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Association of Breast and Ovarian Cancers With Predisposition Genes Identified by Large-Scale Sequencing

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Invited Commentary page 58

Supplemental content

IMPORTANCE Since the discovery of *BRCA1* and *BRCA2*, multiple high- and moderate-penetrance genes have been reported as risk factors for hereditary breast cancer, ovarian cancer, or both; however, it is unclear whether these findings represent the complete genetic landscape of these cancers. Systematic investigation of the genetic contributions to breast and ovarian cancers is needed to confirm these findings and explore potentially new associations.

OBJECTIVE To confirm reported and identify additional predisposition genes for breast or ovarian cancer.

DESIGN, SETTING, AND PARTICIPANTS In this sample of 11 416 patients with clinical features of breast cancer, ovarian cancer, or both who were referred for genetic testing from 1200 hospitals and clinics across the United States and of 3988 controls who were referred for genetic testing for noncancer conditions between 2014 and 2015, whole-exome sequencing was conducted and gene-phenotype associations were examined. Case-control analyses using the Genome Aggregation Database as a set of reference controls were also conducted.

MAIN OUTCOMES AND MEASURES Breast cancer risk associated with pathogenic variants among 625 cancer predisposition genes; association of identified predisposition breast or ovarian cancer genes with the breast cancer subtypes invasive ductal, invasive lobular, hormone receptor–positive, hormone receptor–negative, and male, and with early-onset disease.

RESULTS Of 9639 patients with breast cancer, 3960 (41.1%) were early-onset cases (≤45 years at diagnosis) and 123 (1.3%) were male, with men having an older age at diagnosis than women (mean [SD] age, 61.8 [12.8] vs 48.6 [11.4] years). Of 2051 women with ovarian cancer, 445 (21.7%) received a diagnosis at 45 years or younger. Enrichment of pathogenic variants were identified in 4 non-*BRCA* genes associated with breast cancer risk: *ATM* (odds ratio [OR], 2.97; 95% CI, 1.67-5.68), *CHEK2* (OR, 2.19; 95% CI, 1.40-3.56), *PALB2* (OR, 5.53; 95% CI, 2.24-17.65), and *MSH6* (OR, 2.59; 95% CI, 1.35-5.44). Increased risk for ovarian cancer was associated with 4 genes: *MSH6* (OR, 4.16; 95% CI, 1.95-9.47), *RAD51C* (OR, not estimable; false-discovery rate–corrected $P = .004$), *TP53* (OR, 18.50; 95% CI, 2.56-808.10), and *ATM* (OR, 2.85; 95% CI, 1.30-6.32). Neither the MRN complex genes nor *CDKN2A* was associated with increased breast or ovarian cancer risk. The findings also do not support previously reported breast cancer associations with the ovarian cancer susceptibility genes *BRIP1*, *RAD51C*, and *RAD51D*, or mismatch repair genes *MSH2* and *PMS2*.

CONCLUSIONS AND RELEVANCE The results of this large-scale exome sequencing of patients and controls shed light on both well-established and controversial non-*BRCA* predisposition gene associations with breast or ovarian cancer reported to date and may implicate additional breast or ovarian cancer susceptibility gene candidates involved in DNA repair and genomic maintenance.

