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ORIGINAL REPORT

Inherited Mutations in 17 Breast Cancer Susceptibility Genes Among a Large Triple-Negative Breast Cancer Cohort Unselected for Family History of Breast Cancer

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Terms in blue are defined in the glossary, found at the end of this article and online at www.jco.org.

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ABSTRACT

Purpose
Recent advances in DNA sequencing have led to the development of breast cancer susceptibility gene panels for germline genetic testing of patients. We assessed the frequency of mutations in 17 predisposition genes, including *BRCA1* and *BRCA2*, in a large cohort of patients with triple-negative breast cancer (TNBC) unselected for family history of breast or ovarian cancer to determine the utility of germline genetic testing for those with TNBC.

Patients and Methods
Patients with TNBC (N = 1,824) unselected for family history of breast or ovarian cancer were recruited through 12 studies, and germline DNA was sequenced to identify mutations.

Results
Deleterious mutations were identified in 14.6% of all patients. Of these, 11.2% had mutations in the *BRCA1* (8.5%) and *BRCA2* (2.7%) genes. Deleterious mutations in 15 other predisposition genes were detected in 3.7% of patients, with the majority observed in genes involved in homologous recombination, including *PALB2* (1.2%) and *BARD1*, *RAD51D*, *RAD51C*, and *BRIP1* (0.3% to 0.5%). Patients with TNBC with mutations were diagnosed at an earlier age ($P < .001$) and had higher-grade tumors ($P = .01$) than those without mutations.

Conclusion
Deleterious mutations in predisposition genes are present at high frequency in patients with TNBC unselected for family history of cancer. Mutation prevalence estimates suggest that patients with TNBC, regardless of age at diagnosis or family history of cancer, should be considered for germline genetic testing of *BRCA1* and *BRCA2*. Although mutations in other predisposition genes are observed among patients with TNBC, better cancer risk estimates are needed before these mutations are used for clinical risk assessment in relatives.

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INTRODUCTION

Triple-negative breast cancer (TNBC), defined by little or no expression of estrogen receptor (ER), progesterone receptor (PR), or human epidermal growth factor receptor 2 (HER2) in tumor material, accounts for 12% to 15% of all breast cancers.[1,2] TNBC occurs most frequently in young or premenopausal women and African Americans. Patients with TNBC often have a worse outcome than patients with other breast cancer subtypes,[3,4] with 5-year survival estimated at 70% for those with TNBC compared with > 80% for all other subtypes.[5] Germline mutations in the *BRCA1* breast and ovarian cancer susceptibility gene have been associated with TNBC, with 60% to 80% of breast tumors from *BRCA1* mutation carriers displaying a TNBC phenotype.[6] Additional studies have identified *BRCA1* mutations in up to 29% of patients of Ashkenazi Jewish ethnicity presenting with TNBC,[7]

Table 1. Sample Demographics

Demographic	Germany		Greece	United States						Finland	United Kingdom		Total
	BBCC*	GENICA	(Demokritos)	DFCI	FCCC	KUMC	MCBCS	OSU	RPCI	(HEBCS)	POSH	SBCS	
Ethnicity													
White	270	48	223	252	108	87	186	205	75	87	190	30	1,761
Hispanic	—	—	—	10	—	—	—	—	—	—	—	—	10
African	—	—	—	18	16	—	—	—	—	—	—	—	34
Asian	—	—	—	8	2	—	—	—	—	—	—	—	10
Mixed	—	—	2	—	—	—	—	—	—	—	—	—	2
Unknown	—	—	—	3	—	—	—	4	—	—	—	—	7
Grade													
1	4	0	4	2	1	0	5	0	0	0	2	2	20
2	70	11	44	20	11	0	32	2	14	0	10	1	215
3	195	30	156	234	108	0	139	17	58	0	174	8	1,119
Family history†													1,510
Yes	75	7	42	114	—	35	66	90	15	27	66	2	
No	159	41	126	174	—	51	67	115	51	55	122	10	
%	32	15	25	40	—	41	50	44	23	33	35	17	
Age at diagnosis, years													
Mean	55	54	54	48	53	53	53	51	55	54	36	59	
Range	26-79	25-79	22-83	26-79	29-81	25-80	25-85	25-83	28-92	27-80	25-41	38-93	

Abbreviations: BBCC, Bavarian Breast Cancer Cases and Controls; DFCI, Dana-Farber Cancer Institute; FCCC, Fox Chase Cancer Center; GENICA, Gene Environment Interaction and Breast Cancer in Germany; HEBCS, Helsinki Breast Cancer Study; KUMC, Kansas University Medical Center; MCBCS, Mayo Clinic Breast Cancer Study; OSU, Ohio State University; POSH, Prospective Study of Outcomes in Sporadic Versus Hereditary Breast Cancer; RPCI, Roswell Park Cancer Institute; SBCS, Sheffield Breast Cancer Study.
*BBCC and SUCCESS C studies combined.
†Includes history of breast or ovarian cancer in first- or second-degree relative; unknowns for each category were excluded.

