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Associations Between Cancer Predisposition Testing Panel Genes and Breast Cancer

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IMPORTANCE Germline pathogenic variants in *BRCA1* and *BRCA2* predispose to an increased lifetime risk of breast cancer. However, the relevance of germline variants in other genes from multigene hereditary cancer testing panels is not well defined.

OBJECTIVE To determine the risks of breast cancer associated with germline variants in cancer predisposition genes.

DESIGN, SETTING, AND PARTICIPANTS A study population of 65 057 patients with breast cancer receiving germline genetic testing of cancer predisposition genes with hereditary cancer multigene panels. Associations between pathogenic variants in non-*BRCA1* and non-*BRCA2* predisposition genes and breast cancer risk were estimated in a case-control analysis of patients with breast cancer and Exome Aggregation Consortium reference controls. The women underwent testing between March 15, 2012, and June 30, 2016.

MAIN OUTCOMES AND MEASURES Breast cancer risk conferred by pathogenic variants in non-*BRCA1* and non-*BRCA2* predisposition genes.

RESULTS The mean (SD) age at diagnosis for the 65 057 women included in the analysis was 48.5 (11.1) years. The frequency of pathogenic variants in 21 panel genes identified in 41 611 consecutively tested white women with breast cancer was estimated at 10.2%. After exclusion of *BRCA1*, *BRCA2*, and syndromic breast cancer genes (*CDH1*, *PTEN*, and *TP53*), observed pathogenic variants in 5 of 16 genes were associated with high or moderately increased risks of breast cancer: *ATM* (OR, 2.78; 95% CI, 2.22-3.62), *BARD1* (OR, 2.16; 95% CI, 1.31-3.63), *CHEK2* (OR, 1.48; 95% CI, 1.31-1.67), *PALB2* (OR, 7.46; 95% CI, 5.12-11.19), and *RAD51D* (OR, 3.07; 95% CI, 1.21-7.88). Conversely, variants in the *BRIP1* and *RAD51C* ovarian cancer risk genes; the *MRE11A*, *RAD50*, and *NBN* MRN complex genes; the *MLH1* and *PMS2* mismatch repair genes; and *NF1* were not associated with increased risks of breast cancer.

CONCLUSIONS AND RELEVANCE This study establishes several panel genes as high- and moderate-risk breast cancer genes and provides estimates of breast cancer risk associated with pathogenic variants in these genes among individuals qualifying for clinical genetic testing.

← Editorial

+ Supplemental content

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Recent improvements in DNA sequencing technology have led to the development of multigene panels for clinical genetic testing of several conditions. In particular, panels targeting genes implicated in cancer susceptibility have increased the likelihood of detecting cancer-predisposing variants and offer advantages in time and cost compared with single gene testing.[1-3] A broad range of cancer susceptibility panels are available from genetic testing laboratories.[1,4-6] These include high-penetrance *BRCA1* and *BRCA2* breast and ovarian cancer genes; mismatch repair genes; high-penetrance *CDH1*, *PTEN*, *STK11*, and *TP53* genes that are associated with hereditary diffuse gastric cancer as well as Cowden disease, Peutz-Jeghers syndrome, and Li-Fraumeni syndrome, respectively; and genes associated with moderate risks of breast cancer (2-fold to 5-fold), such as *CHEK2* and *ATM*.[7,8] Patients with pathogenic variants in any of these genes are eligible for increased surveillance for cancer or other preventive measures. Cancer gene testing panels identify variants in substantial proportions of patients.[1,4,6] However, the frequency of variants in each of the panel genes among individuals qualifying for clinical genetic testing remains to be defined, and the risks of breast and other cancers associated with variants in many panel genes are not established. In this study, we report on the risks of breast cancer associated with inactivating variants in these genes identified by clinical genetic testing of patients with breast cancer by 1 laboratory.

