Original Article

Prevalence of *BRCA* Mutations in an Unselected Population of Triple-Negative Breast Cancer

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BACKGROUND: This study assessed *BRCA1* and *BRCA2* mutation prevalence in an unselected cohort of patients with triple-negative breast cancer (BC). **METHODS:** One hundred ninety-nine patients were enrolled. Triple negativity was defined as <1% estrogen and progesterone staining by immunohistochemistry and HER-2/*neu* not overexpressed by fluorescence in situ hybridization. Having given consent, patients had *BRCA1* and *BRCA2* full sequencing and large rearrangement analysis. Mutation prevalence was assessed among the triple-negative BC patients and the subset of patients without a family history of breast/ovarian cancer. Independent pathological review was completed on 50 patients. **RESULTS:** Twenty-one deleterious *BRCA* mutations were identified—13 in *BRCA1* and 8 in *BRCA2* (prevalence, 10.6%). In 153 patients (76.9%) without significant family history (first-degree or second-degree relatives with BC aged <50 years or ovarian cancer at any age), 8 (5.2%) mutations were found. By using prior National Comprehensive Cancer Network (NCCN) guidelines recommending testing for triple-negative BC patients aged <45 years, 4 of 21 mutations (19%) would have been missed. Two of 21 mutations (10%) would have been missed using updated NCCN guidelines recommending testing for triple-negative BC patients aged <60 years. **CONCLUSIONS:** The observed mutation rate was significantly higher ($P = .0005$) than expected based on previously established prevalence tables among patients unselected for pathology. *BRCA1* mutation prevalence was lower, and *BRCA2* mutation prevalence was higher, than previously described. Additional mutation carriers would have met new NCCN testing guidelines, underscoring the value of the updated criteria. Study data suggest that by increasing the age limit to 65 years, all carriers would have been identified. ***Cancer* 2011;000:000–000.**
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KEYWORDS: breast cancer, *BRCA1* gene, *BRCA2* gene, prevalence.

INTRODUCTION

Substantial resources have been allocated to investigate the epidemiology and pathogenesis of triple-negative or basal subtype breast cancer (BC). Despite this intense focus, significant knowledge gaps exist. Published literature postulates that triple-negative BC constitutes 15% to 20% of all BCs diagnosed in the United States, and 20% of patients with triple-negative BC have a mutation in *BRCA1* (Table 1).[1,2] These previous studies were conducted in cohorts of interest selected by ethnicity, family history, or age, and therefore may not be representative of an unselected population. This study is the largest analysis to date, with independent pathological review, of *BRCA* mutations in an unselected cohort of 199 triple-negative BC patients in a community oncology practice over the past 5 years. The prevalence of mutations associated with other risk factors was also assessed.

The triple-negative BC phenotype is characterized by tumors that that do not express estrogen receptor (ER) or progesterone receptor (PR), or contain an amplified HER-2/*neu* gene. A majority of triple-negative tumors also have a core basal phenotype, characterized by expression of cytokeratins 5 and 6, and further defined by hierarchical clustering of transcriptional profiles.[3,4] With the use of stricter criteria for ER and PR negativity, the true prevalence of triple-negative BC has not been estimated.

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DOI: 10.1002/cncr.26576, **Received:** July 12, 2011; **Revised:** August 22, 2011; **Accepted:** August 30, 2011, **Published online** in Wiley Online Library (wileyonlinelibrary.com)

Table 1. Summary of *BRCA* Mutation Prevalence Among Triple-Negative Breast Cancer Patients in the Literature

Study/Cohort Size	Proportion of Ashkenazi Jews	Family History Status	ER−; <1% Staining	*BRCA* Mutation Prevalence	*BRCA1* Prevalence	*BRCA2* Prevalence
Foulkes 2003,[2] N = 72	100%, all <65 years	Not reported	No	23.6%	23.6%	0
Comen 2008,[7] N = 65	100%		No	39.2%	30%	9.2%
Atchley 2008,[5] N = 93	<10%	~100%; all had genetic testing	No	41.9%	34.4%	7.5%
Young 2009,[14] N = 54	0%; all <40 years	None	Not reported	11.1%	9.2%	1.9%
Collins 2009,[21] N = 144; DFCI spore cohort	Not reported, postulated high	Not reported, postulated high	Yes	14%	14%	Not reported
Myriad study, N = 199	<1%	23%	Yes	10.6%	6.5%	4.0%

Approximately 60% to 80% of tumors in patients that carry a germ-line mutation in *BRCA1* are characterized by triple negativity.[5] Approximately 15% to 25% of patients with triple-negative BC of Ashkenazi ethnicity have a *BRCA1* mutation.[2,6] A recent study evaluating a cohort of 500 Ashkenazi women presenting with triple-negative BC found that 29% had a *BRCA1* mutation.[7] As family history was not known for all patients, the mutation prevalence among patients without a family history is unknown. The studies cited above are limited by their assessment of select triple-negative BC cohorts only, as is also their focus on *BRCA1* mutations for which triple-negative BC is thought to be enriched (Table 1). The incidence of *BRCA* mutations unassociated with family history and the prevalence of *BRCA2* mutations remain unaddressed.

