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Pathogenic Variants in Cancer Susceptibility Genes Predispose to Ductal Carcinoma *In Situ* of the Breast

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ABSTRACT

Purpose: To determine the relationship between germline pathogenic variants (PV) in cancer predisposition genes and the risk of ductal carcinoma *in situ* (DCIS).

Experimental Design: Germline PV frequencies in breast cancer predisposition genes (*ATM*, *BARD1*, *BRCA1*, *BRCA2*, *CDH1*, *CHEK2*, *PALB2*, *RAD51C*, and *RAD51D*) were compared between DCIS cases and unaffected controls and between DCIS and invasive ductal breast cancer (IDC) cases from a clinical testing cohort ($n = 9,887$), a population-based cohort ($n = 3,876$), and the UK Biobank ($n = 2,421$). The risk of contralateral breast cancer (CBC) for DCIS cases with PV was estimated in the population-based cohort.

Results: Germline PV were observed in 6.5% and 4.6% of women with DCIS in the clinical testing and population-based cohorts, respectively. *BRCA1*, *BRCA2*, and *PALB2* PV frequencies

were significantly lower among women with DCIS than those with IDC (clinical cohort: 2.8% vs. 5.7%; population-based cohort: 1.7% vs. 3.7%), whereas the PV frequencies for *ATM* and *CHEK2* were similar. *ATM*, *BRCA1*, *BRCA2*, *CHEK2*, and *PALB2* PV were significantly associated with an increased risk of DCIS (OR > 2.0), but only *BRCA2* PV were associated with high risk (OR > 4) in both cohorts. The cumulative incidence of CBC among carriers of PV in high-penetrance genes with DCIS was 23% over 15 years.

Conclusions: The enrichment of PV in *ATM*, *BRCA1*, *BRCA2*, *CHEK2*, and *PALB2* among women with DCIS suggests that multigene panel testing may be appropriate for women with DCIS. Elevated risks of CBC in carriers of PV in high-penetrance genes with DCIS confirmed the utility of testing for surgical decision-making.

Introduction

Ductal carcinoma *in situ* (DCIS) is a relatively common type of breast cancer and accounts for about 20% to 25% of all new breast cancer cases diagnosed in the United States (1, 2). DCIS is considered a non-obligate precursor to invasive ductal breast cancer (IDC), and understanding predisposing factors to DCIS could have implications for the early detection and prevention of breast cancer.

Similarities in the risk factors for DCIS and IDC, including hormonal factors, mammographic density, and family history of breast cancer, have been identified (3–6). However, the contribution of rare germline pathogenic variants (PV) in cancer predisposition genes to the risk of DCIS is poorly understood. Prior studies of *BRCA1* and *BRCA2*, which focused on those qualifying for germline genetic testing because of young age at diagnosis or family history of cancer, found that PV were less frequent in women with DCIS than

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Translational Relevance

Due to a limited understanding of genetic predisposition to ductal carcinoma *in situ* (DCIS) of the breast, current guidelines for germline genetic testing do not distinguish between DCIS and invasive disease. To personalize germline genetic testing, a comprehensive assessment of genetic predisposition to DCIS was conducted using clinical testing and population-based cohorts. This study found that pathogenic variants (PV) in *ATM*, *BRCA1*, *CHEK2*, and *PALB2* were associated with moderate risk and PV in *BRCA2* were associated with high risk of DCIS. The incidence of contralateral breast cancer among *BRCA1*, *BRCA2*, and *PALB2* PV carriers with DCIS was 23% over 15 years. These findings establish that predisposition gene PV are associated with an increased risk of DCIS and may inform the selection of appropriate risk management strategies in germline PV carriers.

those with invasive breast cancer (7–9) but were more frequent in DCIS relative to low-grade invasive disease (10). In contrast to *BRCA1/2*, the frequencies of PV in other breast cancer predisposition genes in DCIS and the risks of DCIS associated with these PV have not been established (7–11). Furthermore, an increased risk of contralateral breast cancer (CBC) has been established for carriers of PV in *BRCA1*, *BRCA2*, *CHEK2*, or *PALB2* with breast cancer (12–15), but it is unknown whether these risk estimates apply to PV carriers with DCIS. In part due to a lack of understanding of genetic predisposition to DCIS, current guidelines on germline genetic testing and management of PV carriers do not distinguish between DCIS and IDC (16). A comprehensive assessment of genetic predisposition to DCIS is needed to personalize germline genetic testing in women with DCIS and identify appropriate management strategies for PV carriers with DCIS. Herein, we present the results of germline genetic testing among women with DCIS from clinical and population-based cohorts and identify the risk of DCIS and CBC among carriers of PV in cancer predisposition genes.

