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ORIGINAL REPORT

Frequency of Germline Mutations in 25 Cancer Susceptibility Genes in a Sequential Series of Patients With 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

Testing for germline mutations in *BRCA1/2* is standard for select patients with breast cancer to guide clinical management. Next-generation sequencing (NGS) allows testing for mutations in additional breast cancer predisposition genes. The frequency of germline mutations detected by using NGS has been reported in patients with breast cancer who were referred for *BRCA1/2* testing or with triple-negative breast cancer. We assessed the frequency and predictors of mutations in 25 cancer predisposition genes, including *BRCA1/2*, in a sequential series of patients with breast cancer at an academic institution to examine the utility of genetic testing in this population.

Methods

Patients with stages I to III breast cancer who were seen at a single cancer center between 2010 and 2012, and who agreed to participate in research DNA banking, were included (N = 488). Personal and family cancer histories were collected and germline DNA was sequenced with NGS to identify mutations.

Results

Deleterious mutations were identified in 10.7% of women, including 6.1% in *BRCA1/2* (5.1% in non-Ashkenazi Jewish patients) and 4.6% in other breast/ovarian cancer predisposition genes including *CHEK2* (n = 10), *ATM* (n = 4), *BRIP1* (n = 4), and one each in *PALB2*, *PTEN*, *NBN*, *RAD51C*, *RAD51D*, *MSH6*, and *PMS2*. Whereas young age ($P < .01$), Ashkenazi Jewish ancestry ($P < .01$), triple-negative breast cancer ($P = .01$), and family history of breast/ovarian cancer ($P = .01$) predicted for *BRCA1/2* mutations, no factors predicted for mutations in other breast cancer predisposition genes.

Conclusion

Among sequential patients with breast cancer, 10.7% were found to have a germline mutation in a gene that predisposes women to breast or ovarian cancer, using a panel of 25 predisposition genes. Factors that predict for *BRCA1/2* mutations do not predict for mutations in other breast/ovarian cancer susceptibility genes when these genes are analyzed as a single group. Additional cohorts will be helpful to define individuals at higher risk of carrying mutations in genes other than *BRCA1/2*.

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INTRODUCTION

Testing for mutations in high-penetrance breast cancer predisposition genes, particularly *BRCA1* and *BRCA2*, has become standard practice for patients with breast cancer. Lifetime estimates of breast cancer risk in *BRCA1* or *BRCA2* (*BRCA1/2*) carriers range from 36% to 90% and of ovarian cancer risk range from 24% to 59% and 8% to 35% in *BRCA1* and *BRCA2* carriers, respectively.[1-5] Identification of *BRCA1/2* mutations permits the implementation of prevention strategies, including magnetic resonance imaging screening or risk-reducing surgeries, which improves survival.[6,7] Genetic testing for other high-risk breast cancer susceptibility genes, such as *TP53* (Li-Fraumeni syndrome), *PTEN* (Cowden's syndrome), and *CDH1* (hereditary diffuse gastric cancer), is also standard in appropriate patients.

More recently, next-generation sequencing (NGS) has enabled simultaneous testing for mutations in these high-penetrance genes and for other, more moderate-risk genes. Multigene panels are now commercially available and are increasingly being used in cancer risk assessment.

Compared with high-penetrance genes, for which inherited mutations confer a five-fold or greater breast cancer risk, mutations in moderate-penetrance genes are associated with a two- to four-fold increase in risk. Cancer risks associated with mutations in these less potent predisposition genes are still being investigated. For example, mutations in *PALB2*, initially thought to confer a moderate risk of breast cancer, now seem to be associated with a five-fold or greater risk.[8] NGS also allows simultaneous testing for other hereditary cancer risks, such as Lynch syndrome, in individuals with and without a suggestive family history. In addition, germline mutations in DNA repair genes such as *BRIP1*, *RAD51C*, and *RAD51D* are associated with an increased risk of ovarian cancer (Table 1).[9-15]

To date, studies evaluating the prevalence of mutations in moderate-penetrance breast cancer predisposition genes have been conducted in select breast cancer populations including African Americans,[16] patients with triple-negative breast cancer (TNBC),[17] and cases seen in high-risk genetic clinics.[18-21] The prevalence of mutations among patients with breast cancer, who are unselected for specific risk factors such as age at diagnosis, breast cancer subtype, or family cancer history, is unknown.

Table 1. Cancer Susceptibility Genes Other Than *BRCA1/2*

Cancer Susceptibility Gene	Breast Cancer RR (90% CI when available) or Inclusion Criteria
Breast	
ATM	2.8 (2.2 to 3.7)[35]
BARD1	Breast cancer association reported; RR not yet determined[17,46,47]
BRIP1	2.0 (1.3 to 3.0)[48]; ovarian cancer RR 11.2[9]
CDH1	6.6 (2.2 to 19.9)[49]
CHEK2	3.0 (2.6 to 3.5)[35]; most data for 1100delC
NBN	2.7 (1.9 to 3.7)[35]
PALB2	5.3 (3.0 to 9.4)[35]
PTEN	RR 2.0-5.0[50,51]
STK11	RR 2.0-4.0[52,53]
TP53	105 (62 to 165)[35]
Other	
APC	Familial adenomatous polyposis
BMPR1A	Juvenile polyposis syndrome
CDK4	Melanoma syndrome
CDKN2A	Melanoma and pancreas cancer syndrome
EPCAM	Lynch syndrome
MLH1	Lynch syndrome
MSH2	Lynch syndrome
MSH6	Lynch syndrome
*MUTYH**	*MUTYH*-associated polyposis
PMS2	Lynch syndrome
RAD51C	Ovarian cancer RR 5.2-6.3[11-13]
RAD51D	Ovarian cancer RR 6.3-12[12,15]
SMAD4	Juvenile polyposis syndrome

Abbreviations: *APC*, adenomatous polyposis coli; *ATM*, ataxia telangiectasia mutated; *BARD1*, BRCA1-associated RING domain 1; *BMPR1A*, bone morphogenetic protein receptor, type 1A; *BRCA1/2*, early-onset breast cancer genes *BRCA1* and *BRCA2*; *BRIP1*, BRCA1 interacting protein C-terminal helicase 1; *CDH1*, E-cadherin; *CDK4*, cyclin-dependent kinase 4; *CDKN2A*, cyclin-dependent kinase inhibitor 2A; *CHEK2*, checkpoint kinase 2; *EPCAM*, epithelial cell adhesion molecule; *MLH1*, mutL homolog 1; *MSH2*, mutS homolog 2; *MSH6*, mutS homolog 6; *MUTYH*, biallelic mutY homolog; *NBN*, nibrin; *PALB2*, partner and localizer of BRCA2; *PMS2*, PMS2 postmeiotic segregation increased 2; *PTEN*, phosphatase and tensin homolog; *RAD51C*, RAD51 paralog C; *RAD51D*, RAD51 paralog D; RR, relative risk; *SMAD4*, SMAD family member 4; *STK11*, serine/threonine kinase 11; *TP53*, tumor protein 53.
*Only tumors with biallelic *MUTYH* mutations were considered for this analysis.

Evidence-based guidelines for *BRCA1/2* testing in patients with breast cancer have been established. Criteria include young age at diagnosis, TNBC, Ashkenazi Jewish ancestry, or a significant family history of breast, ovarian, or other related cancers.[22,23] Existing recommendations for mutation detection in other high-penetrance genes are based on specific syndrome features.[22] Criteria for testing of moderate-penetrance predisposition genes do not yet exist because predictive factors have not been identified and clinical utility is still being evaluated.

In this study, we assessed the frequency of deleterious germline mutations in 25 cancer susceptibility genes in a population of consecutive patients with breast cancer who presented to an academic cancer center. Our goals were to better understand the contribution of inherited mutations in moderate- and high-risk genes in a breast cancer cohort unselected for family history, breast cancer subtype, ethnicity, or age at diagnosis and to evaluate any clinical or pathologic factors that predict for mutations in moderate-risk genes.

METHODS

Patient Selection

All women with stages I to III breast cancer seen at the Dana-Farber Cancer Institute (Boston, MA) between April 2010 and July 2012, who consented to DNA banking for clinical research, were eligible. Patients with a previous breast cancer were excluded. Cases were identified retrospectively and blood samples were obtained from the Dana-Farber/Harvard Cancer Center Specialized Program of Research Excellence (SPORE) in breast cancer biobank. Clinical and pathologic data abstracted from medical records as part of the Clinical Outcomes for Research Information Service program included personal and family cancer histories, cancer histology, stage and receptor status, ancestry, and history of genetic testing. All breast cancers were reviewed by breast pathologists in the Department of Pathology at Brigham and Women's Hospital. ASCO/College of American Pathologists guidelines were used to define estrogen receptor (ER), progesterone receptor, and human epidermal growth factor receptor 2 (HER2) positivity. Women with bilateral breast cancer were eligible, provided their first breast cancer was diagnosed between April 2010 and July 2012; only features of the initial breast cancer were assessed. Genetic test results from this analysis were considered research and were not returned to study participants or used for clinical decision making. Specimens were collected for research purposes only and therefore did not comply with Clinical Laboratory Improvement Amendments chain of custody regulations for clinical testing. This study was approved by the institutional review board of the Dana-Farber/Harvard Cancer Center.