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Understanding the genetic bases for clinical phenotypes is an important goal of both clinical diagnostics and biomedical research. At least 3% of all cancer cases are attributed to hereditary variation in cancer predisposition genes,[1] and 5% to 20% of breast cancer (BC) or ovarian cancer (OV) cases are linked to genetic abnormalities.[2,3] In the 1990s, family-based genetic linkage studies identified 2 major BC and OV genes, *BRCA1* and *BRCA2*, which account for at least 25% of familial risk,[4] implying a more complex "polygenic" genetic architecture of BC.[5] In the past decade, several more BC and OV susceptibility genes were identified from large case-control association studies.[6-9] Although next-generation sequencing has enabled rapid screening for new cancer susceptibility genes, there are currently only 11 well-characterized BC and OV susceptibility genes (*ATM* [OMIM 607585], *BRCA1* [OMIM 113705], *BRCA2* [OMIM 600185], *BRIP1* [OMIM 605882], *CDH1* [OMIM 192090], *CHEK2* [OMIM 604373], *PALB2* [OMIM 610355], *PTEN* [OMIM 601728], *RAD51C* [OMIM 602774], *RAD51D* [OMIM 602954], and *TP53* [OMIM 191170])[10-13] and 6 others (*BARD1* [OMIM 601593], *CDKN2A* [OMIM 600160], *NF1* [OMIM 162200], *MRE11A* [OMIM 600814], *RAD50* [OMIM 604040], and *NBN* [OMIM 602667])[11,14-17] reported to have associations with BC but for which large-scale robust evidence is lacking (eTable 1 in the Supplement). A recent study of 65 057 patients with BC referred for multigene panel testing supports the role of *BARD1* but not *CDKN2A*, *NF1*, *MRE11A*, *RAD50*, or *NBN* in breast cancer.[18] Moreover, although mismatch repair (MMR) genes *MLH1* (OMIM 120436), *MSH2* (OMIM 609309), *MSH6* (OMIM 600678), and *PMS2* (OMIM 600259) are widely accepted and treated clinically as OV susceptibility genes, data regarding their gene-specific contributions to BC or OV risk are limited.

Further investigation of cancer risks associated with less well-characterized genes is imperative, particularly as multigene panel testing that includes moderate-risk genes are increasingly used in oncology practice.[19] Currently, only approximately 10% to 24% of patients referred for BC or OV risk assessment with genetic testing are found to harbor known pathogenic variants identified by multigene panel testing.[11,14,20,21] To determine whether and to what extent additional cancer genes not currently included in most multigene panel tests contribute to these risks, we performed whole-exome sequencing for 11 416 patients with clinical features of BC or OV and for 3988 controls. The results have the potential to add to our understanding of the genomic landscape of BC and OV predisposition and to inform comprehensive genetic testing in the context of patient care.

Key Points

Question Which non-*BRCA* genes are associated with breast or ovarian cancer and what are the magnitudes of those risks?

Findings In this study assessing whole-exome sequencing results from 11 416 patients with breast cancer, ovarian cancer, or both and 3988 controls, an increased risk of breast cancer was associated with *PALB2, ATM, CHEK2,* and *MSH6* genes, whereas *MSH6, RAD51C*, *TP53*, and *ATM* genes were associated with an increased risk of ovarian cancer.

Meaning In addition to confirming several well-known breast or ovarian cancer gene associations, this study identified *MSH6* and *ATM* as possible moderate-risk breast and ovarian cancer predisposition genes, respectively.

Methods

Samples

The study participants comprised 11 416 unrelated patients with BC, OV, or both referred for genetic testing from 1200 hospitals and clinics across the United States between 2014 and 2015 (**Table 1**; eTable 2 in the Supplement). The in-laboratory controls consisted of 3988 unrelated patients referred for genetic testing for noncancer conditions: 445 patients (11.1%) with cystic fibrosis, 1766 patients (44.3%) with rare inherited cardiovascular conditions, and 1777 reportedly healthy parents of children with epilepsy, congenital anomalies, autism, or psychiatric disorders (44.6%) enrolled in our clinical exome sequencing program. Patients with self-reported personal history of cancer were not included in the in-laboratory control group. Demographic, clinical history, and family history information were collected from documents provided by the ordering clinicians. A consistent set of protocols pertaining to exome library construction, exome capture, sequencing platform, and bioinformatics pipeline (eAppendix in the Supplement) was applied to all case and control samples to eliminate potential biases associated with differences in sequencing platforms and to enable further filtration of artifacts in cancers when compared with a reference population, the Genome Aggregation Database (gnomAD) (eAppendix in the Supplement). This study was submitted to the Solutions Institutional Review Board (Little Rock, Arkansas) and was deemed exempt from review. In addition, this board waived the need for informed patient consent because all data used were from previously existing, deidentified samples gathered for the purpose of research.