Materials and Methods

Study population

The study was approved by the Western Institutional Review Board-Copernicus Group, which exempted review of the clinical testing cohort, and by the Mayo Clinic Institutional Review Board. The “CAnceR RiSk Estimates Related to Susceptibility” (CARRIERS) consortium consisted of 12 population-based and family-based studies of breast cancer from the United States, including 4,628 women with DCIS, 18,193 women with IDC, and 25,007 unaffected women. The population-based cohort within CARRIERS included 3,876 women with DCIS, 13,701 women with IDC, and 22,186 unaffected women matched to breast cancer by age from nine prospective studies. Invasive or *in situ* CBC or second breast cancer (combined ipsilateral and CBC) risk was evaluated among 3,024 women with unilateral DCIS receiving ipsilateral surgery for initial breast cancer. DCIS with no invasive component was confirmed by the underlying cohorts within CARRIERS using data from medical records and cancer registries. Women diagnosed with bilateral breast cancer within 1 year of initial diagnosis, undergoing bilateral mastectomy for initial breast cancer, or those missing age at initial breast cancer or CBC status were excluded from the CBC

evaluation. The details of the CARRIERS study have been previously published (15, 17–19) and are also provided in Supplementary Methods.

The clinical testing cohort consisted of a nationwide sample of 9,887 women with DCIS who underwent clinically indicated hereditary cancer multigene panel testing at Ambry Genetics from March 2012 to December 2016. Data on patient characteristics were collected from requisition forms at the time of testing and clinical notes provided by ordering clinicians. For comparison, information on 38,057 women with IDC who underwent germline genetic testing during the same time period at Ambry Genetics was extracted. The diagnoses and tumor pathology for 10% of the patients with DCIS and invasive disease were confirmed in medical records as part of a validation study. Women who had previously undergone testing for *BRCA1* or *BRCA2* prior to multigene panel testing were excluded.

The UK Biobank (UKB; RRID: SCR_012815) is a population-based prospective cohort study of more than 500,000 subjects (20). DCIS cases ($n = 2,339$) were defined by International Classification of Diseases-10 code D05, as determined by linkage to the UK National Cancer Registration and Analysis Service, or self-reported breast cancer. Both prevalent and incident cases were included. The control group consisted of females without breast cancer ($n = 203,896$).

Germline genetic testing and variant classification

For the population-based cohort, germline DNA samples were subjected to multiplex amplicon-based analysis of 746 target regions covering all coding regions and consensus splice sites in cancer predisposition genes using a QIAseq custom panel (17). For the clinical testing cohort, testing of five to 49 genes, depending on the multigene panel ordered, was performed by targeted custom capture and sequencing of all coding domains and flanking 5'- and 3'-ends of all the introns and untranslated regions (21, 22). For both cohorts, germline genetic testing results from 18 cancer predisposition genes were assessed, and results for 10 breast cancer genes (*ATM*, *BARD1*, *BRCA1*, *BRCA2*, *CDH1*, *CHEK2*, *PALB2*, *RAD51C*, *RAD51D*, and *TP53*) were analyzed (Supplementary Table S1; refs. 16, 23, 24). For UKB, gene-specific sequencing data and called variants for these genes from whole-exome sequencing data were accessible through the UKB DNAnexus platform. Quality control metrics for genotype depth and quality were applied (25, 26). A five-tier system was used to classify variants using an American College of Medical Genetics framework (27). PV and likely PV were analyzed together. Known low-penetrance variants were excluded from all analyses (*CDKN2A*^{149T}; *CHEK2*^{H371Y}, *CHEK2*^{I157T}, and *CHEK2*^{S428F}; *TP53*^{R156H} and *TP53*^{R181H}).