NGS Assay

Sample preparation for NGS was performed from frozen DNA using the RainDance microdroplet polymerase chain reaction (PCR) system (RainDance Technologies, Billerica, MA). Briefly, PCR products representing exons and proximal splicing elements of patient DNA were amplified in merged droplets consisting of fragmented patient DNA and select target enrichment primers. These PCR products were subsequently tagged with barcodes and sequencing adaptors for NGS on the Illumina HiSeq platform (Illumina, San Diego, CA). To circumvent highly homologous pseudogenes, modified sample preparation with long-range and nested PCR, followed by NGS on the Illumina MiSeq platform, was used for portions of the *CHEK2* and *PMS2* genes. All clinically actionable variants identified by NGS, as well as regions that did not meet our preset NGS quality metrics, were independently confirmed with orthogonal site-specific Sanger sequencing.

To detect exonic deletions and duplications, NGS dosage, microarray comparative genomic hybridization, multiplex ligation-dependent probe amplification, or a combination of these analyses was performed,[24] with all positive results confirmed by an orthogonal method. Gene variants deemed deleterious or suspected as deleterious were considered mutations. Analyzed genes were categorized into two groups (Table 1).

Variant Classification

Variants were classified using American College of Medical Genetics and Genomics recommendations, with supporting linkage, biochemical, clinical, functional, and statistical data used for specific missense and intronic alterations.[25-27]

Statistics

Participant characteristics and sequencing results were summarized with descriptive statistics, including medians, means, and standard deviations for continuous data. For categorical data, proportions with 95% CIs were calculated by the Clopper-Pearson method. Demographic, clinical, and pathologic characteristics were compared using the χ^2 test (categorical variables) and the *t* test/analysis of variance (continuous variables). *P* values $< .05$ were considered significant.

RESULTS

Study Population

During the study period, 69.8% of patients with breast cancer seen at Dana-Farber Cancer Institute agreed to use of their clinical data and specimens for research. Sixty-one percent of blood samples were collected within 90 days of the initial breast cancer diagnosis and 94% within 1 year of diagnosis. The median time from diagnosis to blood sample collection was 77 days. Blood samples from 582 cases were analyzed and 87 failed due to insufficient DNA quantity or poor DNA quality. Six cases were excluded due to a prior breast cancer diagnosis and one was excluded for lack of clinical data, resulting in 488 cases which comprised the study cohort. Clinical and tumor pathologic features for study participants are provided in Table 2. The mean age at diagnosis was 50.3 years (range, 28 to 88 years); 7.8% of the study population were Ashkenazi Jewish and 81.4% were non-Ashkenazi white. Nearly 18% of women had TNBC, and 82.6% had stage I or II disease. Further, 49.0% of patients reported having a first- or second-degree relative with breast or ovarian cancer.

Table 2. Clinical and Tumor Characteristics in Study Cohort (N = 488)

Study Characteristic	No.	%
Age at diagnosis, years		
Mean ± SD	50.3 ± 11.3	
Median	49	
Range	28-88	
≤ 45	180	36.9
46-60	199	40.8
> 60	109	22.3
Race/ethnicity		
Ashkenazi Jewish	38	7.8
Non-Hispanic white (not Ashkenazi Jewish)	397	81.4
Hispanic	17	3.5
African American	12	2.5
Asian	10	2.0
Other	14	2.9
Ashkenazi Jewish ethnicity		
Yes	38	7.8
No	450	92.2
Breast cancer subtypes, receptor status		
TNBC	87	17.8
HR-positive/HER2-negative	301	61.7
HR-negative/HER2-positive	37	7.6
HR-positive/HER2-positive	63	12.9
Histology		
Ductal	357	73.2
Lobular	36	7.4
Ductal and lobular	68	13.9
Other	27	5.5
Grade*		
1	60	12.3
2	181	37.2
3	246	50.5
Stage		
I	185	37.9
II	218	44.7
III	85	17.4
Bilateral disease		
Yes	9	1.8
No	479	98.2
Patient history of prior cancer†		
Yes	41	8.4
No	447	91.6
First-degree relative with any cancer†‡		
Yes	271	56.7
No	207	43.3
First-/second-degree relative with any cancer†‡		
Yes	403	84.3
No	75	15.7
First-/second-degree relative with breast or ovarian cancer†‡		
Yes	234	49.0
No	244	51.0
First-/second-degree relative with breast cancer (< 50 years of age), male breast cancer, or ovarian cancer (any age)†‡		
Yes	89	18.6
No	389	81.4

Abbreviations: HER2, human epidermal growth factor receptor 2; HR, hormone receptor; SD, standard deviation, TNBC, triple-negative breast cancer.
*Tumor grade was missing for one patient.
†Excludes in situ cancers and nonmelanoma skin cancers.
‡Ten patients were missing family history information. These patients were excluded from analysis. If age at diagnosis was unavailable, it was conservatively considered to be older than 50 years.

Frequency of Deleterious Mutations

Among 488 patients with breast cancer, 55 deleterious mutations were identified in 52 (10.7%) women (Table 3; Appendix Table A1, online only). Thirty (6.1%) women had a germline *BRCA1/2* mutation; 18 in *BRCA1* and 12 in *BRCA2*. In addition, 20 (4.1%) women had a total of 21 deleterious mutations in non-*BRCA1/2* breast cancer predisposition genes including *CHEK2* (n = 10), *ATM* (n = 4), *BRIP1* (n = 4), and one each in *PALB2*, *PTEN*, and *NBN*. One *ATM* mutation was identified in a woman with a *BRCA2* mutation, and one patient had both an *ATM* and a *CHEK2* mutation. Four (0.8%) women carried deleterious mutations in genes unrelated to breast cancer; two in Lynch-related genes (one each in *MSH6* and *PMS2*), and one each in *RAD51C* and *RAD51D*. The patient with a *PMS2* mutation also had a *BRCA1* mutation. Thus, 49 (10.0%) women had an inherited mutation in a breast cancer predisposition gene and 52 (10.7%) in a gene associated with breast or ovarian cancer risk. Eight of the 10 *CHEK2* mutations identified were 1100delC. No mutations in

Table 3. Germline Mutations Identified (N = 488)

Genes	No. of Patients With Mutation	Percent With Mutation	95% CI
Any deleterious mutation*	52 (55 total mutations)	10.7	8.1 to 13.7
Genes related to breast cancer*	49	10.0	7.5 to 13.1
BRCA1 or *BRCA2*	30	6.1	4.2 to 8.7
*BRCA1**	18	3.7	2.2 to 5.8
*BRCA2**	12	2.5	1.3 to 4.3
Other genes related to breast cancer*	20 (21 total mutations)	4.1	2.5 to 6.3
*ATM**	4	0.8	0.2 to 2.1
BRIP1	4	0.8	0.2 to 2.1
*CHEK2**†	10	2.1	1.0 to 3.7
NBN	1	0.2	0.01 to 1.1
PALB2	1	0.2	0.01 to 1.1
PTEN	1	0.2	0.01 to 1.1
Genes not clearly related to breast cancer*	4	0.8	0.2 to 2.1
MSH6	1	0.2	0.01 to 1.1
*PMS2**	1	0.2	0.01 to 1.1
RAD51C	1	0.2	0.01 to 1.1
RAD51D	1	0.2	0.01 to 1.1

NOTE. No mutations were identified in the following genes: *BARD1*; *CDH1*; *STK11*; *TP53*; *APC*; *BMPR1A*; *CDK4*; *CDKN2A p14*; *CDKN2A p16*; *EPCAM*; *MLH1*; *MSH2*; *MUTYH* (biallelic); and *SMAD4*.
*One patient had deleterious mutations in both *BRCA2* and *ATM*. Another patient had deleterious mutations in both *BRCA1* and *PMS2*. Another patient had deleterious mutations in both *ATM* and *CHEK2*.
†Eight of 10 *CHEK2* mutations were 1100delC (Appendix Table A1).

