However, changes in management are limited to additional cancer screening for the moderate-risk predisposition genes.

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. 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. Third, more VUS will be detected, which may lead to anxiety and unwarranted interventions. 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, including a recent position statement by the American College of Medical Genetics and Genomics.

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. The recently updated NCCN criteria (v1.2020) recommend against genetic testing in women > 65 years of age with no family history of cancer. 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. 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. 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 (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 and a population-based study of women with breast cancer. 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, these models have not been validated in clinical practice. 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.

AFFILIATIONS
1. Department of Oncology, Mayo Clinic, Rochester, MN
2. Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN
3. Department of Health Sciences Research, Mayo Clinic, Rochester, MN
4. Department of Medicine, Mayo Clinic, Rochester, MN
5. Perelman School of Medicine, University of Pennsylvania, and Basser Center for BRCA, Philadelphia, PA
6. Department of Surgery, Mayo Clinic, Rochester, MN

CORRESPONDING AUTHOR
Fergus J. Couch, PhD, Department of Laboratory Medicine and Pathology, 200 First St SW, Mayo Clinic, Rochester, MN 55905; e-mail: couch.fergus@mayo.edu.

SUPPORT
Supported in part by National Institutes of Health (NIH) Grants No. CA116167, CA176785, CA192393, and CA225662, an NIH Specialized Program of Research Excellence in Breast Cancer (CA116201), and the Breast Cancer Research Foundation.

AUTHORS’ DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST AND DATA AVAILABILITY STATEMENT
Disclosures provided by the authors and data availability statement (if applicable) are available with this article at DOI https://doi.org/10.1200/JCO.19.02190.

AUTHOR CONTRIBUTIONS
Conception and design: Siddhartha Yadav, Steven N. Hart, Eric C. Polley, Nicole Sandhu, Matthew P. Goetz, Judy C. Boughhey, Fergus J. Couch
Financial support: Matthew P. Goetz, Fergus J. Couch
Administrative support: Patrick Fitz-Gibbon, Fergus J. Couch
Provision of study materials or patients: Daniela L. Stan, Lonzetta Neal, Janet E. Olson, Matthew P. Goetz, Fergus J. Couch
Collection and assembly of data: Siddhartha Yadav, Chunling Hu, Kun Y. Lee, Tricia Lindstrom, Sebastian Armasu, Patrick Fitz-Gibbon, Kartik Ghosh, Sandhya Pruthi, Lonzetta Neal, Christine Klassen, Janet E. Olson, Tanya L. Hoskin, Matthew P. Goetz, Kathryn J. Ruddy, Fergus J. Couch
Data analysis and interpretation: Siddhartha Yadav, Chunling Hu, Steven N. Hart, Nicholas Boddicker, Eric C. Polley, Jie Na, Rohan Gnanaiilvi, Kun Y. Lee, Kartik Ghosh, Daniela L. Stan, Sandhya Pruthi, Nicole Sandhu, Deborah J. Rhodes, Prema P. Peethambaram, Tufa C. Haddad, Janet E. Olson, Tanya L. Hoskin, Matthew P. Goetz, Susan M. Dornchek, Judy C. Boughhey, Fergus J. Couch
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

REFERENCES
1. National Comprehensive Cancer Network: NCCN Clinical Practice Guidelines in Oncology. Genetic/familial high-risk assessment: Breast, ovarian, and pancreatic. Version 1. https://www.nccn.org/professionals/physician_gls/pdf/genetics_bop.pdf
2. Beitsch PD, Whitworth PW, Hughes K, et al: Underdiagnosis of hereditary breast cancer: Are genetic testing guidelines a tool or an obstacle? J Clin Oncol 37: 453-460, 2019
3. Yang S, Axilbund JE, O’Leary E, et al: Underdiagnosis of hereditary breast and ovarian cancer in Medicare patients: Genetic testing criteria miss the mark. Ann Surg Oncol 25:2925-2931, 2018
4. Manahan ER, Kuerer HM, Sebastian M, et al: Consensus guidelines on genetic testing for hereditary breast cancer from the American Society of Breast Surgeons. Ann Surg Oncol 26:3025-3031, 2019
5. Pal T, Agnese D, Daly M, et al: Points to consider: Is there evidence to support BRCA1/2 and other inherited breast cancer genetic testing for all breast cancer patients? A statement of the American College of Medical Genetics and Genomics (ACMG). Genet Med 10.1038/s41436-019-0712-x [Epub ahead of print on December 13, 2019]
6. Milliron KJ, Griggs JJ: Advances in genetic testing in patients with breast cancer, high-quality decision making, and responsible resource allocation. J Clin Oncol 37:445-447, 2019