20% of those with TNBC diagnosed at a young age and/or with a family history of breast cancer,[8] and 8% to 14% of those with TNBC unselected for family history.[9-11] In addition, three (3.9%) of 77 patients with TNBC with a median age at diagnosis of 51 years,[12] six (9%) of 64 patients with TNBC of Ashkenazi Jewish ancestry,[13] and 5.2% of patients with TNBC without a significant family history of breast or ovarian cancer[14] have been shown to carry germline *BRCA2* mutations. Although a substantial proportion of TNBCs arise as a result of inherited mutations in *BRCA1* and *BRCA2*, the contribution of mutations in these genes to TNBC, not specifically selected for age at diagnosis or enriched family history of breast or ovarian cancer, remains to be established. Furthermore, although the development of panel-based testing has revealed that 10% of high-risk patients with no *BRCA1* or *BRCA2* mutation may carry inherited deleterious mutations in other breast cancer predisposition genes,[15] the frequency of inherited mutations in the non-*BRCA1/2* predisposition genes among patients with TNBC has not been determined. In this study, we conducted panel-based mutation screening of breast cancer predisposition genes in a large cohort of patients with TNBC in an effort to better understand the contribution of inherited mutations in moderate- and high-risk predisposition genes to TNBC and determine the best parameters for selection of patients with TNBC for *BRCA* testing.

PATIENTS AND METHODS

Study Populations

The Triple-Negative Breast Cancer Consortium has access to DNA and phenotypic information from consecutive patients with TNBC recruited through oncology clinics from 11 clinical centers in the United States (Mayo Clinic Breast Cancer Study, Dana-Farber Cancer Institute, Ohio State University, Roswell Park Cancer Institute, Kansas University Medical Center, and Fox Chase Cancer Center), Germany (Bavarian Breast Cancer Cases and Controls and Gene Environment Interaction and Breast Cancer in Germany), Finland (Helsinki Breast Cancer Study), Greece (Demokritos), and the United Kingdom (Sheffield Breast Cancer Study; Table 1; Data Supplement). Patients with TNBC from the POSH (Prospective Study of Outcomes in Sporadic Versus Hereditary Breast Cancer) multicenter United Kingdom trial of women diagnosed at age < 40 years were also included (Data Supplement). Selection of patients with TNBC was independent of family history of breast or ovarian cancer and age at diagnosis. All 1,824 patients with TNBC were recruited to institutional review board–approved studies.

Panel-Based Mutation Analysis

Germline DNA samples from 1,824 patients with TNBC underwent custom capture (eArray; Agilent, Santa Clara, CA) of all coding sequences and intron/exon boundaries of coding exons from 122 DNA repair genes, including 17 breast cancer predisposition genes (*BRCA1*, *BRCA2*, *PALB2*, *BARD1*, *BRIP1*, *RAD51C*, *RAD51D*, *RAD50*, *NBN*, *MRE11A*, *XRCC2*, *ATM*, *CHEK2*, *TP53*, *PTEN*, *STK11*, and *CDH1*). Products from each capture reaction were sequenced on a HiSeq 2000 (Illumina, San Diego, CA; Data Supplement), and all likely deleterious mutations were validated by Sanger sequencing.

Bioinformatic Analysis

Paired end reads (100 bp) were aligned to the hg19 reference human genome using Novoalign (Novocraft Technologies, Selangor, Malaysia). Realignment and recalibration were performed using GATK software (version 1.6-7; https://www.broadinstitute.org/gatk). Germline variations were called with a combination of GATK Unified Genotyper[16] and Samtools (version 0.1.18; http://www.htslib.org).[17] Annotations were defined using SnpEFF (version 3.0c; http://snpeff.sourceforge.net/index.html)[18] and ANNOVAR (http://www.openbioinformatics.org/annovar).[19] Population allele frequencies were extracted from the Exome Variant Server (http://evs.gs.washington.edu/EVS), 1000 Genomes (http://www.1000genomes.org), and dbSNP (version 137; http://www.ncbi.nlm.nih.gov/projects/SNP). Known deleterious