BARD1, *CDH1*, *STK11*, *TP53*, *APC*, *BMPR1A*, *CDK4*, *CDKN2A p14*, *CDKN2A p16*, *EPCAM*, *MLH1*, *MSH2*, *MUTYH* (biallelic), or *SMAD4* were detected. Four patients with I1307K *APC* variants and nine women with monoallelic *MUTYH* mutations were identified but not included in this analysis due to lower associated cancer risk. Specific mutations identified and associated patient characteristics are provided in Appendix Table A1.

Of the 30 patients with a *BRCA1/2* mutation, four (13.3%) had not been clinically identified after diagnosis but did meet National Comprehensive Cancer Network (NCCN) criteria for *BRCA1/2* testing.

Variants of Uncertain Significance

At least one variant of uncertain significance (VUS) was identified in 162 (33.2%) women, with as many as three variants found per patient. Fifteen patients with a VUS also had a deleterious mutation. All VUSs identified are listed in Appendix Table A2 (online only).

Predictors of Deleterious Mutations

Age. For *BRCA1/2*, the prevalence of deleterious mutations decreased with age at breast cancer diagnosis, with a frequency of 12.2%, 3.0%, and 1.8% for women diagnosed at age ≤ 45 years, 46 to 60 years, and older than 60 years, respectively (Table 4). In contrast, for these same age groups, the frequency of mutations in other genes related to breast cancer ranged from 3.7% to 4.4%, irrespective of age at diagnosis (Table 4).

Breast cancer subtype. Table 5 illustrates the prevalence of deleterious mutations according to breast cancer subtype. The highest prevalence of *BRCA1/2* or of any mutations was in women with TNBC. Among 87 women with TNBC, 15 (17.2%) had a deleterious germline mutation, with 12 of these (13.8%) in *BRCA1/2* (11 *BRCA1*, 1 *BRCA2*). Two (2.3%) women had a mutation in another breast cancer predisposition gene (one each in *BRIP1* and *NBN*) and one (1.1%) in *RAD51D*. Among 301 women with ER-positive HER2-negative breast cancer, 26 (8.6%) had at least one mutation, with 15 (5.0%) in *BRCA1/2* (5 *BRCA1*, 10 *BRCA2*) and 11 (3.7%) in another breast/ovarian cancer predisposition gene including *CHEK2* (n = 4), *ATM* (n = 3), and one each in *PALB2*, *BRIP1*, *PTEN*, *RAD51C*, and *MSH6*. One woman had mutations in both *ATM* and *CHEK2*. Among 37 women with ER-negative HER2-positive disease, two (5.4%) had a *BRCA1* mutation and two (5.4%) had a *CHEK2* mutation. Eleven percent of 63 women with ER-positive HER2-positive breast cancer had a mutation, one (1.6%) in *BRCA2* (also in *ATM*) and six (9.5%) in other breast cancer predisposition genes including *CHEK2* (n = 4) and *BRIP1* (n = 2).

All predictors. Factors that significantly predicted for a *BRCA1/2* mutation included younger age at breast cancer diagnosis ($P < .01$); Ashkenazi Jewish heritage ($P < .01$); TNBC ($P = .01$); tumor histologic grade 3 ($P < .01$); and family history of breast cancer diagnosed at age younger than 50 years, male breast cancer, or ovarian cancer ($P < .01$; Table 6). No factors predicted for a mutation in other breast cancer predisposition genes when these genes were analyzed as a single group.

DISCUSSION

To our knowledge, this is the first study of the frequency of germline mutations in *BRCA1/2* and other breast cancer predisposition genes retrospectively done in a prospectively collected, sequential series of patients with breast cancer who consented to DNA banking for clinical research. Among 488 patients, we found that 6.1% had a *BRCA1/2* mutation (18.4% among Ashkenazi Jewish and 5.1% among non-Ashkenazi) and an additional 3.9% had a mutation in another breast cancer predisposition gene. In addition, four (0.8%) patients had a mutation in a gene linked to ovarian cancer, *RAD51C*, *RAD51D*, or a Lynch syndrome gene, one of whom also had a *BRCA1* mutation. In total, 10.7% of patients had a deleterious mutation in at least one cancer predisposition gene. The only other report of germline mutations among unselected patients with breast cancer is from The Cancer Genome Atlas, which found that among 507 cases, 5.5% had a germline *BRCA1/2* mutation and 4.3% had a mutation in another cancer predisposition gene. The mutation distribution was almost identical to that found in our study.[28] Likewise, the prevalence of mutations in non-*BRCA1/2* predisposition genes was 3.7% in more than 1,800 TNBC cases[17] and 4.5% in 289 African American women with breast cancer.[16]

Previous studies of multigene panel testing in patients with breast cancer, who were identified through high-risk clinics, have also consistently reported a prevalence of germline mutations in non-*BRCA1/2* breast cancer predisposition genes of approximately 4%, with up to an additional 1% in other cancer susceptibility genes if heterozygous *MUTYH* mutations are excluded.[18-21] The prevalence of mutations in moderate-penetrance breast cancer genes seems to be similar in this sequential series of breast cancer cases and in cases from high-risk clinics. Predictive factors for *BRCA1/2* and other high-penetrance genes that lead to referral to

Table 4. Frequency of Deleterious Mutations by Age at Breast Cancer Diagnosis

Genes	Patients ≤ 45 Years of Age With DM (n = 180)		Patients 46-60 Years of Age With DM (n = 199)		Patients > 60 Years of Age With DM (n = 109)	
	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)
Any deleterious mutation*	30	16.7 (11.5 to 22.9)	15	7.5 (4.3 to 12.1)	7	6.4 (2.6 to 12.8)
Genes related to breast cancer*	29	16.1 (11.1 to 22.3)	14	7.0 (3.9 to 11.5)	6	5.5 (2.1 to 11.6)
BRCA1 or *BRCA2*	22	12.2 (7.8 to 17.9)	6	3.0 (1.1 to 6.5)	2	1.8 (0.2 to 6.5)
*BRCA1**	15	8.3 (4.7 to 13.4)	2	1.0 (0.1 to 3.6)	1	0.9 (0.02 to 5.0)
*BRCA2**	7	3.9 (1.6 to 7.9)	4	2.0 (0.6 to 5.1)	1	0.9 (0.02 to 5.0)
Other genes related to breast cancer*	8	4.4 (1.9 to 8.6)	8	4.0 (1.8 to 7.8)	4	3.7 (1.0 to 9.1)
*ATM**	3	1.7 (0.4 to 4.8)	1	0.5 (0.01 to 2.8)	0	0.0 (0.0 to 3.3)
BRIP1	1	0.6 (0.01 to 3.1)	2	1.0 (0.1 to 3.6)	1	0.9 (0.02 to 5.0)
*CHEK2**	4	2.2 (0.6 to 5.6)	3	1.5 (0.3 to 4.3)	3	2.8 (0.6 to 7.8)
NBN	0	0.0 (0.0 to 2.0)	1	0.5 (0.01 to 2.8)	0	0.0 (0.0 to 3.3)
PALB2	1	0.6 (0.01 to 3.1)	0	0.0 (0.0 to 1.8)	0	0.0 (0.0 to 3.3)
PTEN	0	0.0 (0 to 2.0)	1	0.5 (0.01 to 2.8)	0	0.0 (0.0 to 3.3)
Genes not clearly related to breast cancer*	2	1.1 (0.1 to 4.0)	1	0.5 (0.01 to 2.8)	1	0.9 (0.02 to 5.0)
MSH6	0	0.0 (0.0 to 2.0)	1	0.5 (0.01 to 2.8)	0	0.0 (0.0 to 3.3)
*PMS2**	1	0.6 (0.01 to 3.1)	0	0.0 (0.0 to 1.8)	0	0.0 (0.0 to 3.3)
RAD51C	0	0.0 (0.0 to 2.0)	0	0.0 (0.0 to 1.8)	1	0.9 (0.02 to 5.0)
RAD51D	1	0.6 (0.01 to 3.1)	0	0.0 (0.0 to 1.8)	0	0.0 (0.0 to 3.3)

NOTE. No mutations were identified in the following genes: *BARD1*; *CDH1*; *STK11*; *TP53*; *APC*; *BMPR1A*; *CDK4*; *CDKN2A p14*; *CDKN2A p16*; *EPCAM*; *MLH1*; *MSH2*; *MUTYH* (biallelic); and *SMAD4*.

Abbreviation: DM, deleterious mutation.