7. Tung N, Domchek SM, Stadler Z, et al: Counselling framework for moderate-penetrance cancer-susceptibility mutations. Nat Rev Clin Oncol 13:581-588, 2016

8. Copur MS, Jongiertham P, Zuzan T: Should all patients with a diagnosis of breast cancer undergo expanded panel testing? J Clin Oncol 37:2175-2176, 2019

9. Rajagopal PS, Catenacci DV, Olopade OI: The time for mainstreaming germline testing for patients with breast cancer is now. J Clin Oncol 37:2177-2178, 2019

10. Beitsch P, Hughes K, Whitworth P: Reply to M.S. Copur et al, A. Taylor et al, and P.S. Rajagopal et al. J Clin Oncol 37:2178-2180, 2019

11. Sorscher S: Universal multigene panel testing in all breast cancer patients. Am J Med 132:e765-e766, 2019

12. Robson M, Domchek S: Broad application of multigene panel testing for breast cancer susceptibility—Pandora’s box is opening wider. JAMA Oncol 5:1687, 2019

13. Wood ME, Bedrosian I: Hot topic: Should all women with breast cancer undergo genetic testing? Curr Breast Cancer Rep 11:381-384, 2019

14. John EM, Hopper JL, Beck JC, et al: The Breast Cancer Family Registry: An infrastructure for cooperative multinational, interdisciplinary and translational studies of the genetic epidemiology of breast cancer. Breast Cancer Res 6:R375-R389, 2004

15. Hu C, Hart SN, Polley EC, et al: Association between inherited germline mutations in cancer predisposition genes and risk of pancreatic cancer. JAMA 319:2401-2409, 2018

16. Yadav S, Couch FJ: Germline genetic testing for breast cancer risk: The past, present, and future. Am Soc Clin Oncol Educ Book 39:61-74, 2019

17. Couch FJ, Shimelis H, Hu C, et al: Associations between cancer predisposition testing panel genes and breast cancer. JAMA Oncol 3:1190-1196, 2017

18. Buys SS, Sandbach JF, Gammon M, et al: A study of over 35,000 women with breast cancer tested with a 25-gene panel of hereditary cancer genes. Cancer 123:1721-1733, 2017

19. Shimelis H, LaDuca H, Hu C, et al: Triple-negative breast cancer risk genes identified by multigene hereditary cancer panel testing. J Natl Cancer Inst 110:855-862, 2018

20. Kurian AW, Hughes E, Handorf EA, et al: Breast and ovarian cancer penetrance estimates derived from germline multi-gene sequencing results in women. JCO Precis Oncol 10.1200/PO.16.00066

21. Tyer J, Duffy SW, Cuzick J: A breast cancer prediction model incorporating familial and personal risk factors. Stat Med 23:1111-1130, 2004

22. IBIS Breast Cancer Risk Evaluation Tool. http://www.ems-trials.org/riskevaluator/

23. Chompret A, Abel A, Stoppa-Lyonnet D, et al: Sensitivity and predictive value of criteria for p53 germline mutation screening. J Med Genet 38:43-47, 2001

24. Fitzgerald RC, Hardwick R, Huntsman D, et al: Hereditary diffuse gastric cancer: Updated consensus guidelines for clinical management and directions for future research. J Med Genet 47:436-444, 2010 [Erratum: J Med Genet 48:216, 2011]

25. Kurian AW, Ward KC, Hamilton AS, et al: Uptake, results, and outcomes of germline multiple-gene sequencing after diagnosis of breast cancer. JAMA Oncol 4:1066-1072, 2018

