original reports

Germline Pathogenic Variants in Cancer Predisposition Genes Among Women With Invasive Lobular Carcinoma of the Breast

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abstract

PURPOSE To determine the contribution of germline pathogenic variants (PVs) in hereditary cancer testing panel genes to invasive lobular carcinoma (ILC) of the breast.

MATERIALS AND METHODS The study included 2,999 women with ILC from a population-based cohort and 3,796 women with ILC undergoing clinical multigene panel testing (clinical cohort). Frequencies of germline PVs in breast cancer predisposition genes (*ATM*, *BARD1*, *BRCA1*, *BRCA2*, *BRIP1*, *CDH1*, *CHEK2*, *PALB2*, *PTEN*, *RAD51C*, *RAD51D*, and *TP53*) were compared between women with ILC and unaffected female controls and between women with ILC and infiltrating ductal carcinoma (IDC).

RESULTS The frequency of PVs in breast cancer predisposition genes among women with ILC was 6.5% in the clinical cohort and 5.2% in the population-based cohort. In case-control analysis, *CDH1* and *BRCA2* PVs were associated with high risks of ILC (odds ratio [OR] > 4) and *CHEK2*, *ATM*, and *PALB2* PVs were associated with moderate (OR = 2-4) risks. *BRCA1* PVs and *CHEK2* p.Ile157Thr were not associated with clinically relevant risks (OR < 2) of ILC. Compared with IDC, *CDH1* PVs were > 10-fold enriched, whereas PVs in *BRCA1* were substantially reduced in ILC.

CONCLUSION The study establishes that PVs in *ATM*, *BRCA2*, *CDH1*, *CHEK2*, and *PALB2* are associated with an increased risk of ILC, whereas *BRCA1* PVs are not. The similar overall PV frequencies for ILC and IDC suggest that cancer histology should not influence the decision to proceed with genetic testing. Similar to IDC, multigene panel testing may be appropriate for women with ILC, but *CDH1* should be specifically discussed because of low prevalence and gastric cancer risk.

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INTRODUCTION

Invasive lobular carcinoma (ILC) of the breast accounts for approximately 10%-15% of all invasive breast carcinomas.[1] ILC is a distinct subtype of breast cancer with unique biologic characteristics and clinical outcomes.[2] Although several predisposition genes for breast cancer have been well-established, these studies primarily evaluated women with infiltrating ductal carcinoma (IDC) and there are very few studies specifically focused on genetic predisposition to ILC.[3] Germline pathogenic variants (PVs) in *CDH1* have been associated with hereditary diffuse gastric cancer and ILC.[4] However, the magnitude of ILC risk related to PVs in *CDH1* varies substantially between studies because of small numbers of carriers. In addition, the risk of ILC among carriers of PVs in other genes from multigene hereditary cancer testing panels has not been adequately defined. Some studies have reported that PVs in *BRCA1* and *TP53* do not predispose to ILC,[5,6] whereas PVs in *BRCA2*[7] and the *CHEK2* I157T missense variant have been associated with ILC.[8-10] Furthermore, previous studies have primarily evaluated the frequency of germline PVs in *CDH1* and other genes among high-risk women with family history of breast cancer or young age at diagnosis. Thus, the frequency of germline PVs and the associated risk for ILC in the high-risk and general populations are not currently established. Therefore, in one of the largest studies of ILC involving population-based and clinical testing cohorts, we describe the frequency of germline PVs in cancer predisposition genes in women with ILC and estimate the magnitude of risk of ILC in PV carriers.

ASSOCIATED CONTENT

Data Supplement

Author affiliations and support information (if applicable) appear at the end of this article.

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CONTEXT

Key Objective

Women diagnosed with invasive lobular carcinoma (ILC) of the breast rarely benefit from hereditary cancer testing because the involvement of pathogenic variants (PVs) from cancer predisposition genes in ILC is not well-defined. In this study, population-based and clinical high-risk ILC cohorts were used to assess the risks of ILC conferred by inherited PVs.

Knowledge Generated

The frequency of PVs in breast cancer predisposition genes was 6.5% in the clinical cohort and 5.2% in the population-based cohort. PVs in *CDH1*, *BRCA2*, *CHEK2*, *ATM*, and *PALB2* were associated with increased risk of ILC, whereas PVs in *BRCA1* were not.