*One patient diagnosed in the ≤ 45 years old group had deleterious mutations in both *BRCA2* and *ATM*. Another patient diagnosed in the ≤ 45 years old group had deleterious mutations in both *BRCA1* and *PMS2*. Another patient diagnosed in the ≤ 45 years old group had deleterious mutations in both *ATM* and *CHEK2*.

high-risk clinics do not seem to predict for mutations in moderate-penetrance genes. For example, as expected, the frequency of *BRCA1/2* mutations decreased with increasing age at breast cancer diagnosis. However, the frequency of deleterious mutations in non-*BRCA1/2* predisposition genes is independent of age at diagnosis (Table 4). As a result, among women diagnosed with breast cancer between ages 46 and 60 years in our cohort, more than half the mutations are in genes other than *BRCA1/2*. Among women diagnosed after 60 years of age, 6.4% had a deleterious mutation, with almost three-fourths in genes other than *BRCA1/2*.

Table 5. Deleterious Mutations by Breast Cancer Subtype (N = 488)

Genes	Patients With TNBC Mutation (n = 87)		Patients With ER-Positive/ HER2-Negative Mutation (n = 301)		Patients With ER-Negative/HER2-Positive Mutation (n = 37)		Patients With ER-Positive/HER2-Positive Mutation (n = 63)	
	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)
Any deleterious mutation*	15	17.2 (10.0 to 26.8)	26	8.6 (5.7 to 12.4)	4	10.8 (3.0 to 25.4)	7	11.1 (4.6 to 21.6)
Genes related to breast cancer*	14	16.1 (9.1 to 25.5)	24	8.0 (5.2 to 11.6)	4	10.8 (3.0 to 25.4)	7	11.1 (4.6 to 21.6)
BRCA1 or *BRCA2*	12	13.8 (7.3 to 22.9)	15	5.0 (2.8 to 8.1)	2	5.4 (0.7 to 18.2)	1	1.6 (0.04 to 8.5)
*BRCA1**	11	12.6 (6.5 to 21.5)	5	1.7 (0.5 to 3.8)	2	5.4 (0.7 to 18.2)	0	0.0 (0.0 to 5.7)
*BRCA2**	1	1.1 (0.03 to 6.2)	10	3.3 (1.6 to 6.0)	0	0.0 (0.0 to 9.5)	1	1.6 (0.04 to 8.5)
Other genes related to breast cancer*	2	2.3 (0.3 to 8.1)	9	3.0 (1.4 to 5.6)	2	5.4 (0.7 to 18.2)	7	11.1 (4.6 to 21.6)
*ATM**	0	0.0 (0.0 to 4.2)	3	1.0 (0.2 to 2.9)	0	0.0 (0.0 to 9.5)	1	1.6 (0.04 to 8.5)
BRIP1	1	1.1 (0.03 to 6.2)	1	0.3 (0.01 to 1.8)	0	0.0 (0.0 to 9.5)	2	3.2 (0.4 to 11.0)
*CHEK2**	0	0.0 (0.0 to 4.2)	4	1.3 (0.4 to 3.4)	2	5.4 (0.7 to 18.2)	4	6.3 (1.8 to 15.5)
NBN	1	1.1 (0.03 to 6.2)	0	0.0 (0.0 to 1.2)	0	0.0 (0.0 to 9.5)	0	0.0 (0.0 to 5.7)
PALB2	0	0.0 (0.0 to 4.2)	1	0.3 (0.01 to 1.8)	0	0.0 (0.0 to 9.5)	0	0.0 (0.0 to 5.7)
PTEN	0	0.0 (0.0 to 4.2)	1	0.3 (0.01 to 1.8)	0	0.0 (0.0 to 9.5)	0	0.0 (0.0 to 5.7)
Genes not clearly related to breast cancer*	2	2.3 (0.3 to 8.1)	2	0.7 (0.1 to 2.4)	0	0.0 (0.0 to 9.5)	0	0.0 (0.0 to 5.7)
MSH6	0	0.0 (0.0 to 4.2)	1	0.3 (0.01 to 1.8)	0	0.0 (0.0 to 9.5)	0	0.0 (0.0 to 5.7)
*PMS2**	1	1.1 (0.03 to 6.2)	0	0.0 (0.0 to 1.2)	0	0.0 (0.0 to 9.5)	0	0.0 (0.0 to 5.7)
RAD51C	0	0.0 (0.0 to 4.2)	1	0.3 (0.01 to 1.8)	0	0.0 (0.0 to 9.5)	0	0.0 (0.0 to 5.7)
RAD51D	1	1.1 (0.03 to 6.2)	0	0.0 (0.0 to 1.2)	0	0.0 (0.0 to 9.5)	0	0.0 (0.0 to 5.7)

NOTE. No mutations were identified in the following genes: *BARD1*; *CDH1*; *STK11*; *TP53*; *APC*; *BMPR1A*; *CDK4*; *CDKN2A p14*; *CDKN2A p16*; *EPCAM*; *MLH1*; *MSH2*; *MUTYH* (biallelic); and *SMAD4*.

Abbreviations: HER2, human epidermal growth factor receptor 2; HR, hormone receptor; TNBC, triple-negative breast cancer.

*One HR-positive/HER2-positive patient had deleterious mutations in both *BRCA2* and *ATM*. One TNBC patient had deleterious mutations in both *BRCA1* and *PMS2*. One HR-positive/HER2-negative patient had deleterious mutations in both *ATM* and *CHEK2*.

Table 6. Clinical and Pathologic Predictors of Germline Mutations in *BRCA1/2* and Other Breast Cancer Predisposition Genes*

Variable	No Mutation (n = 436)		*BRCA1/2* Mutation (n = 30)†		Other BC Mutation (n = 19)†*		*P*	
	No.	%	No.	%	No.	%	No Mutation *v BRCA1/2* Mutation	No Mutation *v* Other BC Mutation
Patient characteristic								
Age at BC diagnosis, years							< .01	.72
Mean ± SD	50.7 ± 11.2		42.6 ± 9.7		51.6 ± 10.9			
Median	49		40		53			
Range	28-88		31-66		34-68			
≤ 45	150	34.4	22	73.3	7	36.8	< .01	.96
46-60	184	42.2	6	20.0	8	42.1		
> 60	102	23.4	2	6.7	4	21.1		
Ashkenazi Jewish heritage								
Yes	29	6.7	7	23.3	2	10.5	< .01	.51
No	407	93.3	23	76.7	17	89.5		
History of cancer‡								
Yes	37	8.5	1	3.3	3	15.8	.32	.27
No	399	91.5	29	96.7	16	84.2		
BC characteristic								
Subtype								
TNBC	72	16.5	12	40.0	2	10.5	.01	.11
HR-positive/HER2-negative	275	63.1	15	50.0	9	47.4		
HR-negative/HER2-positive	33	7.6	2	6.7	2	10.5		
HR-positive/HER2-positive	56	12.8	1	3.3	6	31.6		
Histology								
Ductal	325	74.5	22	73.3	10	52.6	.50	.08
Lobular	33	7.6	1	3.3	2	10.5		
Ductal and lobular	58	13.3	4	13.3	4	21.1		
Other	20	4.6	3	10.0	3	15.8		
Histologic grade§								
1	57	13.1	0	0.0	3	15.8	< .01	.94
2	167	38.4	4	13.3	7	36.8		
3	211	48.5	26	86.7	9	47.4		
Stage								
I	169	38.8	12	40.0	4	21.1	.03	.12
II	198	45.4	8	26.7	9	47.4		
III	69	15.8	10	33.3	6	31.6		
Bilateral disease								
Yes	8	1.8	0	0.0	1	5.3	.45	.29
No	428	98.2	30	100.0	18	94.7		
Family history of cancer and prior genetic testing								
First-degree relative with any cancer‡‖								
Yes	242	56.8	15	50.0	12	63.2	.47	.58
No	184	43.2	15	50.0	7	36.8		
First- or second-degree relative with any cancer‡‖								
Yes	356	83.6	30	100.0	15	78.9	.02	.60
No	70	16.4	0	0.0	4	21.1		
First- or second-degree relative with BC or ovarian cancer‡‖								
Yes	202	47.4	22	73.3	9	47.4	.01	1.0
No	224	52.6	8	26.7	10	52.6		
First- or second-degree relative < 50 years of age with BC, ovarian cancer, or male BC‡‖								
Yes	71	16.7	12	40.0	5	26.3	< .01	.27
No	355	83.3	18	60.0	14	73.7		

Abbreviations: BC, breast cancer; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; SD, standard deviation; TNBC, triple-negative breast cancer.
*Three patients with mutations not associated with breast cancer were not included in this analysis.
†One patient with *BRCA2* and *ATM* mutations is included with *BRCA1/2* Mutation (not with Other BC Mutation).
‡Excludes in situ cancers and nonmelanoma skin cancers.
§Tumor grade was missing for one patient without a mutation.
‖Ten patients were missing family history information. These patients were excluded from analysis. If age at diagnosis was unavailable, it was conservatively considered to be older than 50 years.