Statistical Analysis

After data cleaning and filtering (eAppendix in the Supplement), we collapsed pathogenic variants (loss of function and known pathogenic[22]) by gene and performed burden tests for 625 cancer genes (eAppendix, eTable 1, and eFigure 1 in the Supplement) among 9639 BC and 2051 OV cases vs 3988 in-laboratory controls. We excluded truncations located beyond the last 55 base pairs of the penultimate exon and not residing in functional domains (and thus may not be influenced by nonsense-mediated messenger RNA decay).[18,23] We also examined the distribution of pathogenic variants in 11 characterized BC or OV genes (eAppendix and eFigure 2 in the Supplement). Although the coverage of our sequencing data was comparable to that for the gnomAD database, we excluded sites with 10× coverage (per-site sequencing depth) or less in gnomAD or a missing rate of 30% or more at 10× coverage, which avoided inflation of signals in either direction.

For both case vs in-laboratory control comparisons and case vs gnomAD comparisons (eTables 3 and 4 in the Supplement), we used the cohort allelic sums test,[24] a burden test

widely used for association analysis of rare variants.[10,25-27] Odds ratios (ORs) and 2-sided *P* values were computed using the Fisher exact test, followed by the Benjamini-Hochberg false-discovery rate (FDR) multiple testing correction. An FDR-corrected $P < .05$ was considered statistically significant. For genes with variants observed in cases but absent in controls, ORs could not be estimated. Genes with 2 or fewer detectable pathogenic variants were excluded. Suspected pathogenic variants in BC- or OV-associated genes were reviewed and confirmed by Sanger sequencing.

To assess the contribution of candidate genes to specific clinical features of BC or OV, we tested their associations with breast cancer subtypes. Moreover, we performed 5 sensitivity analyses to evaluate the robustness of our findings and 2 case-case analyses to explore genetic associations with these clinical features (eTables 5-11 and eAppendix in the Supplement). Pathogenic variants carried by cases and in-laboratory controls are given in eTables 12 through 14 in the Supplement. All statistical analyses were conducted using R, version 3.3.3.

Results

Patient Characteristics

The overall racial/ethnic distribution for 11 416 patients with BC, OV, or both is shown in eFigure 3 in the Supplement. Of 9639 patients with BC (Table 1), 3960 (41.1%) were early-onset cases (diagnosis age ≤45 years) and 123 (1.3%) were male, with men having an older age at diagnosis than women (mean [SD] age, 61.8 [12.8] vs 48.6 [11.4] years). In addition, 1321 patients (13.7%) had bilateral or multiple BC, with age at first onset similar to the diagnosis age for patients with 1 BC (mean [SD] age, 49.0 [10.7] vs 48.7 [11.6] years), and 641 patients (6.6%) had additional cancer primaries. Of 2015 patients with OV, 445 (21.7%) received a diagnosis at 45 years or younger.

Most patients with BC (6829 [70.8%]) had invasive ductal breast cancer (IDC) (Table 1). Invasive lobular breast cancer (ILC) was less common (493 patients [5.1%]) and developed in patients at a slightly older age than those with IDC or other histologic types of BC. Of the 4447 patients with BC who also had information on tumor pathologic features (46.1%) (eTable 2 in the Supplement), 2476 (55.7%) were hormone (estrogen and progesterone) receptor-positive, 278 (6.3%) were human epidermal growth factor receptor-positive but hormone receptor-negative, 539 (12.1%) were triple-positive (TPBC), and 1154 (26.0%) were triple-negative (TNBC). The mean [SD] age of patients who were TPBC (45.3 [10.7] years) and hormone receptor-negative (45.4 [10.9] years) was less than that of patients who were TNBC (48.8 [11.1] years) and hormone receptor-positive (50.1 [11.6] years). Demographics and BC-associated gene frequencies for patients with BC without pathology information were similar to those described above.