26. Neben CL, Zimmer AD, Stedden W, et al: Multi-gene panel testing of 23,179 individuals for hereditary cancer risk identifies pathogenic variant carriers missed by current genetic testing guidelines. J Mol Diagn 21:646-657, 2019

27. Kaas R, Verhoef S, Wesseling J, et al: Prophylactic mastectomy in BRCA1 and BRCA2 mutation carriers: Very low risk for subsequent breast cancer. Ann Surg 251:488-492, 2010

28. van Grondelle TC, Schmidt MK, Rookus MA, et al: Risk reduction of contralateral breast cancer and survival after contralateral prophylactic mastectomy in BRCA1 or BRCA2 mutation carriers. Br J Cancer 93:287-292, 2005

29. Metcalfe K, Gershman S, Ghadirian P, et al: Contralateral mastectomy and survival after breast cancer in carriers of BRCA1 and BRCA2 mutations: Retrospective analysis. BMJ 348:g226, 2014

30. Robson M, Im SA, Senkus E, et al: Olaparib for metastatic breast cancer in patients with a germline BRCA mutation. N Engl J Med 377:523-533, 2017 [Erratum: N Engl J Med 377:1700, 2017]

31. Litton JK, Rugo HS, Ettl J, et al: Talazoparib in patients with advanced breast cancer and a germline BRCA mutation. J Natl Cancer Inst 106:628-637, 2018

32. Stoll K, Kubendran S, Cohen SA: The past, present and future of service delivery in genetic counseling: Keeping up in the era of precision medicine. Am J Med Genet C Semin Med Genet 178:24-37, 2018

33. Tung N, Domchek SM, Stadler Z, et al: Counselling framework for moderate-penetrance cancer-susceptibility mutations. JAMA Oncol 5:1687, 2019

34. IBIS Breast Cancer Risk Evaluation Tool. http://www.ems-trials.org/riskevaluator/

35. Culver JO, Brinkerhoff CD, Clague J, et al: Breast cancer risk prediction models: Evaluation of surgical decisions, risk perception, and cancer distress. Clin Genet 84:464-472, 2013

36. Hoffman-Andrews L: The known unknown: The challenges of genetic variants of uncertain significance. JAMA Oncol 5:1687, 2019

37. Chavarri-Guerra Y, Hendrickx CB, Brown S, et al: The burden of breast cancer predisposition variants across the age spectrum among 10,000 patients. J Am Geriatr Soc 67:884-888, 2019

38. Owens DK, Davidson KW, Krist AH, et al: Risk assessment, genetic counseling, and genetic testing for BRCA-related cancer: US Preventive Services Task Force recommendation statement. JAMA 322:652-665, 2019 [Erratum: JAMA 322:1830, 2019]

39. Owens DK, Davidson KW, Krist AH, et al: Risk assessment, genetic counseling, and genetic testing for BRCA-related cancer: US Preventive Services Task Force recommendation statement. JAMA 322:652-665, 2019 [Erratum: JAMA 322:1830, 2019]

40. Domchek S, Robson M: Broadening criteria for BRCA1/2 evaluation: Placing the USPSTF Recommendation in context. JAMA 322:619-621, 2019

41. Rajagopal PS, Nielsen S, Olopade OI: USPSTF recommendations for BRCA1 and BRCA2 testing in the context of a transformative national cancer control plan. JAMA Neu Open 2:e1910142, 2019

42. Newman L: US Preventive Services Task Force breast cancer recommendation statement on risk assessment, genetic counseling, and genetic testing for BRCA-related cancer. JAMA Surg 154:895, 2019

43. National Cancer Institute: Iowa Registry. https://seer.cancer.gov/registries/iowa.html

44. Kim SY, Lee CS, Fey JV, et al: Prevalence of BRCA2 mutations in a hospital based series of unselected breast cancer cases. J Med Genet 42:e5, 2005

45. Tung N, Lin NU, Kidd J, et al: Frequency of germline mutations in 25 cancer susceptibility genes in a sequential series of patients with breast cancer. J Clin Oncol 34:1460-1468, 2016