Table 2. Gene-Based Age at Diagnosis and Family History of Cancer

Gene	No. of Mutations	Age at Diagnosis (years)*			Family History of Cancer†							
					Breast				Ovarian			
		Mean	Range	*P*	Yes	No	Percent Positive	*P*	Yes	No	Percent Positive	*P*
BRCA1	155	44	25-80	< .001	66	66	50	< .001	24	108	18	< .001
BRCA2	49	47	27-79	< .001	16	24	40	.31	5	35	13	< .001
Other	67	48	28-79	.02	20	34	37	.46	1	53	2	1
ATM	2	49	35-62	.83	0	2	0	1	0	2	0	1
BARD1	9	55	45-72	.34	2	3	40	.66	0	5	0	1
BRIP1	8	46	36-68	.12	3	5	38	.72	0	8	0	1
CDH1	0	—	—	—	—	—	—	—	—	—	—	—
CHEK2	0	—	—	—	—	—	—	—	—	—	—	—
MRE11A	2	39	36-41	.11	1	1	50	.54	0	2	0	1
NBN	1	59	59-59	—	1	0	100	.32	0	1	0	1
PALB2	21	49	28-79	.22	5	10	33	1	0	15	0	1
PTEN	1	45	45-45	—	1	0	100	.32	0	1	0	1
RAD50	6	54	42-63	.51	2	3	40	.66	0	5	0	1
RAD51C	6	52	37-71	.92	1	4	20	1	0	5	0	1
RAD51D	7	43	31-66	.14	3	3	50	.39	1	5	17	.14
STK11	0	—	—	—	—	—	—	—	—	—	—	—
TP53	1	38	38-38	—	0	1	0	1	0	1	0	1
XRCC2	3	34	28-40	.04	1	2	33	1	0	3	0	1
WT	1,557	51	22-93	Ref	413	873	32	Ref	32	1,254	3	Ref

NOTE. — indicates no data because of absence of mutation.
Abbreviations: Ref, referent; WT, wild type.
*Associations with age at diagnosis were evaluated by *t* test.
†Associations with family history of breast or ovarian cancer were evaluated by Fisher's exact test.

missense mutations in *BRCA1*, *BRCA2*, and *TP53* were included in all analyses (Data Supplement). Predicted deleterious missense mutations were selected using algorithms in ANNOVAR (eg, SIFT, PolyPhen2, LRT, MutationTaster, PhyloP, GERP)[19,20] and AlignGVGD (http://agvgd.iarc.fr).[21]

Statistical Analysis

Likely deleterious mutations from genes other than *BRCA1* or *BRCA2* were combined in an "other" category. Patients with TNBC without mutations were categorized as wild type. The *t* test, χ^2 test, and Fisher's exact test were used for evaluating associations with mutation status. *P* values < .05 were considered statistically significant.

RESULTS

Study Population

The 1,824 female patients with TNBC in this study were recruited from 12 centers (Table 1). Of the 1,817 patients with established ethnicity, 1,762 (97%) were white, and 34 (1.9%), 10 (0.6%), and 10 (0.6%) were of African, Asian, and Hispanic ethnicities, respectively. Age at diagnosis ranged from 22 to 93 years, with an average age of 51 years. This was similar to the average age of patients with TNBC in the METABRIC (Molecular Taxonomy of Breast Cancer International Consortium) study (52.7 years; $P = .2477$)[22] but younger than that of patients with TNBC in the Cancer Genome Atlas Network (54.2 years; $P = .027$).[23] Of the 1,510 patients with available family history information, 514 (34%) had at least one first- or second-degree relative with breast cancer, and 4% had a relative with ovarian cancer. The TNBCs were predominantly grade 3 (81%; Table 1).

Germline Mutations

All coding exons and consensus splice sites of 17 known cancer predisposition genes were screened for mutations in the 1,824 patients with TNBC. Overall, 271 deleterious mutations were identified in 267 patients (14.6%; Table 2; Data Supplement). Of these, 155 (57%) occurred in *BRCA1*, 49 (18%) in *BRCA2*, and 67 (25%) in 12 of 15 other predisposition genes (Table 1; Fig 1). The frequency of mutations by center ranged from 4% to 24% (Data Supplement). The elevated mutation frequency (24%) in the Dana-Farber Cancer Institute study resulted from 21 Ashkenazi Jewish founder mutations in *BRCA1* and *BRCA2*.

