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ARTICLE

Triple-Negative Breast Cancer Risk Genes Identified by Multigene Hereditary Cancer Panel Testing

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Abstract

Background: Germline genetic testing with hereditary cancer gene panels can identify women at increased risk of breast cancer. However, those at increased risk of triple-negative (estrogen receptor–negative, progesterone receptor–negative, human epidermal growth factor receptor–negative) breast cancer (TNBC) cannot be identified because predisposition genes for TNBC, other than *BRCA1*, have not been established. The aim of this study was to define the cancer panel genes associated with increased risk of TNBC.

Methods: Multigene panel testing for 21 genes in 8753 TNBC patients was performed by a clinical testing laboratory, and testing for 17 genes in 2148 patients was conducted by a Triple Negative Breast Cancer Consortium (TNBCC) of research studies. Associations between deleterious mutations in cancer predisposition genes and TNBC were evaluated using results from TNBC patients and reference controls.

Results: Germline pathogenic variants in *BARD1*, *BRCA1*, *BRCA2*, *PALB2*, and *RAD51D* were associated with high risk (odds ratio > 5.0) of TNBC and greater than 20% lifetime risk for overall breast cancer among Caucasians. Pathogenic variants in *BRIP1*, *RAD51C*, and *TP53* were associated with moderate risk (odds ratio > 2) of TNBC. Similar trends were observed for the African American population. Pathogenic variants in these TNBC genes were detected in 12.0% (3.7% non-*BRCA1/2*) of all participants.

Conclusions: Multigene hereditary cancer panel testing can identify women with elevated risk of TNBC due to mutations in *BARD1*, *BRCA1*, *BRCA2*, *PALB2*, and *RAD51D*. These women can potentially benefit from improved screening, risk management, and cancer prevention strategies. Patients with mutations may also benefit from specific targeted therapeutic strategies.

Triple-negative breast cancer (TNBC) is an aggressive subtype defined by absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression (1). TNBC accounts for an estimated 15% of breast cancer in the Caucasian population and 35% in the African American population (2). TNBC is associated with

advanced disease stage and higher-grade tumors at diagnosis (1) and is associated with an increased recurrence risk and poor five-year survival rates relative to other breast cancers (3). Germline genetic testing of TNBC patients using hereditary cancer gene panels is now common practice because of the high frequency of *BRCA1* and *BRCA2* mutations and the identification of non–*BRCA1/2* predisposition gene mutations in 5% of TNBC patients unselected for age of diagnosis or family history (4), and in 4.5% of TNBC patients receiving clinical genetic testing (5).

National Comprehensive Cancer Network (NCCN) guidelines recommend *BRCA1/2* testing for patients meeting hereditary breast and ovarian cancer testing criteria or with a TNBC diagnosis at age 60 years or younger. However, recommendations for testing of other genes are not fully established because the risks of TNBC associated with mutations in cancer predisposition genes have not been established. Thus, a better understanding of gene-specific risks for TNBC is needed to identify the genes that should be tested in the setting of TNBC. This should lead to improved clinical management of individuals at risk for or diagnosed with this aggressive form of breast cancer. We present germline genetic testing results from two cohorts of TNBC patients and provide estimated TNBC risk associated with pathogenic variants in 21 cancer predisposition genes.

Methods

Study Population

This study involved 10 901 TNBC patients, including 8753 from a cohort of 140 449 individuals subjected to clinical germline cancer panel testing between March 2012 and June 2016 at a clinical testing laboratory (Ambry Genetics, Aliso Viejo, CA). Demographic, clinical, and family history information, as well as TNBC status based on histopathology markers, was provided by the ordering physician (Supplementary Methods and Supplementary Figure 1, available online) (6). The clinical testing study was approved by the Western Institutional Review Board. In addition, results from a previous study of 2148 TNBC patients by a Triple-Negative Breast Cancer Consortium (TNBCC) using a 17-gene panel (4) were included (Supplementary Methods, available online). All TNBCC patients provided informed consent for institutional review board–approved studies.