Relevance

Multigene panel testing is appropriate for women with ILC and to identify women at risk of ILC because PVs in several genes predispose to this form of breast cancer. Predisposing PVs may also inform the selection of therapy for women with ILC.

MATERIALS AND METHODS

Study Populations

The data set from the CAnceR RIsk Estimates Related to Susceptibility (CARRIERS) consortium included 3,437 women with ILC, 25,807 women with IDC, and 35,365 unaffected women from seven breast cancer case-control studies nested within prospective cohorts, two case-cohort studies, and three breast cancer case-control studies, along with five breast cancer case-control and case-cohort studies enriched for young onset disease or family history of breast cancer.[11] A brief description of the contributing studies and the characteristics of the entire cohort is provided in the Data Supplement (online only). For the primary analysis, contributing studies enriched for young age or family history of breast cancer were excluded. Therefore, the primary analysis of this population-based cohort included 2,999 women with ILC, 20,323 women with IDC, and 32,544 unaffected female controls.

The clinical cohort data set included a nationwide sample of 3,796 adult women with ILC and 37,405 with IDC referred to Ambry Genetics, between March 2012 and December 2016, by genetic counselors or clinical care providers across the United States for clinically indicated germline genetic testing because of personal or family history of cancer. Data on patient characteristics were collected from test requisition forms and also from clinical notes and pedigrees provided by ordering clinicians. Women who had previously undergone testing for *BRCA1*, *BRCA2*, or Lynch syndrome genes before undergoing multigene panel testing were excluded from the analysis. The majority (> 85%) of women in this cohort met National Comprehensive Cancer Network (NCCN) guidelines for *BRCA1* or *BRCA2* testing on the basis of personal or family history of cancer.

The study was restricted to adult women with IDC or ILC. Women with mixed ILC and IDC, unknown, or other tumor histology were excluded. The Mayo Clinic institutional review board approved the research study. The analysis of the clinical testing cohort was considered exempt from review by the Western Institutional Review Board.

Genetic Testing and Classification of Variants

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 from 37 cancer predisposition genes using a QIAseq custom panel (Data Supplement).[12,13] For the clinical testing cohort, testing of 5-49 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 as described previously.[14-16] For both tested cohorts, the results for 12 breast cancer predisposition genes (*ATM*, *BARD1*, *BRCA1*, *BRCA2*, *BRIP1*, *CDH1*, *CHEK2*, *PALB2*, *PTEN*, *RAD51C*, *RAD51D*, and *TP53*) and nine other cancer predisposition genes (*CDKN2A*, *MLH1*, *MRE11A*, *MSH2*, *MSH6*, *NBN*, *NF1*, *PMS2*, and *RAD50*) were evaluated. In the clinical cohort, PV frequency in each gene was estimated restricting to women who underwent testing for that gene on the basis of the multigene panel ordered. The pooled frequency across breast cancer predisposition genes was then estimated as the sum of frequencies of PVs in the included genes. A five-tier system was used to classify variants using a framework consistent with the guidelines published by American College of Medical Genetics.[17] PVs and likely PVs were analyzed together. All missense and low-penetrance variants in *CHEK2* (eg, c.1111C>T, c.1169A>C, c.1283C>T, c.1427C>T, c.349A>G, c.433C>T, c.499G>A, and c.917G>C) were excluded from the analysis. The *CHEK2* c.470T>C (p.Ile157Thr) variant was analyzed separately because of a previous association with ILC.[10]

Statistical Analysis

The frequency of PVs in each gene was assessed for women with ILC and IDC in the clinical testing and population-based cohorts, for subsets of women with ILC on the basis of estrogen receptor (ER) status of tumors[18] and