In addition, 8152 patients with BC (84.6%) and 1670 patients with OV (81.4%) had a family history of cancer (Table 1). Also, 2060 patients with BC or OV (18.0%) provided information on *BRCA1/2* testing prior to exome sequencing in our laboratory, of whom 2034 (98.7%) had received a negative result. Thus, the cases described in this report are enriched for non-

Table 1. Clinical Charateristics of Patients With Breast Cancer (BC) or Ovarian Cancer (OV) and In-Laboratory Controls[a]

Charateristic	Patients, No. (%)		
	With BC (n = 9639)	With OV (n = 2051)	Controls (n = 3988)
Race/ethnicity			
White	7208 (74.8)	1718 (83.8)	2765 (69.3)
African American	985 (10.2)	80 (3.9)	323 (8.1)
Hispanic	874 (9.1)	161 (7.8)	561 (14.1)
Asian	572 (5.9)	92 (4.5)	339 (8.5)
Age at diagnosis, y			
Mean (SD)	48.7 (11.5)	55.7 (14.1)	39.7 (14.7)[b]
≤45	3960 (41.1)	445 (21.7)	2671 (67.0)
46-60	3876 (40.2)	768 (37.4)	951 (23.8)
>60	1518 (15.7)	751 (36.6)	332 (8.3)
Not provided[c]	285 (3.0)	87 (4.2)	34 (0.9)
Sex			
Male	123 (1.3)	0	1315 (33.0)
Female	9516 (98.7)	2051 (100.0)	2673 (67.0)
BC histology			
IDC	6829 (70.8)	NA	NA
ILC	493 (5.1)	NA	NA
IDC and ILC	141 (1.5)	NA	NA
Not provided[c]	2176 (22.6)	NA	NA
Bilateral BC			
Yes	1321 (13.7)	NA	NA
Not provided[c]	8318 (86.3)	NA	NA
BC subtypes (receptor status)			
ER^+, PR^+, and $HER2^-$ (HR^+)	2476 (25.7)	NA	NA
ER^-, PR^-, and $HER2^+$ (HR^-)	278 (2.9)	NA	NA
ER^+, PR^+, and $HER2^+$ (TPBC)	539 (5.6)	NA	NA
ER^-, PR^-, and $HER2^-$ (TNBC)	1154 (12.0)	NA	NA
Additional cancer primary			
Colorectal cancer	166 (1.7)	35 (1.7)	NA
Uterine cancer	378 (3.9)	145 (7.1)	NA
Others	97 (1.0)	28 (1.4)	NA
None	8998 (93.3)	1843 (89.8)	NA
1st- or 2nd-Degree relative with any cancer			
Yes	8152 (84.6)	1670 (81.4)	NA
No	282 (2.9)	72 (3.5)	NA
Not provided[c]	1205 (12.5)	309 (15.1)	NA
1st- or 2nd-Degree relative with BC			
Yes	5404 (56.1)	787 (38.4)	NA
No	3030 (31.4)	955 (46.6)	NA
Not provided[c]	1205 (12.5)	309 (15.1)	NA
1st- or 2nd-Degree relative with OV			
Yes	981 (10.2)	245 (11.9)	NA
No	7453 (77.3)	1497 (73.0)	NA
Not provided[c]	1205 (12.5)	309 (15.1)	NA

Abbreviations: ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; PR, progesterone receptor; NA, not applicable; TNBC, triple-negative BC; TPBC, triple-positive BC; +, positive for the receptor; −, negative for the receptor.

[a] Inferred ethnicity using principal component analysis to identify population substructure based on exome-sequencing data.

[b] Age at testing for controls.

[c] Incomplete or unavailable clinical information.

Table 2. Estimated ORs for Associations With Breast Cancer and Its Subtypes for Cases[a]