Therapies including DNA-damaging cytotoxic chemotherapies and poly adenosine diphosphate-ribose polymerase inhibitors have recently been evaluated in patients with triple-negative BC. These studies have demonstrated that triple-negative BC patients, particularly those with underlying *BRCA* mutations, have robust and durable responses to therapies that target underlying defects in DNA repair.[8-11] This genotype information might guide therapy selection for patients as well as preventive measures for affected asymptomatic family members.[12]

MATERIALS AND METHODS

Cohort Identification

Patients presenting with triple-negative BC in a community oncology network from 2005 to 2010 were contacted for enrollment. Eligible patients had to be alive, ≥18 years of age, and consent to genetic testing for *BRCA1* and *BRCA2* if such testing had not occurred previously. Patients diagnosed before 2005 were excluded (there was 1 patient enrolled who was diagnosed in 2004) to minimize mortality ascertainment bias (Neyman bias). Having given consent, patients underwent genetic testing if not tested previously. Patients who were already tested gave consent again for study purposes only. The protocol and the consent form were both institutional review board-approved.

Trial Design

This was a retrospective ascertainment study. Medical records were reviewed with the following information captured on a case report form: demographics (age, date of birth, ethnicity), personal history of cancer, age of diagnosis, recurrence, current status, comprehensive family history from the time of the patient's diagnosis, and diagnostic tests and results including assays for ER/PR and human epidermal growth receptor 2 (HER2).

Pathology samples from 50 (25%) of 199 patients were sent for independent pathological review to ARUP Laboratories, a national clinical and anatomic pathology reference laboratory in Salt Lake City, Utah. An electronic registry and medical record/pathology report review were used to identify patients. Recruitment started with all eligible patients diagnosed from 2010 backward to 2005. Patients were contacted about participation in the study, and those interested provided written informed consent.

Triple-Negative Pathology

Triple-negative phenotype was defined by the following. ER and PR immunohistochemistry (IHC) assessments were based on percentage of cells stained and staining pattern using primarily ER clone SP1 and PR clone 1E2 and a polymer detection system on formalin-fixed, paraffin-embedded sections. Additional antibody clones included for ER: 6F11, SP1 + 6F11, and ID5; and for PR: 1E2, 636, and PR002 + PR003. One percent or more staining was reported as positive, consistent with current guidelines.[13] HER2 was analyzed by fluorescence in situ

hybridization and reported as positive or negative as defined by the HER2:CEP17 ratio (<1.8 is not amplified). HER2 IHC was not evaluated for eligibility in this study.

BRCA *Testing*

Patients had *BRCA1* and *BRCA2* full sequencing and large genomic rearrangement analysis performed by Myriad Genetic Laboratories, Inc. Large rearrangement testing was performed for patients who had only sequencing testing previously.

Statistics

The primary study objective was to estimate germline *BRCA* mutation prevalence among triple-negative BC patients. A secondary objective was to estimate the prevalence in the subpopulation of triple-negative BC patients without a significant family history of BC or ovarian cancer. Assuming a 20% *BRCA* mutation prevalence among triple-negative BC patients, a sample size of 200 was considered sufficient to estimate the true prevalence within a 5% precision with 90% confidence. Assuming a lower 10% prevalence, about 100 patients would be needed for a similar estimate in the subpopulation without family history. If observed prevalence was lower than assumed, the precision of the estimate would improve.

An exact 2-sided 90% confidence interval (CI) around the observed proportion of *BRCA* mutation carriers in the study was computed to meet the primary objective. Mutation prevalence in various subgroups of interest, including those stratified by family history and by affected gene (*BRCA1* or *BRCA2*), were estimated similarly. Logistic regression modeling with *BRCA* mutation status as a response variable was conducted to identify significant predictive variables.

All analyses results were used for estimation purposes. The study was not powered for hypothesis testing and had no randomized comparative arms. There was no multiplicity adjustment.

RESULTS

Cohort Characteristics

Our cohort was identified at Texas Oncology Austin, a large community practice. Approximately 3280 patients were screened to meet the targeted enrollment of 200 patients. Thirty patients declined to participate. A total of 215 patients were eligible and agreed to participate. Upon detailed examination of pathology reports, 16 did not meet the study's pathology definition and were deemed ineligible.