age at diagnosis (> 65 $v \leq$ 65 years), and for unaffected female controls in the population-based cohort and in gnomAD.[19,20] gnomAD controls used in this analysis included > 90,000 alleles from unrelated women without a cancer diagnosis in the v2.1.1 data set (GRCh37/hg19). Copy number variations in all genes and gnomAD filter non-PASS variants were excluded from both cases and controls for analyses of the clinical cohort, as described previously.[21] Case-control association testing for the clinical testing cohort compared frequencies of PVs in cases with gnomAD reference controls using logistic regression and with the gnomAD controls weighted so that the relative frequencies of race and ethnicity subgroups were the same between cases and controls, as described previously.[22] Case-control association testing for PVs in each gene in the population-based cohort was conducted with logistic regression models adjusting for age at diagnosis, race or ethnicity, and study. Enrichment analysis comparing PVs in ILC and IDC in both clinical testing and population-based cohorts was conducted using logistic regression. Sensitivity analyses including all studies within the CARRIERS consortium and restricting to ER-positive cases were also performed. All tests were two-sided, and a P value < .05 was considered statistically significant. All analyses were performed using R version 3.4.

RESULTS

Patient Characteristics

The characteristics of women with ILC or IDC from the clinical testing and population-based cohorts included in this study are detailed in Table 1. The median age at diagnosis of ILC was approximately 54 years in the clinical testing cohort and 64 years in the population-based cohort. In both cohorts, > 95% of ILCs with available hormone receptor status were ER-positive and > 92% were human epidermal growth factor receptor 2–negative (Table 1).

TABLE 1. Characteristics of Patients With ILC and IDC

	Clinical Testing Cohort			Population-Based Cohort		
Characteristic	**IDC (n = 37,405)**	**ILC (n = 3,796)**	**Total (n = 41,201)**	**IDC (n = 20,323)**	**ILC (n = 2,999)**	**Total (n = 23,322)**
Age at diagnosis, years						
Mean (SD)	49.8 (11.6)	53.8 (10.6)	50.2 (11.6)	62.5 (11.3)	63.8 (10.7)	62.6 (11.2)
Range	15-90	19-90	15-90	22-94	29-91	22-94
Race or ethnicity, No. (%)						
White or non-Hispanic White[a]	25,247 (67.5)	2,838 (74.8)	28,085 (68.2)	15,615 (76.8)	2,546 (84.9)	18,161 (77.9)
Black or African American	3,240 (8.7)	221 (5.8)	3,461 (8.4)	2,474 (12.2)	242 (8.1)	2,716 (11.6)
Asian	1,908 (5.1)	108 (2.8)	2,016 (4.9)	1,027 (5.1)	87 (2.9)	1,114 (4.8)
Hispanic	2,350 (6.3)	190 (5.0)	2,540 (6.2)	698 (3.4)	70 (2.3)	768 (3.3)
Others or unknown	4,660 (12.5)	439 (11.6)	5,099 (12.4)	509 (2.5)	54 (1.8)	563 (2.4)
Family history of breast cancer,[b] No. (%)	21,540 (60.8)	2,475 (68.0)	24,015 (61.4)	3,888 (19.7)	615 (21.2)	4,503 (19.9)
ER status, No. (%)						
Negative	8,496 (27.4)	108 (3.6)	8,604 (25.3)	2,879 (18.8)	88 (4.3)	2,967 (17.1)
Positive	22,566 (72.6)	2,867 (96.4)	25,433 (74.7)	12,446 (81.2)	1,956 (95.7)	14,402 (82.9)
Unknown	6,343	821	7,164	4,998	955	5,953
Progesterone receptor status, No. (%)						
Negative	10,404 (35.1)	339 (11.9)	10,743 (33.1)	4,481 (30.0)	399 (20.1)	4,880 (28.8)
Positive	19,238 (64.9)	2,515 (88.1)	21,753 (66.9)	10,474 (70.0)	1,588 (79.9)	12,062 (71.2)
Unknown	7,763	942	8,705	5,368	1,012	6,380
HER2 status, No. (%)						
Negative	20,838 (79.4)	2,339 (92.6)	23,177 (80.6)	8,030 (82.9)	1,232 (93.1)	9,262 (84.1)
Positive	5,404 (20.6)	187 (7.4)	5,591 (19.4)	1,660 (17.1)	91 (6.9)	1,751 (15.9)
Unknown	11,163	1,270	12,433	10,633	1,676	12,309

Abbreviations: ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; IDC, infiltrating ductal carcinoma; ILC, invasive lobular carcinoma; SD, standard deviation.

[a]Ashkenazi-Jewish population was included under non-Hispanic Whites.