Gene	Breast Cancer			Male Breast Cancer		
	Mutated Alleles, No. (Total No. of Alleles)	OR (95% CI)	*P* Value	Mutated Alleles, No. (Total No. of Alleles)	OR (95% CI)	*P* Value
ATM[b]	79 (11 512)	2.97 (1.67-5.68)	4.02×10^{-5}	0 (137)	ND	ND
CHEK2[b]	110 (13 553)	2.19 (1.40-3.56)	2.66×10^{-4}	1 (152)	1.66 (0.04-10.34)	.46
MSH6	65 (17 362)	2.59 (1.35-5.44)	1.69×10^{-3}	0 (246)	ND	>.99
PALB2[b]	61 (15 532)	5.53 (2.24-17.65)	6.50×10^{-6}	3 (180)	22.73 (3.50-118.22)	8.86×10^{-4}
	Invasive Ductal Carcinoma			Invasive Lobular Carcinoma		
ATM[b]	41 (6659)	2.67 (1.42-5.30)	1.01×10^{-3}	6 (738)	3.50 (1.10-9.73)	.02
CHEK2[b]	60 (7190)	2.11 (1.30-3.55)	1.39×10^{-3}	8 (960)	2.49 (0.96-5.74)	.05
MSH6	27 (10 670)	1.79 (0.86-4.01)	.10	7 (1180)	4.23 (1.39-11.97)	5.63×10^{-3}
PALB[b]	45 (9466)	6.91 (2.75-22.33)	4.09×10^{-7}	1 (1131)	1.34 (0.03-11.97)	.56
	ER^+, PR^+, and $HER2^-$			ER^-, PR^-, and $HER2^+$		
ATM[b]	23 (2959)	3.39 (1.67-7.14)	2.96×10^{-4}	0 (316)	ND	ND
CHEK2[b]	33 (3152)	2.66 (1.52-4.71)	3.56×10^{-4}	2 (328)	1.54 (0.18-6.25)	.39
MSH6	13 (4724)	1.95 (0.81-4.81)	.14	2 (534)	2.67 (0.29-12.29)	.20
PALB2[b]	17 (4210)	5.87 (2.08-20.36)	1.68×10^{-4}	1 (451)	3.09 (0.07-27.68)	.31
	Triple-Positive Breast Cancer			Triple-Negative Breast Cancer		
ATM[b]	4 (653)	2.70 (0.65-8.64)	.09	3 (1321)	0.99 (0.18-3.55)	>.99
CHEK2[b]	9 (783)	3.14 (1.28-7.03)	6.50×10^{-3}	3 (1470)	0.51 (0.10-1.70)	.34
MSH6	0 (1032)	0.00 (0.00-3.02)	.63	7 (2222)	2.25 (0.74-6.36)	.09
PALB2[b]	4 (830)	6.61 (1.31-30.77)	.01	11 (1936)	8.27 (2.65-30.37)	5.40×10^{-5}

Abbreviations: ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; PR, progesterone receptor; ND, not determined; OR, odds ratio; +, positive for the receptor; −, negative for the receptor.

[a] Derived from Fisher exact test.

[b] Known breast or ovarian cancer gene.

carriers of the deleterious *BRCA1/2* mutations, which resulted in underestimation of risks for *BRCA1/2* genes.

The 3988 in-laboratory controls were younger than the patients with BC or OV at the time of testing (mean [SD] age, 39.7 [14.7] vs 50.9 [14.8] years). Although the racial/ethnic distribution among controls was somewhat similar to that of cases, the proportion of white individuals was slightly lower among controls and the proportion of Hispanic individuals was slightly higher among controls (eFigure 3 in the Supplement).

Breast Cancer Susceptibility Genes

We identified 4 genes that confer significantly increased BC risk (**Table 2** and eTable 3 in the Supplement), including a newly identified BC association with the Lynch syndrome[28] susceptibility gene *MSH6*. Our findings were supported by comparison with the gnomAD database as a reference population, in which all 4 genes were present and significantly associated with similar or higher estimated risks (eTable 3 in the Supplement). For both comparisons, *PALB2* was associated with high BC risk, consistent with other studies,[10,18] whereas *ATM* and *CHEK2* conferred 2- to 3-fold risks, also consistent with prior reports.[10,18] The *CHEK2* founder mutation c.1100delC was enriched among mutation carriers (eTable 14 in the Supplement) and was significantly associated with BC risk (OR, 4.00; 95% CI, 2.04-8.73; uncorrected $P = 2.66 \times 10^{-6}$, FDR-corrected $P = .003$). Without this variant, the association between *CHEK2* and BC was substantially attenuated and nonsignificant (all other variants combined: OR, 1.42; 95% CI, 0.76-2.81, uncorrected $P = .32$). Although *TP53* is known to confer risk for Li-Fraumeni syndrome, our findings indicated that *TP53* might confer moderate to high risk for BC at marginal significance (eTable 3 in the Supplement), in agreement with a recent report.[29] The MMR gene *MSH6* was associated with moderate BC risk, even after excluding 981 BC cases (Table 1) with first- and second-degree OV-affected relatives (eTable 7 in the Supplement). The *MSH6* mutation c.2945delC, enriched in *MSH6* mutation carriers (eTable 14 in the Supplement), primarily accounted for the observed association (c.2945delC variant-specific OR, 2.73; 95% CI, 1.22-7.18, uncorrected $P = .009$ vs all other variants combined OR, 2.21; 95% CI, 0.74-8.89, uncorrected $P = .18$). Increased risks were observed for 4 genes among white individuals (eTable 4 in the Supplement) and women (eTable 6 in the Supplement), although power was diminished for these stratified analyses.