Methods

Study Population

Study participants included a nationwide sample of 65 057 women with breast cancer referred for hereditary cancer genetic testing by Ambry Genetics Inc between March 15, 2012, and June 30, 2016. The mean (SD) age at diagnosis for the 65 057 women included in the analysis was 48.5 (11.1) years. Demographic, clinical history, and family history of cancer information (eTable 1 and eTable 2 in the Supplement) were collected from test requisition forms, clinic notes, and pedigrees provided by ordering clinicians at the time of testing. Information was collected on current age, personal history, and age at diagnosis of all cancers, ancestry, tumor pathology, family history of cancer with cancer type, and age at diagnosis among relatives. The study was approved by the solutions institutional review board, which also determined that this study was exempt from consent requirements.

Phenotype Data

A potential limitation of this study is the quality and quantity of the clinical history information collected for the panel-tested patients. The variant frequencies and breast cancer risk estimates from this study were derived from probands and were not dependent on family history information. To assess data quality, a review of a random sample of 1200 breast and ovarian cancer patient intake forms was conducted. Of these, 520 (43.3%) forms had additional clinical history documentation available. The accuracy of personal cancer history was greater than 97% (eMethods in the Supplement).

Key Points

Question Which genes on hereditary cancer multigene testing panels are associated with high or moderate risks of breast cancer among patients qualifying for clinical genetic testing?

Findings In a case-control study of 65 057 patients with breast cancer, inherited pathogenic variants in *PALB2* were associated with high risks of breast cancer, and variants in *CHEK2*, *ATM*, *BARD1*, and *RAD51D* were associated with moderate risks of breast cancer. Variants in *MRE11A*, *RAD50*, *NBN*, *BRIP1*, *RAD51C*, *MLH1*, and *NF1* were not associated with increased risks of breast cancer.

Meaning Although pathogenic variants in several panel genes confer increased risks of breast cancer and may qualify patients for increased cancer surveillance, variants in several other cancer panel genes may not predispose to breast cancer.

Multigene Panel Testing

Mutation testing was performed by targeted custom capture and sequencing and targeted chromosomal microarray analysis (eMethods in the Supplement).[1] Results from germline genetic testing of 21 known and candidate breast cancer predisposition genes from custom capture sequencing panels (Breast-Next, OvaNext, PancNext, CancerNext, CancerNext-Expanded, ColoNext, BRCAplus, BRCAplus-Expanded, and GYNplus; all Ambry Genetics Inc) (eTable 3 in the Supplement) were included in this study. A 5-tier variant classification system (eTable 4 in the Supplement)[9] was applied to all alterations. All variants identified by Ambry Genetics Inc are submitted to the ClinVar public database (https://www.ncbi.nlm.nih.gov/clinvar/).

Normalization of Breast Cancer Cases and Controls

Among 65 057 patients with breast cancer who underwent testing, 64 405 were women. Patients tested between July and December 2015 were excluded because of incomplete abstraction of sequential patient records, resulting in 58 798 eligible consecutive breast cancer cases. Of these, 41 611 self-identified as white or Ashkenazi Jewish (subsequently referred to as white) (eTable 1 in the Supplement). Restricting inclusion to patients with breast cancer as the first cancer diagnosis and applying filters for matching with Exome Aggregation Consortium (ExAC) controls, as described below, yielded 54 585 patients with breast cancer of all ethnicities and 38 326 white patients (eFigure in the Supplement). The non-Finn European (NFE) population in the ExAC data set,[10] excluding The Cancer Genome Atlas (TCGA) exomes, were used as reference controls for case-control association studies, consistent with the effective use of this data set for estimation of ovarian and prostate cancer risks in recent studies.[11,12] ExAC variants in the PASS and non-PASS category were defined as having Genome Analysis Tool Kit Variant Quality Score Recalibration sensitivity of 99.6% and 95% sensitivity, respectively (http://exac.broadinstitute.org/terms). Although most ExAC variants in this analysis were in the PASS category, several non-PASS variants also detected by Ambry Genetics Inc were included in the ExAC reference data to avoid inflation of gene-specific breast cancer

risks. All remaining loss-of-function variants and any missense variants (defined as pathogenic in ClinVar by clinical laboratories) in breast cancer cases and ExAC controls were selected for analysis. Filtering steps were applied (eMethods in the Supplement) to normalize differences in the breast cancer cases and ExAC controls. Variants with minor allele frequency greater than 0.3% other than common founder mutations were excluded (eTable 5 and eMethods in the Supplement). All suspected mosaic somatic variants (allele ratio >70:30) and truncating variants in the last 55 base pairs of the penultimate exon or last exon that potentially avoid nonsense-mediated messenger RNA decay and do not influence known functional domains were excluded. Large genomic rearrangements of 1 or more exons were excluded because rearrangements were not validated among reference controls. (eFigure in the Supplement).