[b]Family history of breast cancer in first-, second-, or third-degree relatives was included for clinical testing cohort, whereas family history of breast cancer in first-degree relatives was only included in the population-based cohort.

TABLE 2. Comparison of Frequencies of Germline PVs Between ILC and IDC

	PVs in Breast Cancer Predisposition Genes							
	Clinical Testing Cohort				Population-Based Cohort			
Gene	IDC (%)	ILC (%)	OR (95% CI)[a]	*P*	IDC (%)	ILC (%)	OR (95% CI)[b]	*P*
ATM	314 (1.11)	29 (1.03)	0.71 (0.43 to 1.10)	.144	150 (0.74)	20 (0.67)	1.17 (0.66 to 1.95)	.557
BARD1	79 (0.30)	5 (0.19)	1.52 (0.52 to 3.57)	.390	31 (0.15)	1 (0.03)	ND	ND
BRCA1	844 (2.27)	12 (0.32)	0.37 (0.19 to 0.66)	.002	204 (1.00)	5 (0.17)	0.36 (0.09 to 0.96)	.083
BRCA2	877 (2.36)	81 (2.15)	1.17 (0.89 to 1.50)	.246	276 (1.36)	34 (1.13)	1.01 (0.63 to 1.55)	.959
BRIP1	85 (0.32)	9 (0.34)	1.07 (0.44 to 2.22)	.863	45 (0.22)	6 (0.20)	1.31 (0.44 to 3.14)	.585
CDH1	15 (0.04)	20 (0.54)	10.25 (4.52 to 23.48)	< .001	4 (0.02)	7 (0.23)	14.14 (4.02 to 59.34)	< .001
CHEK2[c]	347 (1.22)	35 (1.25)	0.86 (0.57 to 1.25)	.448	203 (1.00)	33 (1.10)	0.70 (0.40 to 1.15)	.187
CHEK2_I157T	159 (0.56)	22 (0.78)	0.91 (0.60 to 1.32)	.633	125 (0.62)	33 (1.10)	1.47 (0.85 to 2.41)	.145
PALB2	317 (1.05)	11 (0.37)	0.36 (0.16 to 0.68)	.005	107 (0.53)	12 (0.40)	0.97 (0.43 to 1.92)	.935
PTEN	20 (0.05)	4 (0.11)	ND	ND	5 (0.02)	2 (0.07)	ND	ND
RAD51C	57 (0.21)	5 (0.19)	1.46 (0.49 to 3.50)	.435	27 (0.13)	1 (0.03)	ND	ND
RAD51D	26 (0.10)	1 (0.04)	ND	ND	18 (0.09)	1 (0.03)	ND	ND
TP53	69 (0.18)	0 (0.00)	ND	ND	14 (0.07)	0 (0.00)	ND	ND
Total[d]	9.2	6.5			5.9	5.2		

Abbreviations: ER, estrogen receptor; IDC, infiltrating ductal carcinoma; ILC, invasive lobular carcinoma; ND, not determined because of insufficient number (< 5) of PVs except for *CDH1*; OR, odds ratio; PV, pathogenic variant.

[a]ORs adjusted for age at diagnosis, race or ethnicity, and ER status.

[b]ORs adjusted for age at diagnosis, race or ethnicity, ER status of tumor, and study.

[c]Missense and low-penetrance variants in *CHEK2* were excluded, and the *CHEK2_I157T* variant was analyzed separately.

[d]Total frequency is a sum of PV frequencies across all breast cancer predisposition genes except for *CHEK2_I157T*.

Gene-Specific PV Prevalence in ILC

The cumulative frequency of PVs in 12 known breast cancer predisposition genes among women with ILC was 6.5% in the clinical testing cohort and 5.2% in the population-based cohort (Table 2). PVs in *CHEK2*, *BRCA2*, and *ATM* were observed in > 1% of ILCs in the clinical testing cohort, whereas only *CHEK2* and *BRCA2* PVs were found in > 1% of ILC in the population-based cohort. The recurrent c.1100delC *CHEK2* PV was observed in 20 (0.8%) and 23 (0.8%) women with ILC in the clinical testing and population-based cohorts, respectively. *CDH1* PVs were observed in 20 (0.5%) ILCs from the clinical testing cohort and 7 (0.2%) ILCs from the population-based cohort. Of the 20 women with *CDH1* PVs in the clinical testing cohort, 50% had either a personal (1 of 20) or family history (9 of 20) of gastric cancer. Among women older than 65 years in the population-based cohort, PVs in breast cancer predisposition genes were detected in 2.5% with ILC (Data Supplement).