Multigene Panel Testing

Germline genetic testing results for 21 genes (*BRCA1*, *BRCA2*, *PALB2*, *BARD1*, *BRIP1*, *NF1*, *MSH2*, *MSH6*, *PMS2*, *CDKN2A*, *RAD51C*, *RAD51D*, *RAD50*, *NBN*, *MRE11A*, *ATM*, *CHEK2*, *TP53*, *PTEN*, *STK11*, *CDH1*) from the clinically tested cohort of TNBC patients were evaluated. Testing was performed by targeted custom capture and sequencing and chromosomal rearrangement analysis (Supplementary Methods, available online) (6). A five-tier classification system was applied to all alterations (6), and variants from Ambry Genetics were subsequently submitted to ClinVar. Analysis of 17 predisposition genes (*BRCA1*, *BRCA2*, *PALB2*, *BARD1*, *BRIP1*, *RAD51C*, *RAD51D*, *RAD50*, *NBN*, *MRE11A*, *XRCC2*, *ATM*, *CHEK2*, *TP53*, *PTEN*, *STK11*, *CDH1*) in 2148 TNBCC patients, unselected for age of diagnosis, family history, or ethnicity, was performed by custom capture panel and sequencing (Supplementary Methods, available online) (4).

Comparison of TNBC Patient and ExAC Reference Control Subjects

The combined frequencies of pathogenic variants and likely pathogenic variants (PVs) in each of 21 genes in TNBC patients from the clinical cohort and 17 genes in TNBC patients from the TNBCC study were compared with PV frequency in more than 26 000 non-Finnish European population (NFE) Exome Aggregation Consortium (ExAC) reference control subjects (7), excluding The Cancer Genome Atlas (TCGA) exomes (Supplementary Methods, available online) (8,9). Both PASS and non-PASS variants in ExAC (GATK Variant Quality Score Recalibration [VQSR] sensitivity of 99.6% and 95%, respectively) were utilized because many non-PASS variants from ExAC were observed as PVs in TNBC patients. All loss of function and missense variants with consensus definitions as "pathogenic" by clinical laboratories in ClinVar were included. The frequencies of PVs by gene in African American TNBC patients from the clinical cohort were compared with frequencies in Genome Aggregation Database (gnomAD) African and African American reference control subjects (gnomAD_AFR) (Supplementary Methods, available online).

Statistical Analysis

Associations between combined PVs in each gene and phenotypic characteristics of TNBC patients and age of diagnosis were assessed using the Fisher exact test and the Kolmogorov-Smirnov test, respectively. Association studies compared summed PV frequency in Caucasian and African American TNBC patients for each gene with ExAC-NFE non-TCGA and gnomAD AFR, respectively. A P value of less than .05 was considered statistically significant. Odds ratios (ORs) and 95% confidence intervals (CIs) were based on the Fisher exact test. Genes were categorized as high risk (OR > 5.0), moderate risk (OR = 2.0–5.0), or low clinical relevance (OR < 2.0). Enrichment of mutations by gene in TNBC relative to all non-TNBC breast cancers was estimated by logistic regression, with adjustment for age of diagnosis and family history of breast cancer. Lifetime absolute risk for TNBC and overall breast cancer were estimated by combining the odds ratio estimates with age-adjusted subtype-specific incidence rates from the Surveillance, Epidemiology, and End Results (SEER) registry in the competing risk component of the absolute risk equation (Supplementary Methods, available online). False discovery rate–adjusted P values were calculated as previously described (10). All statistical tests were two-sided.

Results

Characteristics of Study Population

Among the 8753 TNBC patients from the clinical cohort, 5498 (62.8%) were Caucasian and 1271 (14.5%) were African American (Table 1; Supplementary Table 1, available online). In contrast, 2095 (97.5%) from the TNBCC cohort were Caucasian (Table 1). The mean age (range) at diagnosis was 49.8 (18–90) years for the clinical cohort and 50.8 (20–93) years for the TNBCC cohort (Table 1). In addition, 51.9% of the clinical cohort but only 21.9% of the 2148 TNBCC patients reported a first- or second-degree relative with breast cancer (Table 1).