46. Ziegler A, Gildea M, Cohen P, et al: Cancer risk estimates for family members of a population-based family registry for breast and ovarian cancer. Cancer Epidemiol Biomarkers Prev 9:103-111, 2000
47. Manchanda R, Patel S, Gordeev VS, et al: Cost-effectiveness of population-based BRCA1, BRCA2, RAD51C, RAD51D, BRIP1, PALB2 mutation testing in unselected general population women. J Natl Cancer Inst 110:714-725, 2018
48. Manchanda R, Gaba F: Population based testing for primary prevention: A systematic review. Cancers (Basel) 10:E424, 2018
49. Sun L, Brentnall A, Patel S, et al: A cost-effectiveness analysis of multigene testing for all patients with breast cancer. JAMA Oncol 5:1718, 2019
50. Wentzensen N, Berg CD: Population testing for high penetrance genes: Are we there yet? J Natl Cancer Inst 110:687-689, 2018
AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated unless otherwise noted. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO’s conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/journal/jco/site/ifc.

Open Payments is a public database containing information reported by companies about payments made to US-licensed physicians (Open Payments).

Eric C. Polley
Research Funding: Grail

Sandhya Pruthi
Patents, Royalties, Other Intellectual Property: Mytonomy (Inst)

Tufta C. Haddad
Consulting or Advisory Role: Tersera
Research Funding: Takeda (Inst)

Matthew P. Goetz
Consulting or Advisory Role: Lilly, bioTheranostics, Genomic Health, Novartis, Eisai, Sermonix, Context Therapeutics, Pfizer
Research Funding: Lilly, Pfizer

Patents, Royalties, Other Intellectual Property: Methods and materials for assessing chemotherapy responsiveness and treating cancer, methods and materials for using butyrylcholinesterases to treat cancer, development of human tumor xenografts from women with breast cancer treated with neoadjuvant chemotherapy (Inst).

Travel, Accommodations, Expenses: Lilly

Susan M. Domchek
Honoraria: AstraZeneca, Clovis Oncology, Bristol-Myers Squibb
Research Funding: AstraZeneca (Inst), Clovis Oncology (Inst), Pharmamar (Inst)

Judy C. Boughey
Research Funding: Myriad Genetics (Inst)
Patents, Royalties, Other Intellectual Property: Patent pending: Methods and materials for assessing chemotherapy responsiveness and treating cancer (Inst).

Kathryn J. Ruddy
Stock and Other Ownership Interests: Merck, Pfizer
Patents, Royalties, Other Intellectual Property: My husband is a co-inventor of technology licensed by Mayo Clinic to AliveCor (Mountain View, CA), which makes a smartphone-enabled remote ECG monitoring system (I).

Fergus J. Couch
Consulting or Advisory Role: AstraZeneca
Speakers’ Bureau: Ambry Genetics, Qiagen
Research Funding: Grail
Travel, Accommodations, Expenses: Grail, Qiagen
Other Relationship: Ambry Genetics

No other potential conflicts of interest were reported.
Information

QIAseq 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. 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 200x, with 99.7% of target regions yielding > 20x 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: 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).

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 200x. 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). Annation 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:

\[ \text{Sensitivity} = \frac{\text{Total number of pathogenic variant carriers designated as qualifying for genetic testing}}{\text{Total number of pathogenic variant carriers in the study sample}} \]

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:

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

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.
Women seen at Mayo Clinic between May 15, 2000, and May 31, 2016, for a diagnosis of first breast cancer approached for enrollment into MCBCS (N = 7,300)

Women initially consented to participate and were asked to complete personal and family history questionnaire and provide a blood sample (n = 6,198)

Women provided blood sample with adequate DNA for germline genetic testing (n = 4,516)

Exclusions
- Withdrew consent (n = 32)
- Were enrolled > 1 year after initial diagnosis (n = 61)
- Had a prior diagnosis of breast cancer and were first seen at Mayo Clinic for recurrence or second primary (n = 78)
- Had a diagnosis of lobular carcinoma in-situ only (n = 6)

Germline DNA was subjected to genetic sequencing (n = 4,339)