No significant BC associations were observed with *BARD1*, *NF1*, or *PTEN* after correction for multiple tests (all FDR-corrected $P > .10$). Moreover, the MMR genes *MLH1*, *MSH2*, and *PMS2*, the MRN complex genes *MRE11A*, *RAD50*, and *NBN*, the OV susceptibility genes *BRIP1, RAD51C,* and *RAD51D*, and the melanoma predisposition gene *CDKN2A* did not significantly increase BC risk (all FDR-corrected $P > .20$). No increased BC risk was observed for *CDH1* (FDR-corrected $P = .99$) given its primary association with ILC[30] and the enrichment of IDC cases in the present study (Table 1).

We also identified roles for high- and moderate-risk BC genes in disease pathology (**Table 3**). The *PALB2* mutations were associated with greater than 5-fold risks of IDC, hormone receptor–positive BC, TPBC, TNBC, and male BC (MBC); association with TNBC has been previously noted.[31] The *ATM* and *CHEK2* genes conferred moderate IDC, ILC, and hormone receptor–positive risks; *CHEK2* was also associated with moderate TPBC risk. The *MSH6* gene was more closely asso-

ciated with ILC than with IDC. Moreover *ATM*, *CHEK2*, *MSH6*, and *PALB2* were associated with early-onset BC with similar or higher risks than those estimated for overall breast cancer.

In the case-case analyses of BC clinical features, *ATM* and *CHEK2* mutations were enriched among estrogen-progesterone-positive patients compared with estrogen-progesterone-negative patients (eTable 10 in the Supplement); *CHEK2* may contribute to early onset of BC compared with an onset age of at least 45 years (eTable 11 in the Supplement).

Ovarian Cancer Susceptibility Genes

Four genes were significantly associated with increased OV risks in both case vs in-laboratory control and case vs gnomAD comparisons (eTable 3 in the Supplement). We confirmed associations with known OV susceptibility genes *RAD51C* (OR, not estimable; FDR-corrected $P = .004$) and *TP53* (OR, 18.50; 95% CI, 2.56-808.10). An OR for *RAD51C* could not be estimated because of the absence of pathogenic variants among in-laboratory controls; however, *RAD51C* was associated with high OV risk in the comparison with gnomAD, in agreement with previous reports.[32,33] Ovarian cancer risks associated with *MSH6* or *ATM* or both have only recently been estimated.[29,34,35] We observed moderately increased OV risk associated with *MSH6* (OR, 4.16; 95% CI, 1.95-9.47) and *ATM* (OR, 2.85; 95% CI, 1.30-6.32). However, *BRIP1*, *RAD51D*, *CDKN2A*, and the MRN complex genes *MRE11A*, *RAD50*, and *NBN* were not significantly associated with elevated OV risk. All 4 genes increased OV risks among white individuals (eTable 4 in the Supplement) and among women (eTable 6 in the Supplement); 1 gene, *MSH6*, was significantly associated with early-onset OV (Table 3).