Table 1. Study population characteristics

Characteristics	Clinical TNBC cohort		TNBCC	
	No. (%)	% variant	No. (%)	% variant
Total patients	8753 (100)	14.4	2148 (100)	14.51
Sex				
Female	8743 (99.9)	14.4	2148 (100.0)	14.51
Male	9 (0.1)	0	0 (0.0)	0
Unknown	1 (0.0)	0	0 (0.0)	0
Race/ethnicity				
African American	1271 (14.5)	14.6	34 (1.6)	ND
Ashkenazi Jewish	292 (3.3)	15.1	0 (0.0)	ND
Asian	334 (3.8)	16.9	10 (0.5)	ND
Caucasian	5206 (59.5)	14.0	2095 (97.5)	14.3
Hispanic	580 (6.6)	16.2	7 (0.3)	ND
Other/unknown	1070 (12.2)	13.8	2 (0.1)	ND
Personal history of cancer				
Age at diagnosis, y				
$\leq$36	1178 (13.5)	19.8	277 (12.9)	22.4
$\leq$45	3106 (35.5)	17.8	791 (36.8)	19.1
$\leq$50	4532 (51.8)	16.7	1039 (48.4)	18.4
$\leq$60	7287 (83.3)	15.0	1582 (73.6)	16.0
>60	1431 (16.3)	11.4	501 (23.3)	6.9
Unknown	35 (0.4)	ND	65 (3.0)	ND
Multiple breast cancer	1309 (15.0)	19.5	NA	ND
Ovarian	101 (1.2)	37.7	NA	ND
Colorectal	86 (1.0)	18.1	NA	ND
Pancreatic	24 (0.3)	ND	NA	ND
Family history of cancer*				
Breast (no ovarian)	3983 (45.5)	15.4	470 (21.9)	16.4
Breast and ovarian	560 (6.4)	23.9	NA	ND
Ovarian (no breast)	403 (4.6)	25.8	64 (3.0)	ND
Colorectal	1862 (21.3)	13.8	NA	ND
Pancreatic	753 (8.6)	16.5	NA	ND
No breast, ovarian, colorectal, or pancreatic†	2223 (25.4)	10.0	1612 (75.0)	12.0
Age at TNBC diagnosis, mean ± SD (range), y	49.8 ± 11.3	(18–90)	50.8 ± 12.7	(20–93)

*First- and second-degree relatives. % variant = percentage with inactivating variants in all panel genes; NA = not available; ND = not determined; TNBCC = Triple Negative Breast Cancer Consortium.
†No family history of breast or ovarian cancer in TNBCC patients.

Pathogenic Variants Identified by Panel Testing

From the clinical cohort, PVs in all 21 genes were detected in 14.4% (8.4% BRCA1/2, 6.0% non-BRCA) of TNBC patients of any race or ethnicity, 14.0% (7.8% BRCA1/2, 6.2% non-BRCA) of Caucasians, and 14.6% (9.0% BRCA1/2, 5.6% non-BRCA) of African Americans (Tables 1 and 2; Supplementary Tables 1 and 2, available online). Of the 2148 TNBCC patients, 14.5% (10.4% BRCA1/2, 4.0% non-BRCA) had PVs in 17 genes (Supplementary Table 2, available online). PALB2 (1.0%–1.6%) and BARD1 (0.5%–0.7%) were the most commonly mutated non-BRCA1/2 genes in the clinical cohort and TNBCC study (Table 2; Supplementary Table 2, available online). Characteristics of TNBC patients with PVs are shown in Table 1 and Supplementary Tables 1 and 3 (available online).

Associations Between PVs and TNBC

Comparison of Caucasian TNBC patients from the clinical cohort and the TNBCC study with ExAC NFE non-TCGA reference control subjects showed that BRCA1 PVs were associated with high risk of TNBC, consistent with previous studies (Table 3). Similarly, BRCA2 PVs were associated with high risk (OR > 5.0) of TNBC in both the clinical cohort (OR = 5.42, 95% CI = 4.13 to 7.05, P < 2.2×10^{-16}) and the TNBCC study (OR = 6.33, 95% CI = 4.48 to 8.92, P < 2.2×10^{-16}) (Table 3). Likewise, PVs in PALB2 (OR = 14.41, 95% CI = 9.27 to 22.60, P < 2.2×10^{-16}), BARD1 (OR = 5.92, 95% CI = 3.36 to 10.27, P = 2.2×10^{-9}), and RAD51D (OR = 6.97, 95% CI = 2.60 to 18.66, P = 3.1×10^{-4}), were shown to be statistically significantly associated with high risk of TNBC in the clinical cohort (Table 3). PVs in BRIP1 and RAD51C, both of which were previously excluded as breast cancer predisposition genes (6,11,12), were associated with moderate risk (OR > 2) of TNBC. PVs in NF1 were not statistically significantly associated with moderate risk of TNBC in the clinical cohort after adjustment for multiple testing (Supplementary Table 4, available online). PVs in MSH6 were associated with moderate risk of TNBC in this study, consistent with a twofold increased risk of overall breast cancer (standardized incidence ratio = 2.11, 95% CI = 1.56 to 2.86) for MSH6 PVs in a recent study (13), but the TNBC association was not statistically significant after adjustment for multiple testing