Deleterious *BRCA1* mutations were detected in 8.5% of patients with TNBC, including 145 truncating (frameshift, nonsense, and splice) mutations and 10 known deleterious missense mutations.[24] The 185delAG (c.68_69delAG) Ashkenazi Jewish founder mutation was identified in 18 patients with TNBC, and the 5382insC (c.5266dupC) Eastern European founder mutation was found in 19 patients with TNBC (Data Supplement). Another 21 recurrent *BRCA1* mutations were observed in 64 other patients with TNBC (Data Supplement). The 49 deleterious *BRCA2* mutations (2.7%) included 41 truncating mutations, of which six were the 6174delT (c.5946delT) Ashkenazi Jewish founder mutation, three were splice mutations, and five were known deleterious missense mutations in the *BRCA2* DNA binding domain.[24] Three recurrent mutations in *BRCA2* accounted for 16 patient cases of TNBC (Data Supplement). Likely deleterious mutations in non-*BRCA1/2* predisposition genes were identified in 3.7% of all unselected patients with TNBC. In particular, 21 patients (1.2%) with TNBC had deleterious *PALB2* truncating mutations, including 15 diagnosed at age ≤ 50 years. Three patients from Finland were found to carry the *PALB2* c.1592delT founder mutation.[25] In addition, deleterious mutations were detected in *BARD1* (n = 9), *BRIP1* (n = 8), *RAD51D* (n = 7), *RAD50* (n = 6), and *RAD51C* (n = 6; Data Supplement). In contrast, no mutations