Exclusions
- Samples failed sequencing (n = 5)

High-quality germline sequencing results were obtained from samples (n = 4,334)

Exclusions
- Did not complete personal or family history questionnaire in sufficient detail to allow for evaluation of NCCN criteria (n = 427)

Women included in the final analysis (n = 3,907)

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         | 32896111       | 32973805     | 1      | ENSG00000136818 |
| BRIP1       | NM032043.2         | ENST00000259008        | 17         | 59758627       | 59940882     | 1      | ENSG00000136492 |
| CDH1        | NM004360.4         | ENST00000261769        | 16         | 68771128       | 68869451     | 1      | ENSG00000039068 |
| CDKN2A      | NM000077.4         | ENST00000304494        | 9          | 2196751        | 21995300     | 1      | ENSG00000147899 |
| CHEK2       | 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        | NM004985.3         | ENST00000311936        | 12         | 25357723       | 25403870     | 1      | ENSG00000133703 |
| MEN1        | NM130799.2         | ENST00000312049        | 11         | 64570982       | 64578766     | 1      | ENSG00000133895 |
| MRE1A       | 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       | NM001124825.1      | 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       | NM03620.3          | ENST00000305921        | 17         | 58677544       | 58741849     | 1      | ENSG00000170836 |
| PRSS1       | NM002769           | ENST00000311737        | 7          | 142457319      | 142460923    | 1      | ENSG00000020498 |
| PTEN        | NM000314.6         | ENST00000371953        | 10         | 89622870       | 89731687     | 1      | ENSG00000171862 |
| RAD50       | NM005732.3         | ENST00000378823        | 5          | 131891711      | 131903013    | 1      | ENSG00000113522 |
| RAD51C      | NM058216.2         | ENST00000337432        | 17         | 56769934       | 56811703     | 1      | ENSG00000108384 |
| RAD51D      | NM001142571        | ENST00000345365        | 17         | 33426811       | 33448541     | 1      | ENSG00000185379 |
| RECQL       | NM023907           | ENST00000444129        | 12         | 21621845       | 21654603     | 1      | ENSG00000004700 |
| RINT1       | NM021930.4         | ENST00000257700        | 7          | 105172532      | 105208124    | 1      | ENSG00000135249 |
| SLX4        | NM032444.2         | ENST00000294008        | 16         | 3631182        | 3661599      | 1      | ENSG00000188273 |
| 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. (%) |
|----------------------------------------------------------------------------|---------|
| 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)  |
| 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.8256A>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)
| 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)
| 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               | 1         |
| 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.2223A>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\* | 9 Breast Cancer Genes\* |
|----------------|----------------|---------------------|------------------------|
| 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.

\*BRCA1, BRCA2, CDH1, PALB2, PTEN, and TP53.

\*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 | 9 Breast Cancer Genes |
|---------------|----------------|-------------------|----------------------|
| 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.

*BRCA1, BRCA2, CDH1, PALB2, PTEN, and TP53.*

*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) | No. Additional Tested (% total) | No. Not Tested (% total) | No. of Carriers Not Identified (% total carriers) | Sensitivity (%) | Specificity (%) | VUS to Pathogenic Variant Ratio |
|------------------|----------------------|--------------------------------|--------------------------|-----------------------------------------------|----------------|----------------|-------------------------------|
| 12 breast cancer genes | 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.

*Total number of patients tested in each evaluated criterion.*

*Additional number of patients tested compared with NCCN criteria.*

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

*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.*
| Gene                                      | Frequency of Germline Pathogenic Variants in Age Groups (years) | Total |
|------------------------------------------|----------------------------------------------------------------|-------|
| Overallb                                 | 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 cancerb | 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.
*With or without family history of breast cancer.
**Family history of breast cancer in first- or second-degree relatives.
| 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 genesd | | | | | | | | | | |
| 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 geneses | | | | | | | | | | |
| 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 (6.6) | 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 |

(continued on following page)
**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 (19.1)        | 63 (10.0)           |
| ≤ 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**                        |                  |                     |
| 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.

*Among patients with known estrogen receptor, progesterone receptor, and HER2 status.