Statistical analysis

The frequency of PV in each gene was assessed among women with DCIS in the clinical testing and population-based cohorts and among subsets of women with DCIS based on estrogen receptor (ER) status, age, and family history of breast and ovarian cancers. To identify the genes associated with the increased risk of DCIS, the frequency of PV in each gene in women with DCIS in the clinical testing cohort was compared with the frequency from gnomAD 2.1 reference non-female, noncancer controls (<http://gnomad.broadinstitute.org/>; RRID: SCR_014964; ref. 28) with weighting for the relative frequency of different races and ethnicities utilizing a logistic regression model (17). Copy-number variations and gnomAD filter non-PASS variants were excluded (29). For the population-based cohort, case-control association testing for the risk of DCIS among PV carriers in each gene

Table 1. Characteristics of DCIS cases.

Characteristic	Clinical testing cohort (N = 9,887)	Population-based cohort (N = 3,876)
Age at diagnosis, years		
Median (SD)	55 (10.5)	62 (11.2)
Range	17–90	23–94
≤36	713 (7.2%)	52 (1.4%)
37–45	2,954 (30.0%)	418 (11.0%)
46–50	2,106 (21.4%)	498 (13.1%)
51–60	2,350 (23.9%)	1,158 (30.4%)
>60	1,720 (17.5%)	1,677 (44.1%)
Race/ethnicity		
Non-Hispanic White	6,352 (64.2%)	3,130 (81.2%)
African American	766 (7.7%)	613 (15.9%)
Hispanic	571 (5.8%)	54 (1.4%)
Asian	564 (5.7%)	30 (0.8%)
Others ^a	1,634 (16.6%)	27 (0.7%)
Family history of breast cancer ^b		
Yes	6,308 (66.1%)	841 (22.2%)
No	3,231 (33.9%)	2,951 (77.8%)
Unknown	348	84
ER status		
Negative	887 (15.1%)	391 (16.0%)
Positive	5,000 (84.9%)	2,051 (84.0%)
Unknown	4,000	1,434

^aIncludes 407 (4.1%) participants of Ashkenazi Jewish ancestry.

^bFamily history of breast cancer in first- or second-degree relatives was included for the clinical testing cohort, whereas family history of breast cancer in first-degree relatives only was included in the population-based cohort.

was conducted with logistic regression models adjusting for age at diagnosis, self-reported race and ethnicity, and study. For the UKB, DCIS case–control association analysis was performed using the Fisher exact test. Enrichment analysis comparing PV in DCIS and IDC in the clinical testing and population-based cohorts was conducted using logistic regression adjusting for age at diagnosis, family history of breast or ovarian cancer, and ER status. Among women with DCIS in the population-based cohort, the cumulative incidence of CBC for PV carriers in *BRCA1* and *BRCA2* combined, as well as in the three high-risk genes (*BRCA1*, *BRCA2*, and *PALB2*), was evaluated. This time-to-event analysis began at the time of the first breast cancer diagnosis and adjusted for the competing risk of death using the Fine and Gray method (30). Censoring occurred at the last follow-up or at contralateral prophylactic mastectomy, whichever came first. All tests were two-sided; $P < 0.05$ was considered statistically significant. All analyses were performed using R version 3.6 (RRID: SCR_001905).

Data availability

All data presented in the article and supplementary materials are available directly from the authors. CARRIERS population-based data are available at dbGAP (phs002820).

Results

Patient characteristics

The median age at diagnosis of DCIS was 55 years in the clinical testing cohort and 62 years in the population-based cohort. More than 81% of the women in the population-based cohort and 64% in the clinical cohort were non-Hispanic White, and >80% of the tumors with available information on receptor status were ER positive (Table 1).

Frequency of PV in DCIS

The pooled frequency of PV in 10 known breast cancer predisposition genes among women with DCIS was 6.5% in the clinical testing cohort, whereas frequencies from nine genes (excluding *TP53* because of the absence of DCIS cases) were 4.6% in the population-based cohort (Table 2; Supplementary Table S1). In both datasets, PV in *CHEK2* and *BRCA2* were the most common with frequencies >1%. PV in *ATM* had a mean frequency of 1%, and *PALB2* PV ranged from 0.31% to 0.47% in the two cohorts. However, *BRCA1* PV were relatively infrequent with a frequency range of 0.28% to 0.71% (Table 2).