Table 2. Gene-based frequency of mutated alleles in TNBC patients of all races/ethnicities*

Genes	Clinical cohort			TNBCC cohort		
	PV	No. cases	Frequency, %	PV	No. cases	Frequency, %
Established breast cancer predisposition genes						
ATM	17	6652	0.26	4	2148	0.19
BARD1	48	6464	0.74	10	2148	0.47
BRCA1	513	8537	6.01	166	2148	7.73
BRCA2	201	8537	2.35	58	2148	2.70
CHEK2	22	6639	0.33	2	2148	0.09
PALB2	111	6980	1.59	22	2148	1.02
PTEN	4	8719	0.05	1	2148	0.05
RAD51D	16	6095	0.26	8	2148	0.37
TP53	14	8741	0.16	2	2148	0.09
Total frequency			11.75			12.71
Other cancer predisposition genes						
BRIP1	27	6464	0.42	10	2148	0.47
CDH1	5	8505	0.06	NA	NA	NA
CDKN2A	5	1790	0.28	NA	NA	NA
MLH1	5	3497	0.14	NA	NA	NA
MRE11A	6	6464	0.09	4	2148	0.19
MSH2	3	3497	0.09	0	372	ND
MSH6	9	3497	0.26	1	372	0.27
NBN	12	6464	0.19	2	2148	0.09
NF1	9	6097	0.15	NA	NA	NA
PMS2	9	3497	0.26	NA	NA	NA
RAD50	14	6464	0.22	6	2148	0.28
RAD51C	31	6464	0.48	8	2148	0.37
XRCC2	NA	NA	NA	2	2148	0.09
Total frequency			2.62			1.76

*Frequency = pathogenic variant frequency; NA = not genotyped; PV = pathogenic variant; TNBCC = Triple Negative Breast Cancer Consortium.

(Supplementary Table 4, available online). PVs in *ATM*, *CHEK2*, *NBN*, and *RAD50* yielded no clinically relevant risks (OR > 2) of TNBC (Supplementary Table 4, available online). Similar associations with TNBC for all of these genes were observed in the TNBCC study (Table 3; Supplementary Table 4, available online).

Risk estimates for most genes were not substantially altered 1) when using gnomAD Caucasian control subjects (Supplementary Table 5, available online); 2) when using ExAC PASS controls (Supplementary Table 6, available online); 3) when restricting to patients with TNBC as the first cancer (Supplementary Table 7, available online); and 4) when considering all races and ethnicities (Supplementary Table 8, available online). Exclusion of TNBC patients with personal or family history of ovarian or colorectal cancer had little impact (Supplementary Tables 9 and 10, available online), except for a slightly attenuated association between *RAD51C* and TNBC (OR = 1.96, 95% CI = 0.93 to 4.07, P = .09) when excluding ovarian cancer (Supplementary Table 9, available online). Furthermore, PVs in *BRCA1*, *RAD51C*, *BARD1*, and *RAD51D* were enriched more than threefold in TNBC patients relative to all non-TNBC patients, suggesting that these are predominantly TNBC predisposition genes (Table 4). *PALB2* and *BRCA2* also displayed statistically significant enrichment (Table 4).

PV Frequency in African American TNBC Patients

PVs in TNBC genes were identified in 12.7% of 1271 African American TNBC patients, including 3.7% in non-BRCA genes (Supplementary Table 2, available online). No major differences in the PV frequencies in genes between African American and Caucasian TNBC patients were observed (Supplementary Table 2, available online). An exploratory case–control analysis of African American TNBC patients from the clinical cohort and gnomAD-AFR suggested that PVs in *BRCA1*, *BRCA2*, *BARD1*, and *PALB2* were statistically significantly associated with high or moderate risk of TNBC (Supplementary Table 11, available online). Similarly, *RAD51C* PVs were statistically significantly associated with very high risk of TNBC, although these findings will need to be replicated because of limited numbers of PVs in TNBC patients and control subjects. Small numbers of PVs in other genes yielded unstable estimates.