As expected, factors known to enrich for *BRCA1/2* mutations (such as Ashkenazi heritage, TNBC subtype, and strong family history of breast or ovarian cancer) predicted for *BRCA1/2* mutations in our study. However, these factors were not significantly associated with mutations in other breast cancer predisposition genes when these genes were analyzed as a single group. We were unable to identify any factors that predicted for a mutation in non-*BRCA1/2* breast cancer genes. Larger cohorts of women with mutations in each gene are required to identify gene-specific predictors. For example, the prevalence of ER-positive breast cancer is higher among germline *CHEK2* 1100delC mutation carriers than noncarriers.[29]

We found that among non-*BRCA1/2* genes, mutations in *CHEK2* were most common. This is consistent with results from most studies that have evaluated multigene panels in breast cancer cohorts not enriched for TNBC.[19,20,30] The clinical significance of mutations in moderate-risk breast cancer genes such as *CHEK2*, *ATM*, and *NBN* is still being evaluated. An increased risk of contralateral breast cancer has been associated with germline mutations in *PALB2* and *CHEK2*, suggesting that subsequent screening with breast magnetic resonance imaging may be indicated in these patients.[29,31-33] We identified one mutation in *PTEN*, a high-penetrance cancer predisposition gene associated with Cowden syndrome and an increased risk of breast, thyroid, endometrial, and other cancers. This result would allow initiation of appropriate surveillance and risk-reduction strategies.

We also identified six mutations in three genes (*BRIP1*, *RAD51C*, and *RAD51D*) that are associated with a six- to 12-fold increased risk of ovarian cancer.[10-13,15] Mutations were found even in women with breast cancer diagnosed after 60 years of age. Only two of the six mutation carriers had a personal or family history of ovarian cancer. It seems that identification of mutations in more moderate-penetrance ovarian cancer susceptibility genes will require testing of families with less notable cancer histories.[34] Finally, we found two mutations in genes for Lynch syndrome. Identification of such mutations can lead to increased surveillance for and identification of colorectal, endometrial, ovarian, and other cancers in these patients with breast cancer and their relatives.[18]

The use of multigene panels for assessment of cancer susceptibility has been increasing rapidly in clinical practice. In addition to including high-penetrance genes with established clinical utility, these panels contain genes for which clinical validity or significance is less certain at this time.[35] Proper interpretation of results is critical so that appropriate recommendations for risk management are offered. This presents challenges for clinicians, who often lack genetic training, and their patients, who face decisions about screening and prevention strategies.

Whereas the significance of mutations in several of the non-*BRCA1/2* predisposition genes is still being studied, the benefit of identifying *BRCA1/2* mutations is well established. In addition to cancer prevention strategies, *BRCA*-associated cancers have a greater response to therapies such as poly-(ADP) ribose polymerase inhibitors and platinum agents than sporadic cancers.[36-39] Studying a sequential series of patients with breast cancer allowed us to evaluate how often *BRCA* mutations might be missed in clinical practice, at least in an academic setting. Only four (13.3%) of the *BRCA1/2* mutation carriers were first identified through this study, and all 30 carriers fulfilled NCCN 2015 genetic testing criteria. Thus, clinicians seem to be recognizing patients with breast cancer who are appropriate for *BRCA1/2* testing, and the NCCN criteria seem to perform well. Increased clinician education about testing criteria might decrease the frequency of unidentified carriers even further.

Consistent with previous studies, we found the highest prevalence of *BRCA1/2* mutations among cases with TNBC. For patients with ER-negative or ER-positive breast cancer, we found the frequency of *BRCA* mutations to be lower if the tumor also overexpressed HER2, consistent with previous reports (Table 5).[40-43]

Approximately one-third of patients had at least one VUS, as has been reported in other series evaluating NGS panels.[18] Most of these variants will eventually be reclassified, primarily as benign, but some will likely be deleterious.[27,44] VUSs should not be used to make clinical decisions.

Our study has limitations. Cases were ascertained from an academic center and may not reflect the breast cancer population or prevalence of mutations in the community. In our cohort, the median age at diagnosis was 50 years compared with 61 years in the United States.[45] Likewise, compared with the general population, a higher proportion of individuals (7.8%) were of Ashkenazi descent. However, although these factors may increase the prevalence of *BRCA1/2* mutations, our results show that they do not seem to increase the frequency of mutations in moderate-penetrance breast cancer susceptibility genes. Indeed, one report found a lower frequency of non-*BRCA1/2* mutations in the Ashkenazi population.[19] Finally, only 11% of women in the study were nonwhite, limiting the generalizability of our findings in more diverse populations. Large population-based studies are needed to establish the true frequency of mutations in these genes.

In conclusion, this is the largest prospective study to date to assess the prevalence of mutations in cancer susceptibility genes among a sequential series of breast cancer cases seen at an academic institution and not otherwise selected for age, family history, ethnicity, or breast cancer subtype. We identified a mutation in 10.7% of patients, 6.1% in *BRCA1/2* (5.1% in patients of non-Ashkenazi descent) and 4.6% in other breast/ovarian cancer predisposition genes. The prevalence of mutations in genes other than *BRCA1/2* seems to be relatively consistent regardless of breast cancer population tested, when these genes are assessed as a single group. Although clinicians seem to be identifying the majority of patients with breast cancer who have *BRCA1/2* mutations, the lack of predictive factors for mutations in other breast/ovarian cancer predisposition genes presents a challenge for identifying these carriers. Until better predictors emerge, it will be necessary to continue casting a wider net for identification of mutations in non-*BRCA1/2* genes. This is despite the unclear clinical significance at present for several genes that are included in many commercially available, broad gene panels.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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

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GLOSSARY TERMS

germline mutation: an inherited variation in the lineage of germ cells. Germline mutations can be passed on to offspring.

NextGen Sequencing: a non-Sanger rapid DNA sequencing method that can be done with greater speed, developed after the first methodologic articles describing relatively rapid DNA sequencing produced by Sanger et al (1977).

penetrance: the likelihood that a given gene mutation will produce disease. This likelihood is calculated by examining the proportion of people with the particular genetic mutation that show symptoms of disease.

triple-negative breast cancer (TNBC): breast tumors that are negative for estrogen and progesterone receptor expression and that also underexpress *HER-neu*.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Frequency of Germline Mutations in 25 Cancer Susceptibility Genes in a Sequential Series of Patients With Breast Cancer