Statistical Analysis

Associations between pooled pathogenic variants in each gene (eTable 6 in the Supplement) and phenotypic characteristics of breast cancer cases were assessed using the Fisher exact test. Associations with age at diagnosis were estimated using the Kolmogorov-Smirnov test. The observed frequency of all pathogenic variants within each gene was compared between white patients with breast cancer and ExAC-NFE non-TCGA reference controls. Strength of associations with breast cancer was estimated by odds ratios (ORs) and corresponding 95% CIs based on the Fisher exact test (Table; eTable 7 in the Supplement). $P < .05$ was considered statistically significant. Genes were categorized as high risk (OR, >5.0), moderate risk (OR, 2.0-5.0), or no clinical relevance (OR, <2.0). A series of sensitivity analyses were performed for truncating variants only; cases with pathogenic variants in more than 1 gene; BreastNext-tested cases; all ethnicities combined; and ExAC-NFE nonTCGA PASS reference controls; exclusion of cases with prior testing of *BRCA1* (GenBank, NM_007294.3) and *BRCA2* (GenBank, NM_000059.3); exclusion of ductal carcinoma in situ; and exclusion of cases with a personal or family history of ovarian or colorectal cancer (eTables 8-17 in the Supplement).

Results

Characteristics of Study Population

Clinical and phenotypic characteristics of 121 197 patients subjected to multigene testing, including 65 057 (53.7%) individuals with breast cancer, are reported in eTable 1 in the Supplement. Among the patients with breast cancer, 38 844 (59.7%) developed breast cancer at age 50 years or younger, and 8851 (13.6%) had bilateral disease. Most patients with breast cancer reported a family history of breast cancer (39 878 [61.3%]), colorectal cancer (14 959 [23.0%]), or ovarian cancer (8589 [13.2%]) (eTable 1 in the Supplement). Of the remaining patients not reporting any family history of breast, ovarian, colorectal, or pancreatic cancer (8599 [13.2%]), 7320 (85.1%) developed breast cancer at 50 years or younger or reported bilateral or triple-negative disease.

Variants Identified by Panel Testing

The frequencies of pathogenic variants in each of the 21 genes were estimated among the 58 798 eligible consecutive women with breast cancer, including 41 611 white patients. Because a subset of patients was not tested for all genes, the frequencies of pathogenic variants from each of the 21 genes were combined to estimate the overall frequency of pathogenic variation. Thus, the combined frequency of pathogenic variants among 41 611 white women with breast cancer was 10.2% (eTable 6 in the Supplement). Exclusion of *BRCA1*, *BRCA2*, and the common lower-risk p.Ile157Thr and p.Ser428Phe *CHEK2* (GenBank, NM_007194.3) founder variants yielded a variant frequency of 6.18%. The most commonly mutated non-*BRCA1* and non-*BRCA2* genes among white women with breast cancer were *CHEK2* (1.73%), *ATM* (GenBank, NM_000051.3) (1.06%), and *PALB2* (GenBank, NM_024675.3) (0.87%) (eTable 6 in the Supplement).