Frequency of PVs Based on Age of Onset and Family History

PVs in moderate-risk and high-risk TNBC genes were observed in 12.0% of TNBC patients from the clinical cohort and 13.2% from TNBCC, including 3.7% in non-BRCA genes in both cohorts (Table 2; Supplementary Table 2, available online). PV frequencies varied based on age at diagnosis and total number of relatives with breast or ovarian cancer among Caucasian and African American TNBC patients (Table 5; and Supplementary Table 12, available online, respectively). For example, PV frequencies ranged from 5% in patients diagnosed older than age 60 years with no family history of breast or ovarian cancer to 35.3% in patients diagnosed younger than age 35 years with a family history of ovarian cancer (Table 5). BRCA1 mutations accounted for a larger proportion of early-onset TNBC whereas, other TNBC genes accounted for a larger proportion of later-onset TNBC. In addition, PVs in TNBC genes were identified in

Table 3. Estimated risks of TNBC in Caucasian patients associated with mutations in hereditary cancer panel genes

TNBC-associated genes	Clinical cohort		TNBCC cohort		ExAC controls		Clinical TNBC risk			TNBCC TNBC risk		
	PV	No. cases	PV	No. cases	PV	No. controls	OR (95% CI)	P*	FDR-adjusted P	OR (95% CI)	P*	FDR-adjusted P
BARD1	25	4090	9	2003	27	26 079	5.92 (3.36 to 10.27)	2.20×10^{-9}	1.26×10^{-8}	4.35 (2.02 to 9.30)	7.60×10^{-4}	.002
BRCA1	255	5265	158	2003	82	26 911	16.27 (12.65 to 20.95)	$<2.2 \times 10^{-16}$	$<2.2 \times 10^{-16}$	26.90 (20.51 to 35.51)	$<2.2 \times 10^{-16}$	$<2.2 \times 10^{-16}$
BRCA2	115	5265	51	2003	109	26 791	5.42 (4.13 to 7.05)	$<2.2 \times 10^{-16}$	$<2.2 \times 10^{-16}$	6.33 (4.48 to 8.92)	$<2.2 \times 10^{-16}$	$<2.2 \times 10^{-16}$
BRIP1	17	4090	9	2003	49	26 841	2.28 (1.30 to 4.00)	.006	.01	2.46 (1.18 to 5.08)	.02	.04
PALB2	70	4383	17	2003	30	26 869	14.41 (9.27 to 22.60)	$<2.2 \times 10^{-16}$	$<2.2 \times 10^{-16}$	7.63 (4.05 to 14.08)	7.05×10^{-9}	4.00×10^{-8}
RAD51C	15	4090	8	2003	37	26 647	2.64 (1.44 to 4.80)	3.09×10^{-3}	.01	2.88 (1.21 to 6.29)	.01	.03
RAD51D	8	3814	7	2003	8	26 555	6.97 (2.60 to 18.66)	3.10×10^{-4}	.001	11.62 (3.79 to 31.91)	3.23×10^{-5}	1.10×10^{-4}
TP53	10	5423	2	2003	18	26 790	2.75 (1.18 to 6.16)	.02	.04	1.49 (0.25 to 6.30)	.65	.73
TP53†	6	1055	2	504	18	26 790	8.49 (3.30 to 21.53)	2.19×10^{-4}	8.40×10^{-4}	5.92 (0.98 to 25.16)	.05	.10

*Statistical significance of case-control associations was estimated using the Fisher exact test. All tests were two-sided. CI = confidence interval; FDR = false discovery rate; NA = not genotyped; OR = odds ratio from case-control analysis comparing PV frequencies in clinical cohort cases or TNBCC cohort cases with frequencies in ExAC controls; PV = pathogenic variant; TNBCC = Triple Negative Breast Cancer Consortium.
†Age at diagnosis of 40 years or younger.

12.4% of TNBC patients from the clinical cohort meeting NCCN BRCA1/2 testing criteria and in 4.3% of TNBC patients not meeting criteria. When expanding to other breast cancer predisposition genes, overall detection of PVs increased to 13.1% and 4.6%, respectively.