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Appendix

Table A1. Mutations Identified in Study Cohort

Study ID	Ashkenazi Decent	Bilateral Breast Cancer	Gene	Mutation	Mutation Effect	Age at Dx (Years)	Breast Cancer Subtype	Family History
BOB16452	N	N	*ATM*	c.5623C>T (p.Arg1875*)	Truncation	60	HR+/HER-	None
BOB17213	N	N	*BRCA1*	c.5467+3A>C	Splice site	34	TNBC	CO, LK, Ovary
BOB17227	Y	N	*BRCA1*	c.68_69del (p.Glu23Valfs*17)	Frameshift	45	HR-/HER+	Breast, ES, UNP
BOB17377	Y	N	*BRCA2*	c.1754del (p.Lys585Argfs*29)	Frameshift	62	HR+/HER-	BL, LG, Ovary, LYM, CO
BOB17390	N	N	*BRCA1*	c.4327C>T (p.Arg1443*)	truncation	32	TNBC	Breast, Breast, Ovary, PAN
BOB17443	Y	N	*BRCA1*	c.68_69del (p.Glu23Valfs*17)	Frameshift	66	TNBC	Other, Ovary
BOB17447	Y	N	*BRCA1*	c.68_69del (p.Glu23Valfs*17)	Frameshift	34	HR-/HER+	Breast, CNS, LK
BOB17521	N	N	*CHEK2*	c.1368dupA (p.Glu457Argfs*33)	Frameshift	42	HR+/HER+	None
BOB17625	N	N	*PALB2*	c.599del (p.Leu200*)	Frameshift	34	HR+/HER-	Breast, UNP, UNP, LG
BOB17832	N	N	*RAD51D*	del exon 1	Large rearrangement	40	TNBC	STO, PR, Other, PR, Breast
BOB17843	N	N	*CHEK2*	c.1100del (p.Thr367Metfs*15)	Frameshift	36	HR-/HER+	Breast, HD, Breast, LK
BOB17913	Y	N	*BRIP1*	c.2392C>T (p.Arg798*)	Truncation	40	HR+/HER+	PR
BOB18197	N	N	*BRCA2*	c.5682C>A (p.Tyr1894*)	Truncation	37	HR+/HER-	Breast, STO, CO
BOB18292	N	N	*BRCA1*	c.4964_4982del (p.Ser1655Tyrfs*16)	Frameshift	37	HR-/HER+	Ovary
BOB18356	N	N	*BRCA1*	c.220C>T (p.Gln74*)	Truncation	59	TNBC	LG, LG, LG
BOB18732	N	N	*CHEK2*	c.1100del (p.Thr367Metfs*15)	Frameshift	68	HR+/HER+	PR, ES, Breast, FG, Breast
BOB18736	N	N	*BRCA1*	del exons 1-2	Large rearrangement	43	TNBC	Breast, Breast
BOB18885	N	N	*BRIP1*	c.2392C>T (p.Arg798*)	Truncation	50	HR+/HER+	PR, Other, Ovary, STO, Breast, ENDO
BOB200145	N	N	*CHEK2*	c.1100del (p.Thr367Metfs*15)	Frameshift	58	HR-/HER+	None
BOB20037	N	N	*BRCA1*	c.4675+1G>A	Splice site	39	HR+/HER-	UNP
BOB20040	N	N	*CHEK2*	c.1100del (p.Thr367Metfs*15)	Frameshift	64	HR+/HER+	CO, CO, LG, PAN
BOB20049	N	N	*NBN*	c.127C>T (p.Arg43*)	Truncation	56	TNBC	LG, LG, LG, CNS, PAN
BOB20068	N	N	*BRCA1*	c.5266dupC (p.Gln1756Profs*74)	Frameshift	43	HR+/HER-	Breast, CO, Breast, LYM
BOB20216	N	N	*BRCA2*	c.6644dupA (p.Tyr2215*)	Frameshift	34	HR+/HER+	Breast
BOB20297	N	N	*CHEK2*	c.1100del (p.Thr367Metfs*15)	Frameshift	56	HR+/HER-	MM, CO, LG, WT
BOB20304	Y	N	*BRCA2*	c.5946del (p.Ser1982Argfs*22)	Frameshift	58	HR+/HER-	PR
BOB20356WB	Y	N	*CHEK2*	c.1100del (p.Thr367Metfs*15)	Frameshift	46	HR+/HER-	Breast, ENDO, CLL, Breast, STO, PR
BOB20421WB	N	N	*ATM*	c.7705del (p.Asp2569Metfs*4)	Frameshift	39	HR+/HER-	Other
BOB20467	N	N	*ATM*	c.3381_3384del (p.Gln1128Lysfs*3)	Frameshift	33	HR+/HER+	Breast, ES, THY, PAN, Breast
BOB20467	N	N	*BRCA2*	c.6267_6269delinsC (p.Glu2089Aspfs*2)	Frameshift	33	HR+/HER+	Breast, ES, THY, PAN, Breast
BOB20605WB	N	N	*BRCA2*	c.658_659del (p.Val220Ilefs*4)	Frameshift	47	TNBC	OCMEL
BOB20702WB	N	N	*BRIP1*	c.2392C>T (p.Arg798*)	Truncation	66	HR+/HER-	CO
BOB20756	N	N	*BRCA1*	c.5137del (p.Val1713*)	Frameshift	31	HR+/HER-	Breast, Breast, BL, PR, LG, BL
BOB20822WB	N	N	*CHEK2*	c.1100del (p.Thr367Metfs*15)	Frameshift	45	HR+/HER+	KID, Breast, Breast, KID, LYM, PR
BOB20858WB	N	N	*BRCA1*	c.5266dupC (p.Gln1756Profs*74)	Frameshift	38	TNBC	STO, BL, Breast, CO, PR
BOB20980WB	N	N	*BRCA2*	c.5946del (p.Ser1982Argfs*22)	Frameshift	53	HR+/HER-	PR, BL
BOB20988WB	N	N	*BRCA2*	c.7618-1G>A	Splice site	48	HR+/HER-	Breast, LG
BOB21280WB	N	N	*BRCA2*	c.8585dupT (p.Glu2863Argfs*6)	Frameshift	38	HR+/HER+	FT, Breast, Ovary, PR

(continued on following page)

Table A1. Mutations Identified in Study Cohort (continued)

Study ID	Ashkenazi Decent	Bilateral Breast Cancer	Gene	Mutation	Mutation Effect	Age at Dx (Years)	Breast Cancer Subtype	Family History
BOB21299WB	N	N	*BRCA1*	c.5503C>T (p.Arg1835*)	Truncation	33	HR-/HER+	LG, Breast
BOB21299WB	N	N	*PMS2*	c.137G>T (p.Ser46Ile)	Missense	33	HR-/HER+	LG, Breast
BOB21399WB	N	N	*BRCA1*	c.415C>T (p.Gln139*)	Truncation	45	TNBC	Breast, CO, BL, CO, Other, CO
BOB21568WB	N	N	*CHEK2*	c.1100del (p.Thr367Metfs*15)	Frameshift	68	HR+/HER+	Ovary, PAN
BOB21578WB	N	N	*BRCA2*	c.8537_8538del (p.Glu2846Glyfs*22)	Frameshift	41	HR+/HER-	Breast, Breast, Breast, PR
BOB21663WB	N	N	*BRCA1*	c.5266dupC (p.Gln1756Profs*74)	Frameshift	33	TNBC	Breast, Breast
BOB21668WB	N	N	*BRCA1*	del exons 1-23	Large rearrangement	42	HR+/HER+	HN, Breast, Ovary, Cx
BOB21887WB	N	N	*BRIP1*	c.2255_2256del (p.Lys752Argfs*12)	Frameshift	53	TNBC	BL, Breast, CNS, MEL
BOB21984WB	Y	N	*BRCA2*	c.5946del (p.Ser1982Argfs*22)	Frameshift	39	HR+/HER-	LG
BOB22130WB	N	Y	*ATM*	c.3993+1G>A	Splice site	44	HR+/HER-	CO, Breast, Breast
BOB22130WB	N	Y	*CHEK2*	c.444+1G>A	Splice site	44	HR+/HER-	CO, Breast, Breast
BOB22416WB	N	N	*RAD51C*	c.577C>T (p.Arg193*)	Truncation	77	HR+/HER-	None
BOB23112WB	N	N	*BRCA1*	c.962G>A (p.Trp321*)	Truncation	56	HR-/HER+	Breast, Breast
BOB23117WB	N	N	*PTEN*	c.388C>T (p.Arg130*)	Truncation	56	HR+/HER-	PR, THY, ENDO, LG, PAN
BOB23508WB	N	N	*MSH6*	del exons 3-9	Large rearrangement	50	HR+/HER+	ENDO, ENDO, LG
BOB24634	Y	N	*BRCA2*	c.2808_2811del (p.Ala938Profs*21)	Frameshift	45	HR+/HER+	ES, PR
BOB24943	N	N	*BRCA1*	c.5266dupC (p.Gln1756Profs*74)	Frameshift	34	HR+/HER-	LARYNX, Breast, LK, Breast, CO

Abbreviations: BL, bladder cancer; Breast, breast cancer; CLL, chronic lymphocytic leukemia; CNS, central nervous system cancer; CO, colorectal cancer; Cx, cervical cancer; Dx, diagnosis; ENDO, endometrial/uterine cancer; ES, esophageal cancer; FG, female genital cancer, unspecified; FT, fallopian tube cancer; HD, Hodgkins lymphoma; HER, human epidermal growth factor receptor; HN, head and neck cancer; HR, hormone receptor; KID, kidney cancer unspecified; larynx, laryngeal cancer; LG, lung cancer; LK, leukemia; LYM, lymphoma; MEL, melanoma; MM, multiple myeloma; N, no; OCMEL, ocular melanoma; Other, cancer unspecified; Ovary, ovarian cancer; PAN, pancreatic cancer-exocrine; PR, prostate cancer; STO, stomach cancer; THY, thyroid cancer; TNBC, triple-negative breast cancer; UNP, cancer of unknown primary; WT, wilms tumor; Y, yes.