Phenotypic Associations With Pathogenic Variants

To assess associations between pathogenic variants in non-*BRCA1* and non-*BRCA2* predisposition genes and phenotypic characteristics of patients, we restricted analyses to the 54 585 patients with breast cancer of all ethnicities and 38 326 white patients eligible for association analyses (eTable 2 in the Supplement). Among white patients, pathogenic variants in *CHEK2* (OR, 1.35; 95% CI, 1.12-1.63; $P = 2.00 \times 10^{-4}$), *PALB2* (OR, 1.51; 95% CI, 1.09-2.05; $P = .01$), and *TP53* (GenBank, NM_000546.5) (OR, 2.46; 95% CI, 1.26-4.65; $P = .007$) were associated with bilateral breast cancer, whereas variants in *BRIP1* (GenBank, NM_032043.2) (OR, 5.22; 95% CI, 1.99-12.67; $P = .004$) and *MSH2* (GenBank, NM_000251.2), (OR, 18.44; 95% CI, 3.98-77.80; $P = .001$) were associated with a personal history of ovarian cancer. Only *PALB2* variants were associated (OR, 1.59; 95% CI, 1.15-2.19; $P = .004$) with a family history (1st- or 2nd-degree relatives) of breast cancer. In contrast, *BRIP1* (OR, 2.42; 95% CI, 1.41-4.13; $P = .002$), *RAD51C* (GenBank, NM_058216.2) (OR, 2.89; 95% CI, 1.26-6.45; $P = .01$), and *TP53* (OR, 14.58; 95% CI, 3.02-103.47; $P = .001$) were associated with family history of ovarian cancer. Only patients with breast cancer with pathogenic variants in *CHEK2* (age, 47.7 vs 49.7 years; $P = .003$) and *TP53* (age, 37.1 vs 49.4 years; $P < .001$) had a significantly younger age at diagnosis than did noncarriers.

Breast Cancer Case-Control Association Analysis

Associations between pooled pathogenic variants in 16 panel genes and breast cancer were assessed using sequencing results from 38 326 white patients with breast cancer and 26 911 ExAC-NFE non-TCGA controls (Figure). Pathogenic variants in *PALB2* were associated with high breast cancer risk (OR, 7.46; 95% CI, 5.12-11.19; $P = 4.3 \times 10^{-38}$) (Table), consistent with segregation studies of high-risk families.[13-15] *CHEK2* c.1100delC (OR, 2.31; 95% CI, 1.88-2.85; $P = 3.04 \times 10^{-17}$), pathogenic variants in *CHEK2* (OR, 2.26; 95% CI, 1.89-2.72; $P = 1.75 \times 10^{-20}$) after exclusion of the lower-risk p.Ile157Thr and p.Ser428Phe founder variants, and pathogenic variants in *ATM* (OR, 2.78; 95% CI, 2.22-3.62; $P = 2.4 \times 10^{-19}$) were associated with moderate risks (OR, 2-5)

of breast cancer (Table) consistent with results from a recent review of established predisposition genes.[5]

Several other genes were also associated with increased risks of breast cancer. Pathogenic variants in *BARD1* (GenBank, NM_000465.3) (OR, 2.16; 95% CI, 1.31-3.63; *P* = 2.26 × 10⁻³) and *RAD51D* (GenBank, NM_002878.3) (OR, 3.07; 95%, CI 1.21-7.88; *P* = .01) were significantly associated with moderate risks (Table), whereas *MSH6* (GenBank, NM_000179.2) (OR, 1.93; 95% CI, 1.16-3.27; *P* = .01) was only

marginally below the moderate-risk threshold (OR, ≥2) (Table). Variants in both *MSH2* and *CDKN2A* (GenBank, NM_000249.3) yielded moderate effects, but both associations were nonsignificant due to limited numbers of variants in cases and controls (Table). *BRIP1* mutations conferred only a slightly increased risk of breast cancer (OR, 1.63; 95% CI, 1.11-2.41; *P* = .01), consistent with results from a case-control study involving familial cases (relative risk [RR], 2.0; 95% CI, 1.3-3.0; *P* = .01).[16] Similarly, *RAD51C*, *NF1* (GenBank, NM_000267.3), and the MRN complex genes *NBN* (GenBank, NM_002485.4), *MRE11A* (GenBank, NM_005591.3), and *RAD50* (GenBank, NM_005732.3) were not associated with increased breast cancer risks (Table).[17] Associations between pooled pathogenic variants in *BRCA1*, *BRCA2*, and the *CDH1* (GenBank, NM_004360.4), *PTEN* (NM_000314.6), and *TP53* syndromic genes were also assessed (eTable 7 in the Supplement). However, the attenuated risks associated with *BRCA1* and *BRCA2* pathogenic variants resulting from an enrichment of the cohort for patients who previously tested negative for these genes must be interpreted with care. Similarly, risk estimates for *CDH1*, *PTEN*, and *TP53* were based on very small numbers of patients with pathogenic variants and may also be influenced by limited ascertainment of patients with the associated clinical syndromes. None of the 23 patients with *CDH1* pathogenic variants reported a personal history of gastric cancer.