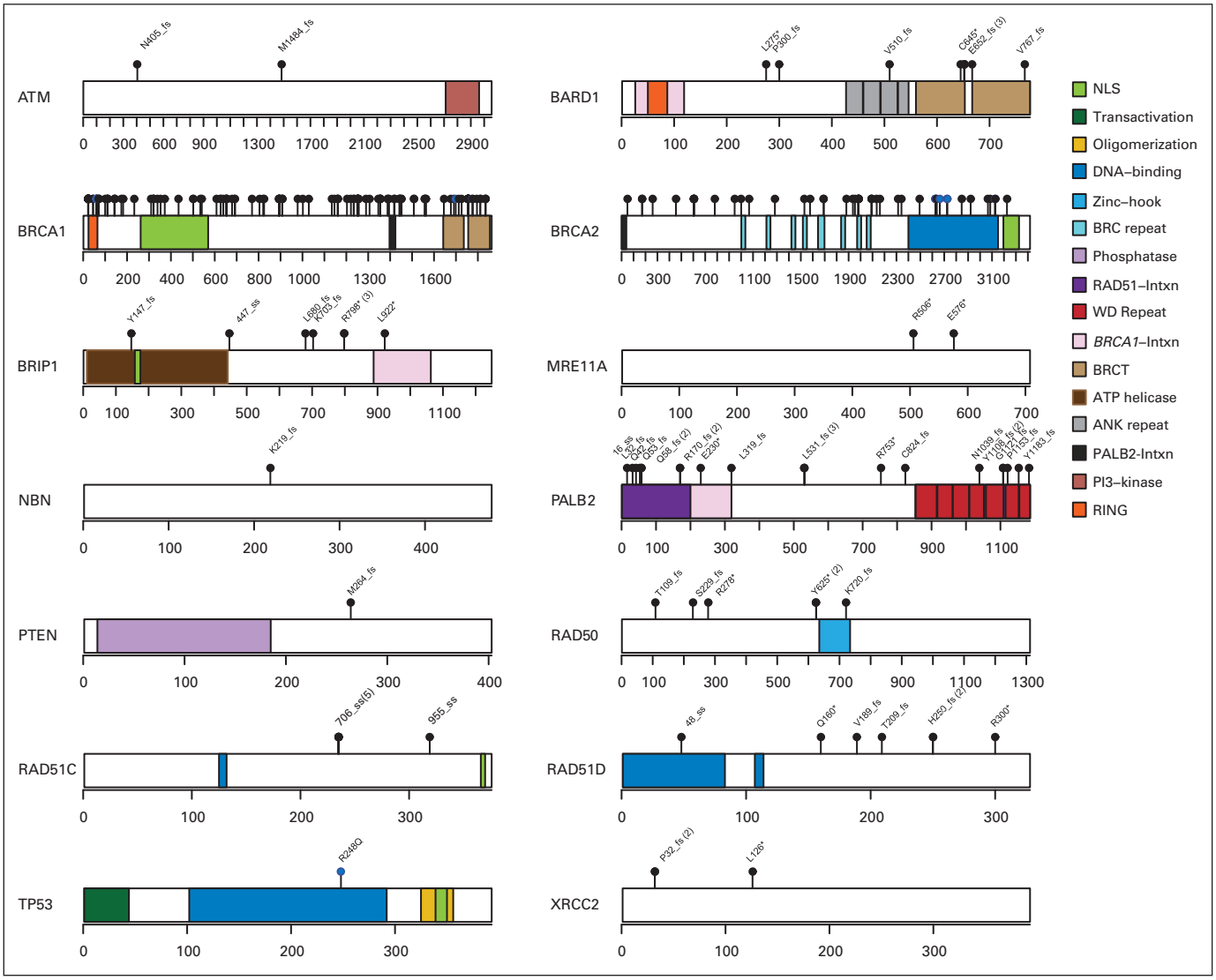

Fig 1. Germline likely deleterious mutations from 14 breast cancer predisposition genes in unselected patients with triple-negative breast cancer. Locations of likely deleterious mutations and domains in proteins encoded by predisposition genes are shown by lollipop structures. Mutations are labeled, other than in *BRCA1* and *BRCA2*. Protein domain patterns are shown in key. Scales on genes reflect number of amino acid residues.

were observed in *CHEK2*, consistent with an association between *CHEK2* mutations and ER-positive breast cancer,[23,26] or in the *CDH1* and *STK11* genes. Four patients with TNBC carried > 1 deleterious mutation (Data Supplement), including an individual with *BRCA1*: c.68_69delAG and *BRCA2*:c.5946delT mutations diagnosed at age 68 years. None had a family history of breast or ovarian cancer.

Age at Diagnosis

In this study, 38% of all deleterious mutations were detected in patients with TNBC diagnosed at age < 40 years. The average age at diagnosis of TNBC was significantly younger for patients with deleterious (45 years; $P < .001$), *BRCA1* (44 years; $P < .001$), *BRCA2* (47 years; $P < .001$), and non-*BRCA1/2* gene mutations (48 years; $P = .02$), relative to those with TNBC with no mutations (ie, wild type; 52 years; Table 2; Fig 2). Consistent with this, the distribution of age at TNBC diagnosis for *BRCA1*, *BRCA2*, and non-*BRCA1/2* gene mutation carriers differed from that among noncarriers (Fig 2). However, 10% (n = 27) of all mutation carriers and 5.5% of all patients with TNBC were diagnosed at age ≥ 60 years. Of these, 37% (n = 10) carried mutations in non-*BRCA1/2* genes, and 50% (n = 13) had no family history of breast or ovarian cancer (Table 3).

Family History

We also evaluated whether patient cases of TNBC with mutations in the 17 predisposition genes were associated with a greater family history of breast and/or ovarian cancers than nonmutated patient cases (Table 2). Patient cases of TNBC with *BRCA1* mutations were enriched for a family history of breast (50%; $P < .001$) and ovarian cancers (18%; $P < .001$), whereas patient cases of TNBC with *BRCA2* mutations were only enriched for a family history of ovarian cancer (Table 2). However, patient cases of TNBC with mutations in the non-*BRCA1/2* genes were not significantly associated with an enriched family history for either breast or ovarian cancer (Table 2). In particular, only five of 21 *PALB2* mutation carriers and 12 of 36

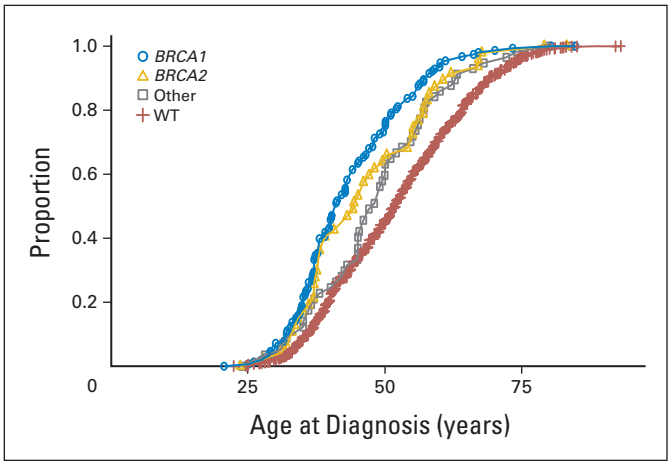

Fig 2. Age at onset of triple-negative breast cancer by mutation status. Distribution shown for patients with triple-negative breast cancer with *BRCA1*, *BRCA2*, and other non-*BRCA1/2* mutations and no mutations (ie, wild type [WT]).

BARD1, *BRIP1*, *RAD50*, *RAD51C*, or *RAD51D* mutation carriers had a family history of breast or ovarian cancer. Thus, many patients with TNBC with mutations in predisposition genes may not be identified by a family history of cancer.