Frequency of PV in DCIS by family history, age at diagnosis, and ER status

Among women in the clinical cohort with first- or second-degree relatives with breast or ovarian cancer, the frequency of PV in 10 predisposition genes was 7.1%, whereas the frequency was 5.1% among those without a family history of these cancers (Supplementary Table S2). Similarly, in the population-based cohort, PV were detected in 7.7% of DCIS cases with first-degree relatives with breast or ovarian cancer and 3.8% of women without a family history (Supplementary Table S2). In both cohorts, PV in *BRCA2* were significantly enriched (clinical $P = 0.02$; population-based $P = 0.002$) among women with a family history. PV in *BRCA1* ($P = 0.004$) and *PALB2* ($P = 0.01$) were significantly enriched in women with a first-degree relative with breast or ovarian cancer in the population-based cohort only. PV in *CHEK2* were observed in >1% of women with DCIS without a family history of breast or ovarian cancer in both cohorts.

Among women diagnosed with DCIS at ≤50 years of age, the frequency of PV was 6.9% in the clinical testing cohort and 6.3% in

Table 2. Associations of gene-specific PV with DCIS in clinical and population-based cohorts.

Gene	DCIS cases			Controls			OR (95% CI)	P value
	Number of PV	Patients tested	Frequency (%)	Number of PV	Subjects tested	Frequency (%)		
Clinical testing cohort ^a								
<i>ATM</i>	73	6,257	1.17	170	44,852	0.38	2.85 (2.16–3.73)	<0.001
<i>BARD1</i>	8	5,815	0.14	32	43,957	0.07	1.80 (0.77–3.71)	0.134
<i>BRCA1</i>	49	8,215	0.60	57	44,925	0.13	4.54 (3.10–6.63)	<0.001
<i>BRCA2</i>	124	8,215	1.51	134	44,217	0.30	4.89 (3.83–6.24)	<0.001
<i>CDH1</i>	0	7,985	0.00	2	43,896	0.005	NA	NA
<i>CHEK2</i>	114	6,254	1.82	178	44,341	0.40	3.29 (2.63–4.11)	<0.001
<i>MSH6</i>	13	3,707	0.35	50	44,138	0.11	2.61 (1.37–4.61)	0.002
<i>PALB2</i>	31	6,668	0.46	83	44,919	0.18	2.28 (1.49–3.38)	<0.001
<i>RAD51C</i>	7	5,834	0.12	30	44,942	0.07	1.66 (0.67–3.55)	0.222
<i>RAD51D</i>	10	5,711	0.18	23	44,875	0.05	3.39 (1.54–6.91)	0.001
Population-based cohort ^b								
<i>ATM</i>	33	3,876	0.85	99	22,186	0.45	1.96 (1.30–2.90)	<0.001
<i>BARD1</i>	9	3,876	0.23	28	22,186	0.13	1.88 (0.83–3.85)	0.101
<i>BRCA1</i>	11	3,876	0.28	30	22,186	0.14	2.07 (0.97–4.13)	0.047
<i>BRCA2</i>	42	3,876	1.08	54	22,186	0.24	4.21 (2.78–6.35)	<0.001
<i>CDH1</i>	4	3,876	0.10	5	22,186	0.02	4.70 (1.15–17.93)	0.022
<i>CHEK2</i>	59	3,876	1.52	101	22,186	0.46	3.25 (2.31–4.52)	<0.001
<i>MSH6</i>	6	3,876	0.15	16	22,186	0.07	2.14 (0.77–5.24)	0.113
<i>PALB2</i>	12	3,876	0.31	26	22,186	0.12	2.61 (1.26–5.12)	0.007
<i>RAD51C</i>	5	3,876	0.13	23	22,186	0.10	1.28 (0.43–3.12)	0.616
<i>RAD51D</i>	3	3,876	0.08	12	22,186	0.05	NA	NA
UKB ^c								
<i>ATM</i>	11	2,421	0.45	289	203,896	0.14	3.32 (1.64–6.04)	<0.001
<i>BRCA1</i>	4	2,421	0.17	169	203,896	0.08	2.06 (0.56–5.39)	0.135
<i>BRCA2</i>	25	2,421	1.03	440	203,896	0.22	4.95 (3.16–7.43)	<0.001
<i>CHEK2</i>	27	2,421	1.11	1,026	203,896	0.50	2.29 (1.50–3.37)	<0.001
<i>PALB2</i>	15	2,421	0.62	278	203,896	0.14	2.28 (1.49–3.38)	<0.001