A series of sensitivity analyses were also performed to assess the influence of various subsets of patients with breast cancer and ExAC control selection on the associations with breast cancer. Effect sizes of associations were consistently inflated for the 16 genes when using ExAC-NFE non-TCGA

Figure. Odds Ratio Between Combined Pathogenic Variants in Each Gene and Breast Cancer Among White Women With Breast Cancer and Reference Controls
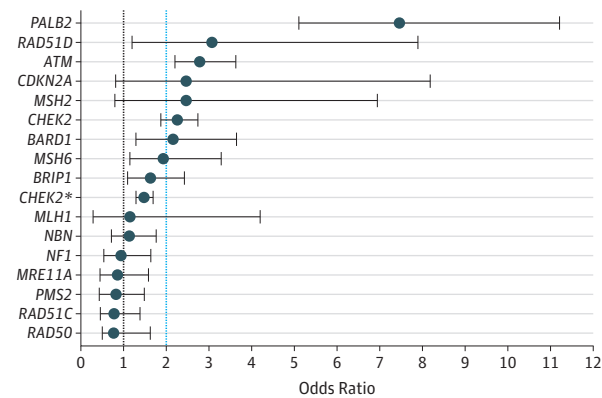
Exome Aggregation Consortium Non-Finn European data set excluding The Cancer Genome Atlas exomes served as the reference control group. Error bars represent 95% CIs.

Table. Estimated Risks of Breast Cancer Associated With Mutations in Selected Panel Genes in Patients of European Ancestry

Gene	Ambry Genetics Inc Cases			ExAC Controls			Cancer Risk	
	Mutated Alleles, No.	Cases, No.	Mutation Frequency, %	Mutated Alleles, No.	Individuals, No.	Mutation Frequency, %	OR (95% CI)	P Value
ATM	274	29 229	0.94	90	26 644	0.34	2.78 (2.22-3.62)	2.42 × 10⁻¹⁹
BARD1	52	28 536	0.18	22	26 078	0.08	2.16 (1.31-3.63)	2.26 × 10⁻³
BRIP1	71	28 536	0.25	41	26 840	0.15	1.63 (1.11-2.41)	.01
CDKN2A	6	8457	0.07	7	24 312	0.03	2.47 (0.83-8.16)	.11
CHEK2	424	29 090	1.46	163	25 215	0.65	2.26 (1.89-2.72)	1.75 × 10⁻²⁰
CHEK2 1100delC	338	29 090	1.16	127	25 215	0.50	2.31 (1.88-2.85)	3.04 × 10⁻¹⁷
CHEK2*	721	29 090	2.48	424	25 215	1.68	1.48 (1.31-1.67)	1.11 × 10⁻¹⁰
MLH1	4	15 475	0.03	6	26 639	0.02	1.15 (0.30-4.19)	>.99
MRE11A	21	28 536	0.07	23	26 767	0.09	0.86 (0.46-1.57)	.65
MSH2	9	15 475	0.06	6	25 329	0.02	2.46 (0.81-6.93)	.11
MSH6	32	15 475	0.21	28	26 151	0.11	1.93 (1.16-3.27)	.01
NBN	48	28 536	0.17	39	26 264	0.15	1.13 (0.73-1.75)	.59
NF1	27	25 950	0.10	29	26 130	0.11	0.94 (0.55-1.62)	.89
PALB2	241	30 025	0.80	29	26 869	0.11	7.46 (5.12-11.19)	4.31 × 10⁻³⁸
PMS2	17	15 475	0.11	33	24 674	0.13	0.82 (0.44-1.47)	.56
RAD50	45	28 536	0.16	54	26 474	0.20	0.77 (0.52-1.61)	.23
RAD51C	26	28 536	0.09	31	26 647	0.12	0.78 (0.47-1.37)	.43
RAD51D	18	25 950	0.07	6	26 555	0.02	3.07 (1.21-7.88)	.01

Abbreviations: ExAC, Exome Aggregation Consortium; OR, odds ratio.