Absolute Risk Estimates for Breast Cancer and TNBC

When combining OR estimates for Caucasian TNBC patients from the clinical cohort with age-adjusted subtype-specific incidence rates from the SEER registry, BRCA1 and PALB2 PVs were associated with 18% and 10% lifetime risks of TNBC, respectively, followed by BARD1 (7%), BRCA2 (6%), and RAD51D (5%) (Figure 1). Estimated TNBC risks contributed substantially to the overall breast cancer risks for genes known to confer greater than 20% lifetime risk for breast cancer (PALB2 56%, BRCA1 46%, BRCA2 42%) (14,15) and to estimated absolute risks of 26% for RAD51D and 21% in BARD1 (Figure 1). Similar exploratory studies based on the African American population within the clinical cohort suggested even greater contributions of TNBC risks to greater than 20% lifetime risk estimates for overall breast cancer for several genes (BRCA1 81%, BRCA2 62%, PALB2 41%, BARD1 39%) (Supplementary Figure 2, available online).

Discussion

This study of 10 901 TNBC patients from a cohort of TNBC patients receiving clinical genetic testing and a separate series of TNBC patients unselected for age of diagnosis or family history of breast or ovarian cancer is the first to establish which cancer predisposition genes on multigene hereditary cancer panels are associated with increased risk of TNBC. Consistent with findings from an independent study of TNBC patients (5), 14.4% of TNBC patients in the clinical cohort had PVs in cancer predisposition genes, whereas 12.0% had PVs in the eight TNBC genes.

Among these TNBC predisposition genes, only BRCA1 has been well characterized as a TNBC susceptibility gene, although BARD1, BRIP1, PALB2, and RAD51C PVs have been reported as enriched in TNBC relative to other breast cancer subtypes (5,16,17). Results from this study further characterize the role of these genes in TNBC and also introduce a novel association between RAD51D and risk for TNBC. Importantly, BRIP1, RAD51C, and RAD51D have been primarily characterized by others as ovarian rather than breast cancer susceptibility genes (18,19); however, results from the present study strongly support an association of these genes with TNBC. It is likely that this association was undetected in some previous studies due to the rarity of PVs in these genes among breast and breast/ovarian cancer families, paired with the lower prevalence of TNBC relative to other breast cancer subtypes.

Despite higher general population TNBC risk in African Americans relative to Caucasians (1.91% vs 1.19%) (20), risk estimates conferred by PVs in predisposition genes have not been reported. Thus, although the analysis was limited by cohort size, an exploratory association study using African American TNBC patients and gnomAD AFR control subjects was undertaken. Similar to the Caucasian population, statistically significant associations between BRCA1, BRCA2, BARD1, and PALB2 PVs and TNBC were observed. Importantly, breast cancer absolute risk estimates for patients with BRCA1, BRCA2, PALB2, and BARD1 PVs ranged from 40% to 80%, which may impact breast cancer risk management decisions. However, larger studies of

Table 4. Enrichment of pathogenic variants in TNBC genes in Caucasian TNBC relative to non-TNBC breast cancer patients

Gene*	TNBC		Non-TNBC		TNBC/non-TNBC associations		
	Mutated alleles	TNBC BC†	Mutated alleles	Non-TNBC BC†	OR (95% CI)	P‡	FDR-adjusted P
BARD1	27	4032	62	34 437	3.73 (2.30 to 5.95)	2.35×10^{-7}	6.27×10^{-7}
BRCA1	292	5208	443	44 503	5.77 (4.96 to 6.71)	$<2.2 \times 10^{-16}$	$<2.2 \times 10^{-16}$
BRCA2	116	5208	695	44 503	1.43 (1.17 to 1.75)	6.57×10^{-4}	.001
BRIP1	18	4032	109	34 437	1.41 (0.84 to 2.35)	.19	.23
PALB2	74	4324	298	36 714	2.12 (1.63 to 2.74)	9.83×10^{-8}	3.15×10^{-7}
RAD51C	21	4032	47	34 437	3.82 (2.23 to 6.39)	3.62×10^{-6}	8.27×10^{-6}
RAD51D	10	3766	27	31 818	3.13 (1.42 to 6.43)	.004	.007
TP53	10	5362	95	46 065	0.90 (0.46 to 1.71)	.87	.93

*Only genes with four or more pathogenic variants in TNBC or non-TNBC BCs are displayed. BC = breast cancer; CI = confidence interval; FDR = false discovery rate; OR = odds ratio; TNBC = triple-negative breast cancer.
†Breast cancers from the clinical breast cancer cohort.
‡Significance of associations were estimated using the Fisher exact test. All tests were two-sided.