Genes Associated With Increased Risk of ILC

In case-control association testing, PVs in *BRCA2*, *CDH1*, and *CHEK2* were significantly enriched in ILC cases compared with controls in both the clinical testing and the population-based cohorts (Fig 1 and Data Supplement). The risk of ILC was highest among *CDH1* PV carriers (odds ratio [OR]: 15.74; 95% CI, 5.08 to 50.22) followed by *BRCA2* (OR: 4.94; 95% CI, 3.22 to 7.41) and *CHEK2* (OR: 2.56; 95% CI, 1.71 to 3.73) in the population-based cohort. By contrast, the *CHEK2* p.Ile157Thr variant was only associated with a mildly increased risk of ILC in the population-based cohort (OR: 1.76; 95% CI, 1.18 to 2.54; $P = .004$) and was not associated with increased risk in the clinical testing cohort (OR: 1.29; 95% CI, 0.79 to 1.97; $P = .27$). PVs in *ATM* and *NBN* were associated with moderate risk (OR > 2) of ILC in the clinical testing cohort only (Fig 1), whereas PVs in *PALB2* were only significantly associated with increased risk of ILC in the population-based cohort (OR: 3.47; 95% CI, 1.72 to 6.55; $P < .001$). Importantly, PVs in *BRCA1* were not associated with an increased risk of ILC in either cohort. Sensitivity analysis restricting to ER-positive cases demonstrated that PVs in *ATM*, *BRCA2*, *CDH1*, and *CHEK2* were associated with increased risk of ER-positive ILC in both cohorts (Data Supplement). Further sensitivity analysis using the entire CARRIERS cohort including the family history–enriched CARRIERS studies demonstrated results similar to those from the primary analysis (Data Supplement).

Comparison of Gene-Specific PV Frequencies in ILC and IDC

The overall frequency of PVs among ILCs was similar to that in women with IDC in the clinical (6.5% *v* 9.2%) and