Association With Tumor Pathology

Overall, patients with TNBC with mutations presented with higher-grade tumors than noncarriers ($P < .001$; Fig 3; Data Supplement). Although 83% (1,117 of 1,351) of all TNBCs with pathology data were grade 3, this increased to 94% (105 of 112) for *BRCA1* carriers ($P < .001$) and 90% (35 of 39) for *BRCA2* carriers ($P = .35$); however, this decreased slightly to 82% (37 of 45) for TNBCs in non-*BRCA1/2* mutation carriers ($P = .84$).

Missense Mutations

A total of 66 unique missense mutations from 91 patients were predicted deleterious by at least six of seven methods, or five of six methods when AlignGVGD was not available (Data Supplement). This included 21 unique variants in *BRCA1* and *BRCA2*, accounting for 31 patients. Of 10 *BRCA1* and *BRCA2* known deleterious variants identified, only p.Ile2627Phe,[27-30] which alters splicing of *BRCA2*, and p.Leu22Ser in *BRCA1* were not predicted as deleterious. These findings suggest that a high proportion (40% to 80%) of the missense mutations predicted as deleterious in this study are likely to predispose individuals to TNBC (Data Supplement) and that an additional 1% to 3% of patient cases of TNBC may be associated with deleterious missense mutations in the predisposition genes.

Mutation Prediction

Patients with TNBC are often considered for breast cancer predisposition gene panel testing, because mutations in predisposition genes are common,[14] and both carriers of mutations and their family members may benefit from informed cancer risk management. Although criteria for testing based on age at diagnosis and presence of family history have been suggested, detailed predisposition gene mutation rates for patients with TNBC based on these phenotypic categories are not currently available. Here we combined mutation results with phenotypic characteristics of patients with TNBC to provide estimates of mutation frequency by categories of age at diagnosis and family history of cancer (Table 3). When including all genes, > 18% of patients with TNBC diagnosed at age < 60 years with or without a family history of cancer carried deleterious mutations. Conversely, 5% (n = 13) diagnosed at age ≥ 60 years and with no family history of cancer carried mutations.

DISCUSSION

We present results from the largest series to date, to our knowledge, of patients with TNBC analyzed for germline mutations in a panel of known breast cancer predisposition genes. We found that 14.6% of 1,824 patients with TNBC unselected for family history of cancer carried germline deleterious mutations in 14 of 17 predisposition genes tested. *BRCA1* and *BRCA2* mutations were found in 11.2% of patients, consistent with other studies of TNBC, whereas mutations in 12 other genes were found in 3.7% of patients. In addition, 1% to 3% of patients carried missense mutations predicted by in silico methods to be deleterious (Data Supplement). Furthermore, the detection of *BRCA1* and *BRCA2* large exonic deletions or duplications in six (2%) of 294 patients with TNBC from the GeparSixto study[31] suggests that approximately 2% of the patients with TNBC in our study may have also carried this type of mutation. Thus, between 14.6% and 20% of patients with TNBC may have deleterious germline mutations in these genes.

The selection of patients with TNBC for clinical genetic testing of *BRCA1* and *BRCA2* is often based on an age-related threshold and the associated probability of finding a mutation. The frequency of mutations in patients presenting with TNBC based on age at diagnosis and family history of breast or ovarian cancer in this study is summarized in Table 3. In our study, only 1.4% of those diagnosed at age > 60 years and with no family history of cancer were found to carry *BRCA1* or *BRCA2* mutations, supporting the current National Comprehensive Cancer Network guidelines for testing of patients diagnosed with TNBC before age 60 years (Table 3). Similarly, our findings verify that the probability of an underlying pathogenic *BRCA1* or *BRCA2* mutation exceeds 10% in those diagnosed before age 40 years; however, this probability is < 10% in older age groups in the absence of a family history of cancer (Table 3). This is consistent with the UK National Institute for Clinical Excellence testing guidelines, which do not recommend testing in patients with TNBC diagnosed at age > 40 years and with no family history of cancer. However, on the basis of our data, this latter approach would overlook 24% of all *BRCA1* and *BRCA2* mutations among TNBCs. Because a relatively high proportion (7.5%) of patients with TNBC with no family history and diagnosed between age 50 and 60 years had mutations, perhaps testing of all patients diagnosed at age < 60 years, or even all patients irrespective of age or family history, should be considered, especially if the cost of mutation screening were to decrease over time.