APC NM_000038.5 ATM NM_000051.3 BARD1 NM_000465.3 BMPR1A NM_004329.2 BRCA1 NM_007294.3 BRCA2 NM_000059.3 BRIP1 NM_032043.2 CDH1 NM_004360.3 CDK4 NM_000075.3 CHEK2 NM_007194.3

EPCAM NM_002354.2 MLH1 NM_000249.3 MSH2 NM_000251.2 MSH6 NM_000179.2 MUTYH NM_001128425.1 NBN NM_002485.4 P14ARF NM_058195.3

P16 NM_000077.4 PALB2 NM_024675.3 PMS2 NM_000535.5 PTEN NM_000314.4 RAD51C NM_058216.2 RAD51D NM_002878.3 SMAD4 NM_005359.5 STK11 NM_000455.4 TP53 NM_000546.5

Table A2. Variants of Unknown Significance Identified in Study Cohort

Study ID	Gene	Variant of Uncertain Significance
BOB21387WB	*APC*	c.5424_5426del (p.Asn1808del)
BOB20704WB	*APC*	c.437C>T (p.Ala146Val)
BOB20752WB	*APC*	c.1276G>T (p.Ala426Ser)
BOB20406WB	*APC*	c.2204C>T (p.Ala735Val)
BOB19922	*APC*	c.8462A>G (p.Asp2821Gly)
BOB21973WB	*APC*	c.420G>C (p.Glu140Asp)
BOB20898WB	*APC*	c.95A>G (p.Asn32Ser)
BOB17786	*APC*	c.7399C>A (p.Pro2467Thr)
BOB21159WB	*APC*	c.3511C>T (p.Arg1171Cys)
BOB21291WB	*APC*	c.4766G>A (p.Arg1589His)
BOB17786	*APC*	c.5026A>G (p.Arg1676Gly)
BOB20049	*APC*	c.5357G>A (p.Arg1786Lys)
BOB21384WB	*APC*	c.5357G>C (p.Arg1786Thr)
BOB24011	*APC*	c.5503A>G (p.Arg1835Gly)
BOB16413	*APC*	c.7589G>A (p.Arg2530Gln)
BOB17440	*APC*	c.2717C>T (p.Ser906Phe)
BOB20365WB	*APC*	c.2725A>G (p.Thr909Ala)
BOB20054	*APC*	c.3374T>C (p.Val1125Ala)
BOB18356	*ATM*	c.1960C>A (p.Gln654Lys)
BOB21660WB	*ATM*	c.2096A>G (p.Glu699Gly)
BOB20412	*ATM*	c.2275A>G (p.Ser759Gly)
BOB21410WB	*ATM*	c.2494C>T (p.Arg832Cys)
BOB22428WB	*ATM*	c.2494C>T (p.Arg832Cys)
BOB20047	*ATM*	c.2552A>G (p.Asp851Gly)
BOB16447	*ATM*	c.2699T>C (p.Met900Thr)
BOB17825	*ATM*	c.3014A>G (p.Asn1005Ser)
BOB20403WB	*ATM*	c.3240C>A (p.Asp1080Glu)
BOB200138	*ATM*	c.3467C>T (p.Thr1156Met)
BOB17688	*ATM*	c.3590T>C (p.Val1197Ala)
BOB20212	*ATM*	c.3925G>A (p.Ala1309Thr)
BOB21585WB	*ATM*	c.3925G>A (p.Ala1309Thr)
BOB21877WB	*ATM*	c.3993+5G>T
BOB21139WB	*ATM*	c.4148C>T (p.Ser1383Leu)
BOB21979WB	*ATM*	c.4324T>C (p.Tyr1442His)
BOB200137	*ATM*	c.4375G>A (p.Gly1459Arg)
BOB22337WB	*ATM*	c.4388T>G (p.Phe1463Cys)
BOB24634	*ATM*	c.4388T>G (p.Phe1463Cys)
BOB20412	*ATM*	c.4420C>G (p.His1474Asp)
BOB20920WB	*ATM*	c.4424A>G (p.Tyr1475Cys)
BOB18118	*ATM*	c.4709T>C (p.Val1570Ala)
BOB17701	*ATM*	c.4949A>G (p.Asn1650Ser)
BOB20521WB	*ATM*	c.5693G>A (p.Arg1898Gln)
BOB17446	*ATM*	c.6067G>A (p.Gly2023Arg)
BOB23405	*ATM*	c.6067G>A (p.Gly2023Arg)
BOB18191	*ATM*	c.6332A>G (p.His2111Arg)
BOB20888WB	*ATM*	c.6543G>T (p.Glu2181Asp)
BOB20885WB	*ATM*	c.6860G>C (p.Gly2287Ala)
BOB17443	*ATM*	c.6919C>T (p.Leu2307Phe)
BOB21067WB	*ATM*	c.6919C>T (p.Leu2307Phe)
BOB21289WB	*ATM*	c.6919C>T (p.Leu2307Phe)
BOB21984WB	*ATM*	c.6919C>T (p.Leu2307Phe)
BOB17646	*ATM*	c.7618G>A (p.Val2540Ile)
BOB20767WB	*ATM*	c.7740A>C (p.Arg2580Ser)
BOB17523	*ATM*	c.7919C>T (p.Thr2640Ile)
BOB18813	*ATM*	c.7988T>C (p.Val2663Ala)
BOB18356	*ATM*	c.8147T>C (p.Val2716Ala)
BOB21385WB	*ATM*	c.8734A>G (p.Arg2912Gly)
BOB20044	*ATM*	c.977T>C (p.Ile326Thr)
BOB21282WB	*BARD1*	c.1042G>A (p.Val348Ile)
BOB20970WB	*BARD1*	c.2183C>T (p.Ser728Phe)
BOB16447	*BARD1*	c.2282G>A (p.Ser761Asn)
BOB20049	*BARD1*	c.2282G>A (p.Ser761Asn)
BOB21068WB	*BARD1*	c.2282G>A (p.Ser761Asn)
BOB21742WB	*BARD1*	c.581G>A (p.Arg194Lys)
BOB20751WB	*BARD1*	c.620A>G (p.Lys207Arg)
BOB20606WB	*BARD1*	c.632T>C (p.Leu211Ser)

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Table A2. Variants of Unknown Significance Identified in Study Cohort (continued)

Study ID	Gene	Variant of Uncertain Significance
BOB22413WB	*BARD1*	c.668A>G (p.Glu223Gly)
BOB200140	*BARD1*	c.716T>A (p.Leu239Gln)
BOB21660WB	*BARD1*	c.841C>T (p.Pro281Ser)
BOB20049	*BARD1*	c.928T>G (p.Ser310Ala)
BOB17708	*BMPR1A*	c.1327C>T (p.Arg443Cys)
BOB21384WB	*BMPR1A*	c.1327C>T (p.Arg443Cys)
BOB213892WB	*BMPR1A*	c.1327C>T (p.Arg443Cys)
BOB20235	*BMPR1A*	c.560G>A (p.Arg187His)
BOB21048WB	*BMPR1A*	c.676-3A>C
BOB18108	*BRCA1*	c.1263A>C (p.Glu421Asp)
BOB21874WB	*BRCA1*	c.5513T>A (p.Val1838Glu)
BOB18622	*BRCA2*	c.4901T>C (p.Phe1634Ser)
BOB23117WB	*BRCA2*	c.7925T>G (p.Phe2642Cys)
BOB18803	*BRCA2*	c.7434A>C (p.Leu2478Phe)
BOB21143WB	*BRCA2*	c.8902A>G (p.Thr2968Ala)
BOB20819WB	*BRIP1*	c.1616G>A (p.Arg539Lys)
BOB17429	*BRIP1*	c.1899C>G (p.Ile633Met)
BOB17713	*BRIP1*	c.1899C>G (p.Ile633Met)
BOB20231	*BRIP1*	c.205+5G>A
BOB18605	*BRIP1*	c.2071A>C (p.Ile691Leu)
BOB22122WB	*BRIP1*	c.2120G>A (p.Arg707His)
BOB20986WB	*BRIP1*	c.262_264del (p.Cys88del)
BOB21880WB	*BRIP1*	c.2830C>G (p.Gln944Glu)
BOB17212	*BRIP1*	c.337A>C (p.Thr113Pro)
BOB17786	*BRIP1*	c.3464G>A (p.Gly1155Glu)
BOB20223	*BRIP1*	c.3651G>T (p.Trp1217Cys)
BOB17776	*BRIP1*	c.380-17T>A
BOB20592WB	*BRIP1*	c.728T>C (p.Ile243Thr)
BOB21143WB	*BRIP1*	c.728T>C (p.Ile243Thr)
BOB21573WB	*BRIP1*	c.778A>G (p.Thr260Ala)
BOB17377	*BRIP1*	c.790C>T (p.Arg264Trp)
BOB20756	*BRIP1*	c.790C>T (p.Arg264Trp)
BOB20988WB	*BRIP1*	c.820A>G (p.Thr274Ala)
BOB18605	*BRIP1*	del exon 7
BOB21139WB	*CDH1*	c.1090A>T (p.Thr364Ser)
BOB18734	*CDH1*	c.1297G>A (p.Asp433Asn)
BOB21048WB	*CDH1*	c.2329G>A (p.Asp777Asn)
BOB18354	*CDH1*	c.499G>A (p.Glu167Lys)
BOB18287	*CDH1*	c.88C>A (p.Pro30Thr)
BOB21284WB	*CDK4*	c.209A>G (p.Asn70Ser)
BOB18539	*CDK4*	c.820-15T>G
BOB21587WB	*CHEK2*	c.1217G>A (p.Arg406His)
BOB17443	*CHEK2*	c.1283C>T (p.Ser428Phe)
BOB17433	*CHEK2*	c.1343T>G (p.Ile448Ser)
BOB20594WB	*CHEK2*	c.1343T>G (p.Ile448Ser)
BOB200150	*CHEK2*	c.1558_1559insC (p.Lys520Thrfs*5)
BOB17184	*CHEK2*	c.190G>A (p.Glu64Lys)
BOB22413WB	*CHEK2*	c.190G>A (p.Glu64Lys)
BOB18002	*CHEK2*	c.275C>T (p.Pro92Leu)
BOB18810	*CHEK2*	c.410G>A (p.Arg137Gln)
BOB17992	*CHEK2*	c.422A>C (p.Lys141Thr)
BOB18283	*CHEK2*	c.432T>G (p.Phe144Leu)
BOB21805WB	*CHEK2*	c.470T>C (p.Ile157Thr)
BOB20237	*CHEK2*	c.499G>A (p.Gly167Arg)
BOB21046WB	*CHEK2*	c.598G>A (p.Val200Ile)
BOB17828	*CHEK2*	c.715G>A (p.Glu239Lys)
BOB21787WB	*CHEK2*	c.787G>C (p.Glu263Gln)
BOB21923WB	*CHEK2*	c.931G>A (p.Asp311Asn)
BOB21384WB	*MLH1*	dup entire *MLH1* gene
BOB17691	*MLH1*	c.226G>A (p.Val76Ile)
BOB18664	*MSH2*	c.982G>C (p.Ala328Pro)
BOB17227	*MSH2*	c.944G>T (p.Gly315Val)
BOB20421WB	*MSH2*	c.2458+6T>C
BOB18197	*MSH2*	c.835C>G (p.Leu279Val)
BOB17446	*MSH2*	c.775C>T (p.Pro259Ser)