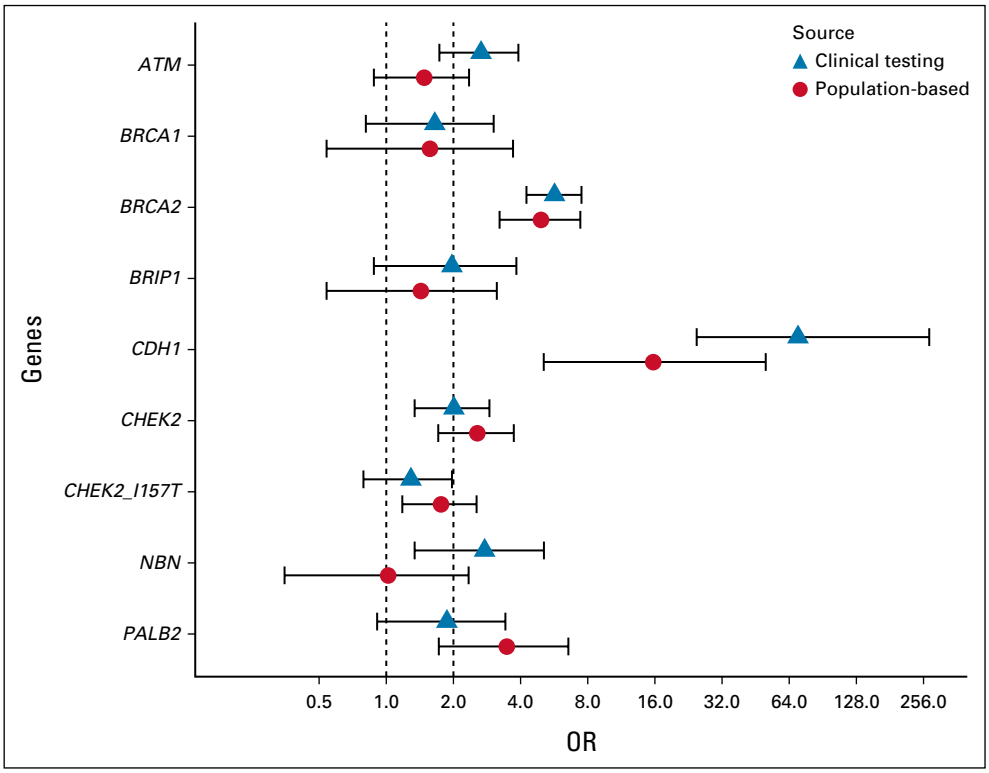

FIG 1. Enrichment of gene-specific pathogenic variants in ILC cases compared with controls. Controls were derived from gnomAD for comparison with the clinical testing cohort and from the CARRIERS consortium for comparison with population-based cases. The Forest plot shows ORs and 95% CIs for each gene. ORs were not determined for genes with less than five PVs in cases or controls. CARRIERS, CAnceR RIsk Estimates Related to Susceptibility; ILC, invasive lobular carcinoma; OR, odds ratio; PV, pathogenic variant.

population-based (5.2% *v* 5.9%) cohorts (Table 2). However, *CDH1* PVs were more than 10-fold enriched in ILCs compared with IDCs in both cohorts (Table 2). By contrast, PVs in *BRCA1* were significantly reduced in ILCs relative to IDCs in the clinical cohort ($P = .002$) and were less frequent in ILCs in the population-based cohort, although the difference was not significant (OR = 0.36; $P = .08$). The frequency of *PALB2* PVs was also significantly lower among ILCs compared with IDCs in the clinical testing cohort (OR = 0.36; $P = .005$), but not in the population-based cohort (Table 2). No other genes yielded a significant difference in the frequency of PVs among IDCs and ILCs in either cohort. Further analysis restricted to ER-positive ILCs and IDCs identified differences in the frequencies of PVs in *BRCA1*, *CDH1*, and *PALB2* between women with ILC and IDC, similar to the primary results (Table 3).

DISCUSSION

We present the results from the largest study of germline PVs in cancer predisposition genes among women with ILC from a clinical testing cohort and a population-based cohort, both of which included large numbers of racially and ethnically diverse women from the United States. The study identifies the breast cancer predisposition genes with PVs associated with ILC and enumerates differences in gene-specific frequencies of PVs in women with IDC and ILC. The confirmation of the findings in two cohorts with distinct ascertainment is a significant strength of the study. Overall, the results of this study have clinical implications for germline testing, counseling of PV carriers for ILC risk, and personalized management of ILC risk among carriers.

The finding that the overall frequency of PVs in established breast cancer predisposition genes is similar between women with ILC and IDC suggests that breast cancer histology should not affect the decision to proceed with genetic testing. However, gene-specific differences in the frequencies of *BRCA1* and *CDH1* were observed between ILC and IDC. In particular, the frequency of *BRCA1* PVs was noted to be significantly lower among women with ILC compared with IDC and *BRCA1* was ruled out as an ILC predisposition gene. In addition, approximately two thirds of PVs in breast cancer predisposition genes among women with ILCs were observed in genes other than *BRCA1* or *BRCA2*, with similar frequency of PVs in *ATM*, *CHEK2*, and *PALB2* between IDC and ILC. These findings support the use of multigene panels for genetic testing of women with ILC, similar to the genetic testing approach commonly used in women with IDC. In addition, the estimates of the overall

TABLE 3. Comparison of Gene-Specific Frequencies of PVs Between ILC and IDC Among ER-Positive Cases