CHEK2*: Inclusion of common missense variants p.Ile157Thr and p.Ser428Phe.

PASS reference controls instead of PASS/non-PASS controls (eTable 8 in the Supplement). For example, *BARD1* variants showed effects ranging from ORs of 2.16 to 3.18, and *PALB2* variants ranged from ORs of 7.46 to 8.66 (Table and eTable 8 in the Supplement). Associations for each gene were also estimated after exclusion of patients with breast cancer reporting prior testing for *BRCA1*, *BRCA2*, or multigene panels. Results were consistent with those from the primary analysis (eTable 9 in the Supplement). In addition, a sensitivity analysis of patients tested only by the BreastNext panel was conducted to assess whether combining results from multiple panels that did not always contain the full complement of genes influenced the combined allele frequencies and the estimated risks of breast cancer. Only minor changes in risk estimates were observed (eTable 13 in the Supplement).

Sensitivity analyses were also conducted when restricting analysis to pathogenic protein-truncating variants (eTable 10 in the Supplement), excluding ductal carcinoma in situ (eTable 11 in the Supplement), and including patients with pathogenic variants in multiple genes (eTable 12 in the Supplement). Results for each gene were highly consistent across all of these analyses. In contrast, no associations with breast cancer were observed for the mismatch repair genes when excluding all patients with personal and family history of ovarian and/or colorectal cancer (eTables 14-16 in the Supplement). Similarly, associations between pathogenic variants in *RAD51D* were attenuated when including patients of all ethnicities and ExAC non-TCGA PASS reference controls due to recurrent variants in the South East Asian reference population (eTable 17 in the Supplement).

Discussion

We present results from multigene panel–based clinical testing for pathogenic variants in inherited cancer genes among 65 057 patients with breast cancer. Pathogenic variants in 21 panel genes were identified in 10.2% of white women with breast cancer and in 6.2% of women with breast cancer after exclusion of *BRCA1* and *BRCA2*. These findings were somewhat consistent with the 3.8%,[6] 3.9%,[17] and 4.6%[18] variant frequencies from other studies of breast cancer cases enriched for a family history of breast and/or other cancers.

This study provides insight into genes with pathogenic variants that predispose to moderate and high risks of breast cancer. In total, 5 of 16 non-*BRCA1/2*, nonsyndromic panel genes were significantly associated with moderate or high (OR, >2) breast cancer risk in the white population (*PALB2*, *ATM*, *CHEK2*, *BARD1*, and *RAD51D*). *PALB2* was confirmed as a high-risk breast cancer gene (OR, 7.46; 95% CI, 5.12-11.19) in this testing population, consistent with a cumulative lifetime risk of up to 58% for breast cancer from family segregation studies.[13] We also confirmed that *CHEK2* and *ATM* are associated with increased breast cancer risk.[5] Several stratified analyses of the patients with breast cancer and the ExAC reference controls in this study also provided consistent results. These findings provide further support for the National Comprehen-

sive Cancer Network recommendations, version 1.2017 (http://www.nccn.org/professionals/physician_gls/f_guidelines.asp#detection) for management of treatment for patients with pathogenic *ATM*, *CHEK2*, and *PALB2* variants.

We establish that pathogenic variants in *BARD1* and *RAD51D* are associated with moderately increased risks of breast cancer. Because pathogenic variants in these genes are rare (<1 in 500 in patients with breast cancer), previous studies had insufficient numbers of breast cancer cases and controls to adequately assess the influence of pathogenic variants in these genes on breast cancer risk.[5] This was possible only by using more than 25 000 patients with breast cancer and reference controls in this study. Additional studies of patients with *BARD1* and *RAD51D* variants are now needed to better understand the related breast cancer phenotypes. Furthermore, *MSH6* pathogenic variants were associated with near-moderate risks (OR, 1.93; 95% CI, 1.16-3.27) of breast cancer, contrary to a previous study suggesting little influence of mismatch repair gene mutations on breast cancer risk.[19] However, excluding patients with breast cancer who had a personal or family history of colorectal cancer removed all evidence of an influence on breast cancer. Family-based segregation studies will be needed to determine whether variants in this gene have no influence on breast cancer risk or predispose to complex phenotypes involving breast and colorectal cancer. Additional studies of the influence of *CDKN2A* and *MSH2* on breast cancer are also needed following the observation that pathogenic variants in these genes may be associated with moderate breast cancer risk.