Table 3. Candidate Genes Associated With Early-Onset Breast or Ovarian Cancer[a]

Gene	Cases, Mutated Alleles, No. (Total No. of Alleles)	Controls, Mutated Alleles, No. (Total No. of Alleles)	OR (95% CI)[b]	*P* Value
Breast cancer				
ATM[c]	34 (4804)	14 (6038)	3.07 (1.60-6.19)	2.26×10^{-4}
CHEK2[c]	57 (5630)	24 (6442)	2.73 (1.67-4.61)	1.69×10^{-5}
MSH6	29 (7601)	11 (7806)	2.71 (1.31-6.03)	3.90×10^{-3}
PALB2[c]	24 (6812)	5 (7314)	5.17 (1.93-17.35)	2.40×10^{-4}
Ovarian cancer				
ATM	1 (492)	14 (6095)	0.88 (0.02-5.84)	>.99
MSH6[c]	8 (852)	11 (7826)	6.73 (2.34-18.43)	2.35×10^{-4}
RAD51C[c]	1 (344)	0 (4804)	ND	.07
TP53[c]	3 (749)	1 (7209)	28.96 (2.32-1506.64)	3.09×10^{-3}

Abbreviation: ND, not determined.

[a] Diagnosis age 45 years or younger.

[b] Derived from Fisher exact test.

[c] Known breast or ovarian cancer gene.

Discussion

We undertook large-scale whole-exome sequencing of 15 404 patients referred for genetic testing to provide new insights into predisposition genes for breast and ovarian cancers. Owing to the lack of significantly powered studies, the number and selection of risk genes for hereditary cancer testing have not been standardized,[36] and robust evidence for association with BC or OV risk is only available for a modest set of characterized genes typically included in most panels.[10,11] This is particularly the case for moderate-penetrance genes, for which there exists either conflicting evidence or insufficient data for reliable estimation.[14]

Many of our findings regarding the known BC or OV or both susceptibility genes are consistent with the literature reported to date.[10,11,14,18] However, some anticipated associations were not observed and other associations were newly identified in this study of case patients and controls. Moreover, we were able to examine associations with clinical features of breast cancer and found that *ATM* and *CHEK2* carriers were more likely to have estrogen-progesterone receptor–positive disease than estrogen-progesterone receptor–negative disease, a finding partially supported by a previous study examining the *CHEK2* association with estrogen receptor–positive breast cancer.[37] The role of *PALB2* as a high penetrance BC gene has been established,[10,18] but evidence for its association with OV is mixed.[13,34] Consistent with Ramus et al,[13] we did not observe elevated OV risk associated with *PALB2* (OR, 2.1; 95% CI, 0.5-9.1; $P = .31$). We also observed increase risk for multiple breast cancer subtypes associated with *PALB2*, including MBC, for which prior evidence is scant.[31] The *RAD51C* gene conferred OV but not BC risk, in agreement with previous reports indicating that it may be a high penetrance OV[32,33] but not BC gene.[2,10] Likewise, *RAD51D* was recently reported to increase BC risk[18]; however, we and others observed no such association.[12] Moreover, we and others[18,38] failed to find any association of *BRIP1* with BC. The MMR genes *MSH2* and *PMS2* were also not associated with increased BC risk, in agreement with recent findings.[18] Despite earlier reports[15-17] of BC and OV associations with MRN complex genes and *CDKN2A*, we and others[29] did not observe higher BC or OV risk associated with those genes.

We also identified BC and OV associations with additional genes involved in genomic stability pathways. Protein kinase produced by *ATM*, a gene known to be associated with BC, plays a crucial role in the early stages of homologous recombination and regulation of cell cycle checkpoints.[39] However, prior evidence linking *ATM* and OV is limited. In both comparisons with in-laboratory and gnomAD controls, among all participants or a subset of white individuals, we observed a 2- to 3-fold increased risk of OV associated with *ATM*, with effect sizes similar to or slightly higher than those in recent observations.[29,34,35] The well-characterized MMR complex also has a role in maintaining genomic stability, repairing small genomic alterations and ensuring error-free homologous recombination repair. Mutations in MMR genes have been shown to increase susceptibility to BC, OV, or both,[39] although evidence to date regarding *MSH6* is inconsistent. Whereas some studies have failed to