Excluded *TP53* because of differences in allele counting between clinical cohort cases and public reference controls.

Abbreviations: NA, not analyzed for genes with less than four PV.

^aOR estimates utilizing weighted logistic regression with gnomAD 2.1 reference control populations (exome noncancer female) weighted for the relative frequency of different races and ethnicities in the cases. Copy-number variations were excluded from the analysis.

^bOR estimates utilizing logistic regression adjusted for study, age, family history of breast cancer, and race/ethnicity.

^cOR estimates using the Fisher exact test.

the population-based cohort (Supplementary Table S3). In comparison, the frequencies of PV among women diagnosed at >50 years were 5.9% and 4.1% in the clinical testing and population-based cohorts, respectively. Further analysis by ER status (Supplementary Table S4) demonstrated a comparable frequency of PV among women with ER-positive and ER-negative DCIS in the clinical testing (6.2% in ER-positive vs. 5.8% in ER-negative) and the population-based (4.8% in ER-positive vs. 4.6% in ER-negative) cohorts. However, in both cohorts, *CHEK2* PV were more than twofold enriched, and the frequency of *BRCA1* PV was substantially lower among women with ER-positive DCIS compared with those with ER-negative DCIS.

Associations of PV in cancer predisposition genes with DCIS

Case-control association analysis showed that PV in *BRCA2* were significantly associated with a high risk (OR > 4) of DCIS, whereas PV in *ATM*, *CHEK2*, and *PALB2* were associated with moderate risk (2 < OR < 4) in both the clinical testing and population-based cohorts (Fig. 1; Table 2). *BRCA1* PV were associated with high risk [OR, 4.5, 95% (confidence interval) CI, 3.1–6.6, $P < 0.001$] of DCIS in the clinical testing cohort and moderate risk (OR, 2.1; 95% CI, 1.0–4.1; $P = 0.047$) in the population-based cohort. Interestingly, PV in *MSH6* were

associated with an increased risk of DCIS (OR, 2.6; 95% CI, 1.4–4.6; $P = 0.002$) in the clinical testing cohort and in the population-based cohort (OR, 2.1; 95% CI, 0.8–5.2; $P = 0.11$), although the latter association was not significant. Results from the UKB were consistent with the findings from the population-based study (Table 2). Further analyses of the clinical and population-based cohorts based on ER status of DCIS cases showed that PV in *ATM*, *BRCA2*, *CHEK2*, and *PALB2* were consistently associated with increased risk of ER-positive DCIS (Supplementary Table S5). In contrast, only *BRCA1* and *BRCA2* PV were significantly associated with increased risk of ER-negative DCIS (Supplementary Table S5). Importantly, the ER-negative findings should be interpreted with caution because the numbers of ER-negative DCIS cases were limited.

Comparison of PV frequencies between DCIS and IDC cases

The frequency of PV in the breast cancer predisposition genes was higher in IDC cases than DCIS cases overall in both the clinical (9.3% vs. 6.5%) and population-based cohorts (6.2% vs. 4.6%; Table 3). PV in *BRCA1*, *BRCA2*, and *PALB2* were significantly enriched twofold or more in IDC cases compared with DCIS in both cohorts, whereas no difference in the frequency of *ATM* and *CHEK2* PV was observed. Similarly, no difference in

Table 3. Comparison of frequency of gene-specific PV between DCIS and invasive ductal breast cancer cases.