Of equal importance, however, are the findings that pathogenic variants in *NF1*, *BRIP1*, *RAD51C*, the *MLH1* (GenBank, NM_000249.3) and *PMS2* (GenBank, NM_000535.6) mismatch repair genes, and the *MRE11A*, *RAD50*, and *NBN* MRN complex genes did not confer any appreciable risks of breast cancer. Although the *BRIP1* ovarian cancer gene was associated with modestly increased risk of breast cancer overall (OR, 1.63; 95% CI, 1.11-2.41), exclusion of cases with a personal or family history of ovarian cancer to account in part for competing risks of cancer substantially reduced the risks of breast cancer (OR, 1.27; 95% CI, 0.81-1.99) to effect sizes observed for the *BRIP1* p.Arg798Ter variant (OR, 1.09; 95% CI, 0.58-2.03) in other large case-control studies.[20] In contrast, the results for *NBN* differed from those in a large study of the Slavic founder variant (c.657del5) that associated the variant with a moderate risk of breast cancer (RR, 2.4; 95% CI, 1.9-3.7).[21] Likewise, pathogenic variants in *NF1* among patients with neurofibromatosis have been associated with moderate risks of breast cancer (RR, 2.6; 95% CI, 2.1-3.2),[22,23] whereas we failed to observe any influence on breast cancer risk. Thus, additional studies of the influence of *NF1* pathogenic variants on breast cancer risk in individuals with and without neurofibromatosis are needed. In contrast, results demonstrating no increased risk for breast cancer for pathogenic variants in *RAD51C*, *MLH1*, *PMS2*, *RAD50*, *MRE11A*, and *NBN* were consistent across all stratified analyses, suggesting that these genes may not be relevant in clinical testing for breast cancer risk. However, it remains to be determined whether specific missense variants in these genes influence risk.

We acknowledge the limitations of the public reference data set; however, extensive data cleaning and filtering were used in an effort to normalize the breast cancer cases and control data.

Limitations

This study was focused on patients qualifying for clinical genetic testing and was not a population-based study. In addition, associations between pathogenic variants in panel genes and breast cancer were evaluated using sequencing results from breast cancer cases and the database of ExAC reference samples. The use of results from unmatched cases and controls that were sequenced on different platforms could have caused inflation of ORs for breast cancer. This limitation could be addressed in the future using combined case-control studies matched on age and race. However, when considering sequence quality, variant allele frequency, race, and ethnicity, and excluding known cancer samples, the ExAC-NFE non-TCGA data set offered a reasonable approximation of white population-based allele frequencies.[24] ExAC controls have been used to identify genes that predispose to ovarian and prostate cancer,[11,12] to exclude other genes from involvement in these cancers, and to approximate risks associated with variants when comparing ExAC data with variants detected on other platforms.[12] Similarly, ExAC data have been used to evaluate genes associated with increased cardiovascular risk.[25] Thus, although variants in ExAC and ovarian or prostate cancer cases were derived from different sequencing platforms and were identified using different algorithms, the studies successfully identified genes associated with increased risks.

Conclusions

We present breast cancer risk estimates in a clinical, multigene panel testing cohort as useful indicators of the clinical relevance of pathogenic variants in breast cancer susceptibility genes. The breast cancer cases qualifying for clinical genetic testing were enriched for a clinical history of early-onset, bilateral, and triple-negative breast disease and a family history of breast cancer. Therefore, the risk estimates derived in this study are likely to be inflated over estimates from population-based studies, as previously reported for *PALB2* pathogenic variants from high-risk families.[13] Although the risks presented herein may not be generalizable to all mutation carriers, they are highly relevant to those with clinical histories suggestive of hereditary breast cancer predisposition. Future studies involving patients with breast cancer unselected for age at diagnosis, tumor pathology, and family cancer history and incorporating a broader range of alterations in addition to segregation studies in families are needed to further inform breast cancer risks for mutation carriers.

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