observe significantly increased BC or OV risks associated with *MSH6*,[28,39] others have reported it to be an OV susceptibility gene.[40,41] Our data suggest that *MSH6* has a strong significant association with OV. We also observed modestly increased risk for BC, early-onset BC, and the ILC subtype associated with *MSH6*, in agreement with a small prior study that reported BC association but failed to identify the *MSH6* pathogenic variants.[42] The *MSH6* c.2945delC variant, which is reported herein with relatively high frequency, is a known pathogenic variant and therefore no upfront orthogonal confirmation was performed, per the research study design. We suspect that at least some of these are false-positive calls. Further analysis of the surrounding sequence suggests a mechanism for the false-positive calls due to polymerase slippage. Our findings support *MSH6* as a weak BC susceptibility gene.[18] These 2 associations were confirmed in multiple sensitivity analyses, including comparisons with gnomAD and analyses among white individuals and women.

Limitations and Strengths

Although many of our findings are supported by the literature, there are some limitations to the present study. First, we had incomplete information on the personal disease history of the in-laboratory controls and cannot rule out the possibility that some individuals may have been affected with BC or OV. The same issue underlies the use of Exome Aggregation Consortium and gnomAD data sets, particularly because approximately 6% of the gnomAD samples were from The Cancer Genome Atlas. However, analysis of affected controls would result in a bias toward null and implies that the true risks are greater than what we reported here. Second, we based our analysis on the aggregation of both protein truncating and known pathogenic variants, which could result in underestimation of risk for less-studied genes. Third, information on hormone receptor status was available for only approximately 46% of cases. Although power was diminished for these analyses because of the reduced sample size, inferences were unlikely to have selection bias because demographics and mutation frequencies of cases with or without pathology data were comparable (eTable 2 in the Supplement). Last, our study was composed of patients referred for genetic testing and may therefore be enriched for individuals with early-onset BC or OV, bilateral and TNBC, Lynch syndrome–associated cancers, and a family history of BC, OV, or other cancers. We showed that BC associations persisted despite exclusion of cases with Lynch syndrome and other cancers and exclusion of cases with close relatives affected with OV. Although the enrichment of personal and family histories for patients with BC may have afforded us an opportunity to identify new associations, particularly those of moderate risk, these characteristics should be considered when generalizing our study findings.

Despite these limitations, our study had several strengths. As a clinical diagnostic laboratory processing hundreds of thousands of samples annually, we were able to generate high-quality and high-sensitivity next-generation sequencing results (eFigures 3-5 in the Supplement) linked to detailed patient phenotype, personal and family history, and demographic information. The high resolution of sequencing data using a large sample of patients and controls enabled us to identify novel associations of BC and OV with susceptibility genes in addition to providing evidence for reported but unconfirmed associations. Although other large studies relying solely on the use of publicly available reference data sets[13,31,34] are susceptible to bias when estimating genetic risks because of systematic differences in sequencing methods among cases and controls,[43,44] we uniformly sequenced and analyzed cases and controls with the same technology and in the same laboratory to avoid this bias. We were then able to support our results by comparisons with the gnomAD database, while requiring 10× minimum site coverage to avoid inflation of estimates due to no or low coverage. Moreover, suspected pathogenic variants in the associated genes were subsequently confirmed by Sanger sequencing, eliminating the false-positives that are commonly present in next-generation sequencing results.[45]

Conclusions

Our findings in a large sample of patients referred for genetic testing confirmed several known or suspected associations with BC or OV and implicate new roles for genes involved in genomic maintenance. These results, therefore, have the potential to serve as the foundation for future epidemiologic, clinical, or functional studies of BC or OV and to inform comprehensive genetic testing and clinical practice.

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Author Contributions: Drs Lu and Li contributed equally and are considered co–first authors. Drs Lu and Li had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
Concept and design: Lu, Black, Lee, Hoiness, Chen, Tippin Davis, Mexal, Dunlop, Elliott.
Acquisition, analysis, or interpretation of data: Lu, Li, Black, Lee, Hoiness, Wu, Mu, Huether, Chen, Sridhar, Tian, McFarland, Dolinsky, Mexal.
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