Clinical testing cohort								
Gene	DCIS cases			IDC cases			OR (95% CI) ^a	P value
	Number of PV	Patients tested	Frequency (%)	Number of PV	Patients tested	Frequency (%)		
<i>ATM</i>	90	7,537	1.19	314	28,399	1.11	0.97 (0.71-1.30)	0.82
<i>BARD1</i>	11	7,009	0.16	79	26,528	0.30	0.39 (0.12-0.95)	0.07
<i>BRCA1</i>	70	9,839	0.71	851	37,222	2.29	0.47 (0.34-0.63)	<0.001
<i>BRCA2</i>	157	9,839	1.60	877	37,222	2.36	0.58 (0.46-0.74)	<0.001
<i>CDH1</i>	1	9,568	0.01	15	36,333	0.04	NA	NA
<i>CHEK2</i>	137	7,533	1.82	473	28,358	1.67	0.91 (0.70-1.17)	0.46
<i>PALB2</i>	38	8,019	0.47	317	30,172	1.05	0.47 (0.30-0.71)	<0.001
<i>RAD51C</i>	9	7,031	0.13	57	26,597	0.21	0.51 (0.15-1.25)	0.19
<i>RAD51D</i>	11	6,892	0.16	26	25,988	0.10	1.61 (0.59-3.76)	0.31
<i>TP53</i>	23	9,887	0.23	67	37,405	0.18	1.30 (0.81-2.09)	0.28
<i>TP53</i> < 31	4	182	0.22	26	1,253	0.21	1.05 (0.37-3.07)	0.91
Total			6.48			9.31		

Population-based cohort						
Gene	DCIS cases		IDC cases		OR (95% CI) ^a	P value
	Number of PV	Frequency (%)	Number of PV	Frequency (%)		
<i>ATM</i>	33	0.85	125	0.91	1.07 (0.65-1.71)	0.77
<i>BARD1</i>	9	0.23	29	0.21	0.92 (0.26-2.50)	0.88
<i>BRCA1</i>	11	0.28	175	1.28	0.19 (0.08-0.41)	<0.001
<i>BRCA2</i>	42	1.08	254	1.85	0.60 (0.40-0.88)	0.01
<i>CDH1</i>	4	0.10	4	0.03	3.57 (0.83-15.26)	0.07
<i>CHEK2</i>	59	1.52	153	1.12	1.17 (0.77-1.72)	0.45
<i>PALB2</i>	12	0.31	75	0.55	0.43 (0.17-0.93)	0.05
<i>RAD51C</i>	5	0.13	21	0.15	1.24 (0.40-3.22)	0.68
<i>RAD51D</i>	3	0.08	14	0.10	NA	NA
Total		4.58		6.20		

Excluded *TP53* from population-based analysis because of the limited number of DCIS cases.

Abbreviations: NA, not analyzed for genes with less than four PV.

^aOR adjusted for age, ER status of breast cancer, family history of breast or ovarian cancer, and race/ethnicity.

However, given the concerns about overdiagnosis of DCIS (34), long-term follow-up studies are needed to understand whether the rates of overdiagnosis may differ between PV carriers and noncarriers.

The higher frequency of germline PV in predisposition genes among women with early-onset DCIS or a family history of breast or ovarian cancer is consistent with prior studies and the observations in IDC (17, 21, 22, 35). The current National Comprehensive Cancer Network (NCCN) and National Institute for Health and Care Excellence (NICE) guidelines rely on family history and age at diagnosis and receptor status of breast cancer for identifying women with breast cancer who may benefit from testing but do not distinguish between IDC and DCIS (16). In addition, there is an ongoing debate in terms of testing women with breast cancer based on age at diagnosis and family history versus testing all women with breast cancer (36-38). However, when considering the lower frequencies of germline PV in high-risk genes among women with DCIS compared with those with IDC and the lack of therapeutic indications with PARP inhibitors for DCIS even with *BRCA1*, *BRCA2*, or *PALB2* PV (39-41) but the substantial risk of CBC and other cancers for PV carriers, perhaps germline genetic testing of women with DCIS is appropriate when based on family history, age at diagnosis, and receptor status.