Table 5. Frequency of mutations by age at TNBC diagnosis and family history of breast and ovarian cancer among Caucasian TNBC patients in the clinical cohort

	Age at TNBC diagnosis, y									
	<35		35–39		40–49		50–60		>60	
Family cancer history	PV carriers	% PV	PV carriers	% PV	PV carriers	% PV	PV carriers	% PV	PV carriers	% PV
No breast, no ovarian										
BRCA1	18	9.2	12	5.9	29	5.2	20	2.8	2	0.9
Other TNBC genes*	6	3.1	7	4.43	23	4.9	28	4.7	7	4.1
All TNBC genes total	24	12.3	19	10.31	52	10.1	48	7.5	9	5.0
Other breast cancer†	2	1.3	0	0.0	6	1.4	2	0.4	0	0.0
All breast cancer genes‡	26	13.6	19	10.3	58	11.5	50	7.9	9	5.0
One relative with breast, no ovarian										
BRCA1	15	12.6	14	10.6	23	5.3	22	3.6	8	2.4
Other TNBC genes*	8	6.9	4	3.1	29	7.6	36	6.7	16	5.4
All TNBC genes total	23	19.6	18	13.7	52	12.9	58	10.3	24	7.8
Other breast cancer†	0	0.0	1	0.8	2	0.6	4	0.7	2	0.8
All breast cancer genes‡	23	19.6	19	14.6	54	13.4	62	11.0	26	8.5
≥2 relatives with breast, no ovarian										
BRCA1	13	28.9	3	6.0	16	8.2	16	5.2	5	2.0
Other TNBC genes*	2	5.2	6	12.4	15	8.2	18	6.5	15	6.3
All TNBC genes total	15	34.1	9	18.4	31	16.4	34	11.7	20	8.3
Other breast cancer†	1	2.6	1	1.8	2	1.1	3	1.1	3	1.5
All breast cancer genes‡	16	36.7	10	20.2	33	17.5	37	12.8	23	9.8
Any relative with ovarian										
BRCA1	14	32.6	7	13.7	18	11.8	15	6.3	11	7.2
Other TNBC genes*	1	2.8	3	6.1	10	7.6	21	9.4	17	12.5
All TNBC genes total	15	35.3	10	19.9	28	19.4	36	15.7	28	19.6
Other breast cancer†	0	0.0	0	0.0	1	0.8	3	1.5	3	2.4
All breast cancer genes‡	15	35.3	10	19.9	29	20.1	39	17.2	31	22.0

*Other TNBC genes: BARD1, BRCA2, BRIP1, PALB2, RAD51C, RAD51D, TP53. PV = pathogenic variant; % PV = % cases with pathogenic variants; TNBC = triple-negative breast cancer.
†Other breast cancer genes: ATM, CHEK2, CDH1, PTEN.
‡All breast cancer genes: ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, PALB2, PTEN, RAD51C, RAD51D, TP53.

African American patients will be needed to develop more precise risk estimates. Interestingly, RAD51C PVs were associated with high risk of TNBC in the African American population but only moderate risk among Caucasians. Whether RAD51C is a specific and important driver of TNBC in the African American population, as suggested by high rates of epigenetic silencing of the RAD51C promoter in African American basal tumors (21), remains to be determined.

NCCN guidelines currently recommend BRCA1 and BRCA2 testing for individuals with TNBC diagnosed at age 60 years or younger or who meet criteria based on personal and family cancer history. However, guidelines for non-BRCA predisposition gene testing have not been established. In this study, PVs in TNBC genes were identified in 4.3% of TNBC patients from the clinical cohort not meeting NCCN BRCA1/2 testing criteria. When considering all breast cancer predisposition genes, PVs
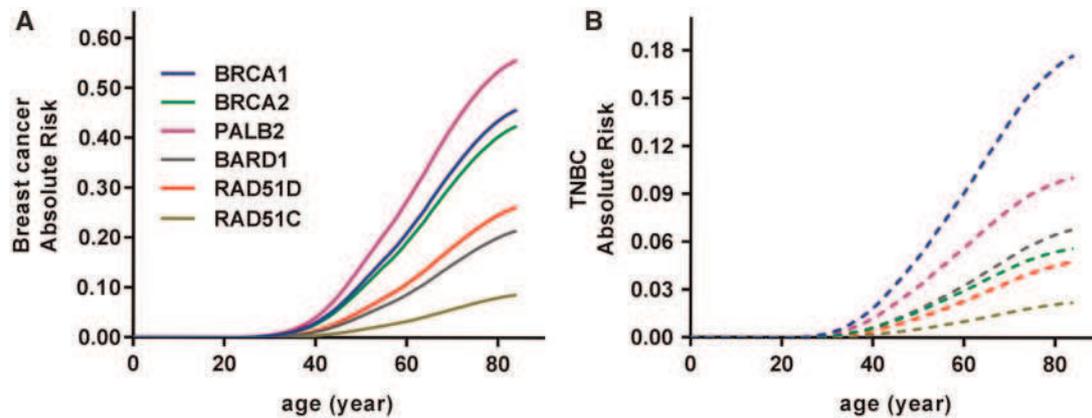
Figure 1. Absolute risk estimates to age 85 for overall breast cancer and triple-negative breast cancer. **A)** Age-related risk curves for overall breast cancer for six genes are shown as **colored lines**. **B)** Age-related risk curves for TNBC for six genes are shown as **colored dashed lines**. TNBC = triple-negative breast cancer.