	PVs in Breast Cancer Predisposition Genes							
	Clinical Testing Cohort				Population-Based Cohort			
Gene	IDC (%)	ILC (%)	OR (95% CI)[a]	*P*	IDC (%)	ILC (%)	OR (95% CI)[b]	*P*
ATM	236 (1.36)	18 (0.84)	0.64 (0.38 to 1.01)	.068	87 (0.70)	17 (0.87)	1.21 (0.68 to 2.01)	.486
BARD1	23 (0.14)	5 (0.25)	1.87 (0.62 to 4.57)	.210	14 (0.11)	1 (0.05)	ND	ND
BRCA1	211 (0.94)	7 (0.25)	0.33 (0.14 to 0.64)	.003	46 (0.37)	3 (0.15)	0.51 (0.12 to 1.41)	.266
BRCA2	485 (2.16)	65 (2.28)	1.21 (0.92 to 1.56)	.157	137 (1.10)	21 (1.07)	1.05 (0.64 to 1.64)	.825
BRIP1	48 (0.30)	7 (0.35)	1.13 (0.46 to 2.35)	.771	22 (0.18)	5 (0.26)	1.42 (0.48 to 3.47)	.477
CDH1	11 (0.05)	12 (0.43)	7.85 (3.32 to 18.53)	< .001	3 (0.02)	7 (0.36)	15.04 (4.14 to 70.24)	< .001
CHEK2[c]	251 (1.45)	27 (1.26)	0.88 (0.58 to 1.29)	.544	135 (1.08)	16 (0.82)	0.72 (0.41 to 1.18)	.222
CHEK2_I157T	117 (0.68)	15 (0.70)	0.97 (0.54 to 1.62)	.923	74 (0.59)	18 (0.92)	1.52 (0.87 to 2.51)	.114
PALB2	186 (1.01)	7 (0.31)	0.33 (0.14 to 0.65)	.07	46 (0.37)	8 (0.41)	1.15 (0.50 to 2.32)	.721
PTEN	10 (0.04)	4 (0.14)	ND	ND	2 (0.02)	1 (0.05)	ND	ND
RAD51C	24 (0.15)	4 (0.20)	ND	ND	13 (0.10)	0 (0.00)	ND	ND
RAD51D	9 (0.06)	0 (0.00)	ND	ND	8 (0.06)	1 (0.05)	ND	ND
TP53	44 (0.19)	0 (0.00)	ND	ND	8 (0.06)	0 (0.00)	ND	ND

Abbreviations: ER, estrogen receptor; IDC, infiltrating ductal carcinoma; ILC, invasive lobular carcinoma; ND, not determined because of insufficient number (< 5) of mutations; OR, odds ratio; PV, pathogenic variant.

[a]ORs adjusted for age at diagnosis and race or ethnicity.

[b]ORs adjusted for age at diagnosis, race or ethnicity, and study.

[c]Missense and low-penetrance variants in *CHEK2* were excluded, and *CHEK2_I157T* variant was analyzed separately.

and gene-specific frequencies of PVs in the known breast cancer predisposition genes in ILC will also aid in discussion on the probability of finding a PV during pretest genetic counseling of women with ILC.

The slight difference in the overall frequency of PVs in breast cancer predisposition genes (5.2% *v* 6.5%) between the population-based and clinical testing cohorts is likely due to the differences in ascertainment. The CARRIERS consortium included women with breast cancer from the general population, whereas the clinical testing cohort primarily (> 85%) included women with breast cancer who underwent clinical germline genetic testing because they met the current NCCN guidelines for genetic testing on the basis of personal or family history of cancer. Therefore, the results also provide insight into testing all women with ILC in the general population as advised by the American Society of Breast Surgeons[23] versus testing women with ILC selectively on the basis of age at diagnosis or family history as advised by the NCCN guidelines.[24]

One of the primary benefits of detecting high-penetrance gene PVs such as *BRCA1* or *BRCA2* in women diagnosed with breast cancer is prevention of ovarian cancer and contralateral breast cancer through risk-reducing surgeries. Cost-effectiveness analysis of expanding genetic testing to all women with breast cancer has often taken this downstream effect of secondary cancer prevention into consideration.[25,26] In this context, several guidelines and studies have advocated for an expansion of the current NCCN guidelines on germline genetic testing[24] to include all women with breast cancer.[23,27-29] However, these cost-benefit ratio analyses may not apply to women with ILC because of the significantly lower frequency of *BRCA1* PVs. Therefore, the differences in genetic testing results between ILC and IDC should be accounted for in studies evaluating cost-effectiveness of expanding genetic testing to all women with breast cancer.