Deleterious mutations (n = 67) in the non-*BRCA1/2* predisposition genes were identified in 3.7% of all patients with TNBC. The frequency of these mutations, especially in *PALB2*, which has recently been associated with a high lifetime risk of breast cancer,[32] was similar to the frequency in high- and moderate-risk breast cancer families,[33] suggesting a distinct enrichment for predisposition gene mutations in unselected TNBCs. Furthermore, genes involved in homologous recombination, including *PALB2*, *BARD1*, *BRIP1*, *RAD51C*, *RAD51D*,

Table 3. Frequency of Mutations by Age at Diagnosis and Family History of Breast or Ovarian Cancer

Family Cancer History	Age at TNBC Diagnosis (years)														
	< 35			35 to 39			40 to 49			50 to 59			≥ 60		
	Mutation Carriers	All Patients	%	Mutation Carriers	All Patients	%	Mutation Carriers	All Patients	%	Mutation Carriers	All Patients	%	Mutation Carriers	All Patients	%
BRCA1															
No breast, no ovarian	14	91	15.4	15	149	10.1	14	209	6.7	13	241	5.4	4	279	1.4
One relative with breast, no ovarian	6	48	12.5	7	50	14	11	103	10.7	3	80	3.8	2	79	2.5
≥ Two relatives with breast, no ovarian	4	12	33.3	5	16	31.3	7	38	18.4	2	28	7.1	1	23	4.3
Any relative with ovarian	3	5	60	6	15	40	6	18	33.3	9	17	52.9	0	7	0
Total	27	156	17.3	33	230	14.3	38	368	10.3	27	366	7.4	7	388	1.8
BRCA2															
No breast, no ovarian	4	91	4.4	8	149	5.4	4	209	1.9	5	241	2.1	2	279	0.7
Any relative with breast, no ovarian	3	60	5	1	66	1.5	4	141	2.8	2	108	1.9	2	102	2
Any relative with ovarian	0	5	0	2	15	13.3	1	18	5.6	1	17	5.9	1	7	14.3
Total	7	156	4.5	11	230	4.8	9	368	2.4	8	366	2.2	5	388	1.3
BRCA1 and *BRCA2*															
No breast, no ovarian	18	91	19.8	23	149	15.4	18	209	8.6	18	241	7.5	6	279	1.4
One relative with breast, no ovarian	7	48	14.6	7	50	14	14	103	13.6	5	80	6.3	4	79	5.1
≥ Two relatives with breast, no ovarian	6	12	50	6	16	37.5	8	38	21	2	28	7.1	1	23	0
Any relative with ovarian	3	5	60	8	15	53.3	7	18	38.9	10	17	58.8	1	7	14.3
Total	34	156	21.8	44	230	19.1	47	368	12.8	35	366	9.6	12	388	3.1
Other genes															
No breast, no ovarian	3	91	3.3	6	149	4	10	209	4.8	7	241	2.9	7	279	2.5
Any relative with breast, no ovarian	2	60	3.3	4	66	6.1	6	141	4.3	6	108	5.6	2	102	2
Any relative with ovarian	0	5	0	0	15	0	0	18	0	1	17	5.9	0	7	0
Total	5	156	3.2	10	230	4.3	16	368	4.3	14	366	3.8	9	388	2.3
All genes															
No breast, no ovarian	21	91	23.1	29	149	19.5	27	209	12.9	25	241	10.4	13	279	4.7
One relative with breast, no ovarian	9	48	18.8	9	50	18	18	103	17.5	9	80	11.3	5	79	6.3
≥ Two relatives with breast, no ovarian	6	12	50	7	16	43.8	10	38	26.3	4	28	14.3	2	23	8.7
Any relative with ovarian	3	5	60	8	15	53.3	7	18	38.9	11	17	64.7	1	7	14.3
Total	39	156	20.0	53	230	23.0	62	368	16.8	49	366	13.4	21	388	5.4

NOTE. Patients with TNBC for whom information was lacking on age at cancer diagnosis or family history of cancer were excluded.
Abbreviation: TNBC, triple-negative breast cancer.

and *XRCC2*, accounted for 54 (81%) of the 67 mutations in non-*BRCA1/2* predisposition genes, suggesting that disruption of homologous recombination repair may be an important event in the development of triple-negative breast tumors. Interestingly, the detection of deleterious mutations in *RAD51C* and *RAD51D*, which have been associated with a low to moderate risk of breast cancer but a higher risk of ovarian cancer, also raises the possibility that mutations in these genes confer higher risks of triple-negative and basal subtypes of breast cancer. In contrast, no mutations were observed in *CHEK2*, *CDH1*, or *STK11*, and only one mutation was identified in *TP53* or *PTEN*, indicating that the syndromic breast cancer predisposition genes are rarely involved in predisposition to TNBC.