were detected in 4.6% of patients. As most of these variants are considered clinically actionable, these findings suggest that testing criteria for TNBC patients should be expanded to include testing of all breast cancer predisposition genes regardless of age of diagnosis or family history of cancer.

Although absolute risks for TNBC in the general population are low (1.19% in the Caucasian population; https://www.seer.cancer.gov), PVs in TNBC predisposition genes substantially increased breast cancer risk. Absolute risk models accounting for risks of different subtypes of breast cancer identified five TNBC predisposition genes (*PALB2*, *BRCA1*, *BRCA2*, *RAD51D*, and *BARD1*) with greater than 20% lifetime risks for overall breast cancer. Thus, the risk assessment and management of patients with PVs in these genes should include some consideration of the increased risk for TNBC. Although NCCN guidelines already recommend additional breast cancer screening via magnetic resonance imaging for women with *PALB2*, *BRCA1*, and *BRCA2* PVs, the results from the current study provide evidence to support similar screening for patients with PVs in *RAD51D* and *BARD1*. Continued study of gene-specific risks for breast cancer subtypes may lead to tailored medical management recommendations for PV carriers. Consistent with this hypothesis, initial studies evaluating intensified screening in high-risk women have suggested that a decrease in mortality from TNBC can be achieved (22).

Although panel-based testing can identify unaffected women at increased risk of TNBC and other cancers who can benefit from risk management strategies, genetic testing can also identify individuals who may benefit from targeted therapeutic strategies. In particular, results from the OlympiAD randomized trial of the olaparib PARP inhibitor in HER2-negative metastatic breast cancer patients suggested that *BRCA1* and *BRCA2* carriers with TNBC derived the greatest benefit from the PARP inhibitor in combination with chemotherapy (hazard ratio = 0.43, 95% CI = 0.29 to 0.63) (23). Moreover, a recent analysis of TNBC patients from the Geparsixto randomized clinical trial showed that *BRCA1* and *BRCA2* carriers exposed to anthracycline, taxane, and bevacizumab with or without carboplatin had high pathological complete response (pCR) rates of 65%–67% compared with patients without mutations (pCR rates of 35%–55%) (24). Whether TNBC patients with other predisposition gene mutations will also benefit from these and other targeted therapies remains to be determined, but future clinical trials involving TNBC patients should consider multigene germline panel testing to provide insight into

a putative relationship between PVs and targeted TNBC treatment responses.

There are several limitations of this study. As noted above, although this study identifies TNBC-associated predisposition genes, larger case–control studies and family-based segregation studies will be needed to refine risks of TNBC and other breast cancer subtypes associated with mutations in these genes. In addition, sequencing results from ExAC and gnomAD public control subjects were used for the case–control association studies reported here. Although these control subjects likely provide reasonable approximations of population-based allele frequencies (7), the lack of study level matching of the TNBC patients and control subjects and the derivation of sequencing data using different sequencing platforms and allele-calling algorithms may have resulted in some inaccuracies in gene-level PV frequencies and associations with TNBC. However, extensive data cleaning and filtering was used to normalize TNBC patient and control subjects, and the ExAC control subjects have been used successfully in multiple studies to evaluate cancer risk (25).

Overall, this study identifies several genes that predispose to TNBC and are associated with high lifetime risks of TNBC and overall breast cancer. The results suggest that all TNBC patients should undergo multigene panel testing, regardless of age at diagnosis or family history of cancer, for improved cancer risk assessment and because of the ongoing development of targeted therapeutic approaches for TNBC patients with mutations in predisposition genes.

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