PVs in *CDH1* have been associated with an increased risk of ILC and hereditary diffuse gastric cancer.[30,31] However, some studies have reported *CDH1* PVs among patients with ILC without a family history of hereditary diffuse gastric cancer.[32-34] Furthermore, previous studies of associations between *CDH1* PVs and breast cancer have primarily evaluated the risk of ILC among women who underwent testing on the basis of personal or family history of breast cancer.[35] To our knowledge, the current study is the first to provide a population-based frequency of *CDH1* PVs in ILC and confirms that PVs in *CDH1* are associated with a high risk (OR > 15) of ILC even among women with no personal or family history of gastric cancer. Although *CDH1* PVs are associated with a high penetrance of ILC, the frequency of *CDH1* PVs, even in ILC, is low (0.2% in the population-based cohort and 0.5% in the clinical testing cohort). NCCN guidelines[36] currently recommend increased breast cancer surveillance for women with PVs in *CDH1* and cite insufficient evidence for risk-reducing mastectomy. The high risk of ILC among *CDH1* carriers in the general population observed

in this study may justify risk-reducing mastectomy in carriers even in the absence of a family history of breast cancer. However, such decisions need to be made on the basis of an individual's personal preference. Furthermore, clinical management of a *CDH1* PV carrier in the absence of a family history of gastric cancer, as noted in approximately 50% of *CDH1* PV carriers in the clinical cohort involved in this study, can be very challenging as the management guidelines are not well-defined.[37]

Significant associations (OR > 2) between PVs in *BRCA2* or *CHEK2* and ILC were observed, as in previous studies.[38,39] Since mammography and breast ultrasound are known to have lower sensitivity for detection of ILC compared with IDC,[40-42] carriers of genes predisposing to ILC may benefit from magnetic resonance imaging screening. Importantly, current guidelines already support surveillance breast magnetic resonance imaging for carriers of PVs in *CHEK2* or *BRCA2* on the basis of > 20% lifetime risks of breast cancer.[36] The association between *CHEK2* and an increased risk of ILC appears to be primarily driven by the c.1100delC variant in this study. Interestingly, the p.Ile157Thr *CHEK2* variant has been previously associated with a low risk of breast cancer overall (OR ≈ 1.4),[43] but an increased risk of ILC.[8,9,44,45] In this study, the variant was only associated with a mildly increased risk of ILC in the population-based cohort and was not significantly associated with increased risk in the clinical testing cohort. This is consistent with a recent study that did not observe association between *CHEK2* p.Ile157Thr variant and ILC. Thus, p.Ile157Thr should not be considered clinically relevant (OR < 2) for either ILC or breast cancer overall and perhaps should not influence surveillance for breast cancer.

In this study, significant associations between PVs in *ATM*, *NBN*, or *PALB2* and ILC were observed in either the clinical testing or population-based cohort, but not both. Importantly, *NBN* has previously been excluded as a breast cancer predisposition gene.[15] Thus, the current association with ILC needs to be investigated further. The association between PVs in *PALB2* and ILC in the population-based cohort but not in the clinical testing cohort is interesting because the frequency of PVs in *PALB2* in both sets of ILC cases was similar. This suggests that the absence of a significant association in the clinical cohort may be related to the quality of *PALB2* variant calling in gnomAD controls. The association between *ATM* PVs and ER-positive ILC in both cohorts may most accurately reflect the known predisposition of *ATM* PVs to ER-positive breast cancer.[18]

Although this is the largest study on multigene panel testing of women with ILC, the major limitation of the present study is still the sample size, which resulted in wide confidence intervals for some associations. For the clinical testing cohort, limitations include inclusion of women from different clinical sites with potential differences in ascertainment, enrollment of women at high risk for PVs in breast cancer predisposition genes, and the procurement of clinical data through test requisition forms rather than the medical records.

In conclusion, the largest study involving multigene panel testing of women with ILC, we describe the frequency of germline PVs in ILC and the differences in gene-specific frequencies between ILC and IDC. This study establishes *BRCA2*, *CDH1*, and *CHEK2* and suggests *ATM* and *PALB2* as genes associated with increased risk of ILC, but rules out *BRCA1* as an ILC predisposition gene. Similar to IDC, multigene panel testing may be appropriate for women with ILC, but *CDH1* should be specifically discussed in the context of its low prevalence and attendant gastric cancer risk.

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DISCLAIMER

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Germline Pathogenic Variants in Cancer Predisposition Genes Among Women With Invasive Lobular Carcinoma of the Breast

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