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Table A2. Variants of Unknown Significance Identified in Study Cohort (continued)

Study ID	Gene	Variant of Uncertain Significance
BOB20874WB	*MSH2*	c.2728C>A (p.Gln910Lys)
BOB17994	*MSH2*	c.440T>G (p.Val147Gly)
BOB17828	*MSH6*	c.3974_3976del (p.Lys1325del)
BOB20594WB	*MSH6*	c.3294C>G (p.Cys1098Trp)
BOB18110	*MSH6*	c.1599G>C (p.Glu533Asp)
BOB16469	*MSH6*	c.3173-10C>A
BOB18399	*MSH6*	c.3801+5G>A
BOB21391WB	*MSH6*	c.1793A>G (p.Lys598Arg)
BOB18268	*MSH6*	c.2225A>G (p.Asn742Ser)
BOB23707WB	*MSH6*	c.41C>T (p.Ser14Phe)
BOB17777	*MYH*	c.1013_1014delinsGC (p.Gln338delinsArg)
BOB20856WB	*MYH*	c.1013_1014delinsGC (p.Gln338delinsArg)
BOB20894WB	*MYH*	c.1013_1014delinsGC (p.Gln338delinsArg)
BOB18198	*MYH*	c.821G>A (p.Arg274Gln)
BOB17696	*MYH*	c.820C>T (p.Arg274Trp)
BOB17212	*MYH*	c.1276C>T (p.Arg426Cys)
BOB18489	*MYH*	c.1276C>T (p.Arg426Cys)
BOB200139	*MYH*	c.305G>A (p.Ser102Asn)
BOB21157WB	*NBN*	c.1036G>A (p.Val346Met)
BOB20888WB	*NBN*	c.1354A>C (p.Thr452Pro)
BOB21973WB	*NBN*	c.1444A>G (p.Arg482Gly)
BOB24387	*NBN*	c.1690G>A (p.Glu564Lys)
BOB17995	*NBN*	c.1720T>A (p.Leu574Ile)
BOB18279	*NBN*	c.1952C>T (p.Pro651Leu)
BOB18481	*NBN*	c.643C>T (p.Arg215Trp)
BOB20974WB	*NBN*	c.643C>T (p.Arg215Trp)
BOB21578WB	*NBN*	c.643C>T (p.Arg215Trp)
BOB17928	*P16*	c.9_32del (p.Ala4_Pro11del)
BOB21134WB	*P16*	c.430C>T (p.Arg144Cys)
BOB20888WB	*PALB2*	c.400G>A (p.Asp134Asn)
BOB20592WB	*PALB2*	c.656A>G (p.Asp219Gly)
BOB20988WB	*PALB2*	c.656A>G (p.Asp219Gly)
BOB18125	*PALB2*	c.3037A>G (p.Ile1013Val)
BOB20870WB	*PALB2*	c.3350+4A>G
BOB21672WB	*PALB2*	c.298C>T (p.Leu100Phe)
BOB21660WB	*PALB2*	c.1564C>T (p.Pro522Ser)
BOB20037	*PALB2*	c.22C>A (p.Pro8Thr)
BOB21587WB	*PALB2*	c.109C>T (p.Arg37Cys)
BOB17440	*PALB2*	c.3296C>G (p.Thr1099Arg)
BOB18667	*PALB2*	c.950C>T (p.Thr317Ile)
BOB18594	*PALB2*	c.1430C>T (p.Thr477Ile)
BOB20237	*PMS2*	c.1092T>A (p.Asp364Glu)
BOB17523	*PMS2*	c.1417G>A (p.Glu473Lys)
BOB17824	*PMS2*	c.86G>C (p.Gly29Ala)
BOB20760WB	*PMS2*	c.86G>C (p.Gly29Ala)
BOB24634	*PMS2*	c.53T>C (p.Ile18Thr)
BOB20970WB	*PMS2*	c.935T>C (p.Met312Thr)
BOB21149WB	*PMS2*	c.1723A>G (p.Asn575Asp)
BOB17523	*PMS2*	c.58C>G (p.Arg20Gly)
BOB20041	*PMS2*	c.1567T>A (p.Ser523Thr)
BOB17196	*PMS2*	c.2149G>A (p.Val717Met)
BOB20894WB	*PMS2*	c.2149G>A (p.Val717Met)
BOB21149WB	*PMS2*	c.2386G>A (p.Val796Ile)
BOB17992	*PTEN*	c.210-7_210-3del
BOB20892WB	*RAD51C*	c.-13A>C
BOB20047	*RAD51C*	c.428A>G (p.Gln143Arg)
BOB20233	*RAD51C*	c.601C>G (p.Leu201Val)
BOB18416	*RAD51C*	c.605A>G (p.Asp202Gly)
BOB18729	*RAD51C*	c.752A>G (p.Asp251Gly)
BOB21394WB	*RAD51C*	c.790G>A (p.Gly264Ser)
BOB18729	*RAD51C*	c.7G>A (p.Gly3Arg)
BOB20042	*RAD51D*	c.491T>C (p.Leu164Pro)
BOB21149WB	*RAD51D*	c.620C>T (p.Ser207Leu)
BOB21391WB	*RAD51D*	c.972G>T (p.Gln324His)
BOB21979WB	*SMAD4*	c.1448-18C>A
BOB18288	*SMAD4*	c.667+3G>A

(continued in next column)

Table A2. Variants of Unknown Significance Identified in Study Cohort (continued)

Study ID	Gene	Variant of Uncertain Significance
BOB20958WB	*STK11*	c.1040C>G (p.Ala347Gly)
BOB17218	*STK11*	c.1211C>T (p.Ser404Phe)
BOB18813	*TP53*	c.139C>T (p.Pro47Ser)
BOB22178WB	*TP53*	c.139C>T (p.Pro47Ser)
BOB17207	*TP53*	c.329G>A (p.Arg110His)
BOB23508WB	*TP53*	c.75-18T>G
BOB20974WB	*TP53*	c.845G>A (p.Arg282Gln)
BOB20610WB	*TP53*	c.877G>T (p.Gly293Trp)

APC NM_000038.5 ATM NM_000051.3 BARD1 NM_000465.3 BMPR1A NM_004329.2 BRCA1 NM_007294.3 BRCA2 NM_000059.3 BRIP1 NM_032043.2 CDH1 NM_004360.3 CDK4 NM_000075.3 CHEK2 NM_007194.3 EPCAM NM_002354.2 MLH1 NM_000249.3 MSH2 NM_000251.2 MSH6 NM_000179.2 MUTYH NM_001128425.1 NBN NM_002485.4 P14ARF NM_058195.3

P16 NM_000077.4 PALB2 NM_024675.3 PMS2 NM_000535.5 PTEN NM_000314.4 RAD51C NM_058216.2 RAD51D NM_002878.3 SMAD4 NM_005359.5 STK11 NM_000455.4

TP53 NM_000546.5