This study provides insights into the magnitude of risk of DCIS in germline PV carriers. PV in *BRCA2* were the only variants associated with a high risk of DCIS (OR > 4) in both the clinical testing and population-based cohorts, whereas PV in *BRCA1* were associated with high risk of DCIS only in the clinical study. Importantly, in contrast to a high risk of IDC (17), *PALB2* PV were associated with a moderate risk for DCIS in both cohorts. PV in *ATM* and *CHEK2* were also consistently associated with moderate risk. The PV frequencies per gene were somewhat consistent with results from a study of 655 DCIS cases diagnosed <50 years of age (9), which had twofold higher rates of PV in *BRCA2*, *PALB2*, and *TP53*, likely because of the younger age at diagnosis, but similar rates of PV in *BRCA1* and *CHEK2*.

Current guidelines support MRI surveillance in addition to mammograms for women with PV in the high-risk *BRCA1*, *BRCA2*, and *PALB2* genes and for *ATM* and *CHEK2* PV carriers (16). The results provided here are consistent with this approach. Although the risk of DCIS may only be moderately elevated in *BRCA1* or *PALB2* PV carriers, prophylactic risk-reducing mastectomy is still appropriate in unaffected *BRCA1* and *PALB2* PV carriers because of the high risk of IDC (17). In addition, chemoprevention with endocrine agents may be a consideration, particularly for PV in genes

such as *ATM* and *CHEK2*, which are primarily associated with an increased risk of ER-positive DCIS. However, the efficacy of endocrine agents for breast cancer prevention in germline PV carriers has not been thoroughly evaluated, with one prior study suggesting that tamoxifen decreased the risk of breast cancer in *BRCA2* but not in *BRCA1* PV carriers (42). As PV in *CDH1*, *MSH6*, and *RAD51D* were significantly associated with an increased risk of DCIS in the clinical cohort, in which ascertainment bias may play a role, these findings will need to be further evaluated in larger studies.

This study is the first to comprehensively assess the second breast cancer risk among women with DCIS from the general population who are carriers of PV in *BRCA1*, *BRCA2*, or *PALB2*. Carriers of germline PV in *BRCA1* and *BRCA2* with DCIS were noted to be at a substantially increased risk of CBC compared with noncarriers. Furthermore, the risk of CBC among PV carriers of these genes was observed to be similar regardless of whether the primary breast cancer is IDC or DCIS (15). Importantly, these findings suggest that contralateral risk-reducing mastectomy may be considered in women with DCIS who are carriers of PV in *BRCA1* and *BRCA2*, similar to the strategy in PV carriers of these genes with IDC (16, 43). There were insufficient *PALB2* carriers identified in this study to inform on the use of contralateral risk-reducing mastectomy. Furthermore, among carriers of PV in high-risk genes with DCIS who do not undergo contralateral prophylactic mastectomy, surveillance for CBC risk with supplemental MRI is warranted based on the >20% 15-year risk of CBC observed in this study (44, 45). Importantly, these surveillance and risk-reducing strategies are already available and recommended even for unaffected carriers of PV in these genes.

Although this is the largest reported study of genetic associations for DCIS, the sample size was still a limitation, in that PV in some of the predisposition genes resulted in associations with wide CI. In addition, the use of test requisition forms rather than medical records for ascertainment of clinical and phenotypic data for the clinical testing cohort and the lack of central pathologic validation of the diagnosis of DCIS across the entire dataset were limitations that may have introduced some uncertainty into the observed associations. Furthermore, this study established associations between PV in breast cancer predisposition genes and risk of DCIS, but prospective studies with long-term follow-up will be needed to estimate the absolute risk and/or RR of DCIS in PV carriers and the subsequent risk of progression to invasive breast cancer. The lack of long-term outcome data limits the demonstration of the clinical benefit of identifying PV carriers among women with DCIS beyond what would be expected for unaffected PV carriers.

Conclusions

PV in *ATM*, *BRCA1*, *BRCA2*, *CHEK2*, or *PALB2* are associated with a moderate to high risk of DCIS. Among women with DCIS undergoing germline genetic testing, multigene panels may be appropriate because of high frequencies of PV in genes other than *BRCA1* and *BRCA2*. Contralateral prophylactic mastectomy may be appropriate in women with DCIS who are carriers of PV in *BRCA1*, *BRCA2*, and possibly *PALB2* because of the high risk of developing CBC.

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