Clinical testing of predisposition gene panels has recently been developed to improve identification of women at increased risk for breast or ovarian cancer. However, the risks of breast and ovarian cancer associated with mutations in the non-*BRCA1/2* predisposition genes are not well defined.[34,35] In our study, the prevalence of mutations in the non-*BRCA1/2* predisposition genes was stable across all age groups and reported cancer family histories (Table 3), consistent with lower penetrance of disease for mutations in many of these genes. Clinical management guidelines for *BRCA1* and *BRCA2* mutation carriers have been developed over the 20 years since these genes were identified, with recent studies suggesting that bilateral mastectomy and bilateral oophorectomy in *BRCA1/2* mutation carriers may reduce breast cancer– and all cause–related mortalities.[36-39] In contrast, management guidelines are not available for carriers of mutations in non-*BRCA1/2* predisposition genes.[34,35] Thus, the clinical utility of results from a broad gene panel of the type used in our study remains controversial. However, with panel-based genetic testing of *BRCA1* and *BRCA2* in combination with other genes now well established, and with testing for *BRCA1* and *BRCA2* mutations likely to increase if poly (ADP-ribose) polymerase inhibitors[40,41] are approved for clinical use in patients with breast cancer with *BRCA1/2* mutations, or if

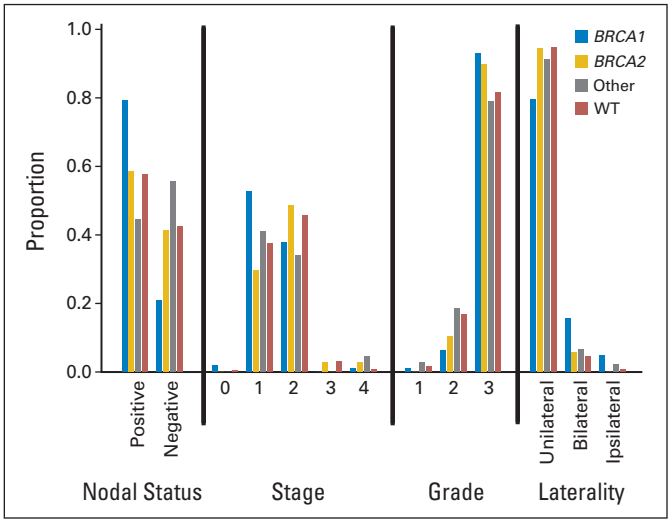

Fig 3. Tumor characteristics of patients with triple-negative breast cancer by mutation status. Proportion of those with *BRCA1*, *BRCA2*, and other non-*BRCA1/2* mutations and no mutations (ie, wild type [WT]) exhibiting specified nodal status, tumor stage, tumor grade, and bilateral breast cancer status.

BRCA1/2 mutations are proven to predict response to platinum-based or other chemotherapies,[42] panel testing will likely continue to expand. Clearly, further research with appropriate consent and curation of clinical data from patients receiving panel testing will be needed to establish the most appropriate application of results from the non-*BRCA1/2* breast cancer susceptibility genes to patient care.

In conclusion, *BRCA1* and *BRCA2* mutation testing has a clear role for patients with TNBC, many of whom will meet the current probability threshold guidelines. However, although inclusion of other susceptibility genes in the genetic testing panel is already a widely adopted strategy, it is important that clinical care providers appreciate the current lack of robust estimates of penetrance for many of these genes.

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Disclosures provided by the authors are available with this article at www.jco.org.

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GLOSSARY TERMS

BRCA1: a tumor suppressor gene known to play a role in repairing DNA breaks. Mutations in this gene are associated with increased risks of developing breast or ovarian cancer.

BRCA2: a tumor suppressor gene whose protein product is involved in repairing chromosomal damage. Although structurally different from BRCA1, BRCA2 has cellular functions similar to BRCA1. BRCA2 binds to RAD51 to fix DNA breaks caused by irradiation and other environmental agents. Also known as the breast cancer 2 early onset gene.

sequencing: a laboratory process that determines the nucleotide sequence of DNA (can involve the whole genome or whole exome or be targeted to as little as one coding sequence). Unlike somatic mutation genotyping, sequencing can detect previously unknown somatic mutations.

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Inherited Mutations in 17 Breast Cancer Susceptibility Genes Among a Large Triple-Negative Breast Cancer Cohort Unselected for Family History of Breast Cancer

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