The median age of our cohort was 54, and 51.5% were postmenopausal (Table 2). Previously reported cohorts were much younger, and the majority of their patients were premenopausal (Table 1).[5,14] Sixty-six percent (n = 131) of our cohort was Caucasian, 15.7% (n = 31) Hispanic, and 13.6% (n = 27) African American. This reflects the current US population. Only 1 (0.5%) patient declared Ashkenazi ancestry. The majority (76.9%) did not have a significant family history of breast and/or ovarian cancer. The study defines a significant family history as BC before the age of 50 years or ovarian cancer at any age in any first-degree or second-degree relative. Forty-four percent of the cohort did not have any incidence of BC or ovarian cancer in their family history.

Pathology Results

In independent validation of TNM classification, discordance was observed in 3 of a total 50 patients (6% discordance with exact 95% CI of 1.3%-16.6%).

Mutation Prevalence

We identified 21 deleterious *BRCA* mutations: 13 in *BRCA1* and 8 in *BRCA2* (Table 3), providing an overall prevalence rate of 10.6%. Of the 153 patients with no significant family history of BC or ovarian cancer, 8 (5.2%) had a mutation. Of the 88 patients with no family history of BC or ovarian cancer, 5 (5.7%) had a mutation. One large rearrangement mutation was identified in a patient diagnosed with triple-negative BC at age 61 years who had only sequence testing previously. This patient is of Central European ancestry and without a significant family history per study definition. This patient also had a previous incidence of BC (unknown pathology) at age 40 years, and would have met both old and new National Comprehensive Cancer Network (NCCN) guidelines criteria for testing. Eight variants of uncertain significance were identified. Five (3.3%) of the observed *BRCA2* mutations occurred in patients with no significant family history of breast and ovarian cancer. When assessed by age at triple-negative BC diagnosis, 13 mutations (8 *BRCA1* and 5 *BRCA2*) were identified among the 86 patients diagnosed at <50 years, and 8 mutations (5 *BRCA1* and 3 *BRCA2*) were identified among the remaining 113 patients diagnosed at ≥50 years. When assessed by age at first BC diagnosis, 15 mutations (10 *BRCA1* and 5 *BRCA2*) were identified among the 91 patients diagnosed

Table 2. Demographic and Baseline Characteristics

Characteristic	Patients, N = 199
Current age, y	
Median	54.0
SD	11.72
Min-max	26-80
Age at TNBC diagnosis, y	
Mean	52.5
SD	11.72
Median	52.0
Min-max	23-79
Age at TNBC diagnosis, No. (%)	
<50 years	86 (43.2%)
≥50 years	113 (56.8%)
Age at 1st BC diagnosis, No. (%)[a]	
<50 years	91 (45.7%)
≥50 years	108 (54.3%)
Menopausal status at time of TNBC diagnosis, No. (%)[b]	
Premenopausal	63 (36.8%)
Perimenopausal	20 (11.7%)
Postmenopausal	88 (51.5%)
Missing	28
Ethnicity, No. (%)[b]	
Black	27 (13.6%)
Native American	1 (0.5%)
Hispanic	31 (15.7%)
Asian	3 (1.5%)
Caucasian	131 (66.2%)
Unknown	1 (0.5%)
Other	4 (2.0%)
Missing	1
Ancestry, No. (%)[c]	
Africa	16 (8.0%)
Ashkenazi	1 (0.5%)
Asia	5 (2.5%)
Central/Eastern Europe	18 (9.0%)
Latin America/Carribean	33 (16.6%)
Near East/Middle East	2 (1.0%)
North America	25 (12.6%)
Other	30 (15.1%)
Western Europe	113 (56.8%)
Missing	2
Family history of any cancer, No. (%)	
Yes	171 (85.9%)
No	25 (12.6%)
Unknown	3 (1.5%)
Family history of breast/ovarian cancer, No. (%)	
Yes	108 (54.3%)
No	88 (44.2%)
Unknown	3 (1.5%)
Significant family history, No. (%)	
Yes	46 (23.1%)
No	153 (76.9%)
Calender year of TNBC diagnosis, No. (%)	
2010	13 (6.5%)
2009	46 (23.1%)
2008	42 (21.1%)
2007	44 (22.1%)
2006	39 (19.6%)
2005	14 (7.1%)
2004	1 (0.5%)

Abbreviations: BC, breast cancer; TNBC, triple-negative breast cancer.
[a] The first BC diagnosis is the subject's first ever diagnosis of any BC (not necessarily TNBC). A total of 9 patients had an incidence of BC (either invasive BC or ductal carcinoma in situ) prior to their most recent diagnosis of BC. The most recent diagnosis was classified as triple negative, and therefore they were considered evaluable for the study.
[b] In computing percentages, the number of missing values is not included in the denominator, as it would cause a downward bias in the percentages.
[c] Categories represented by this subrow grouping are not exclusive, so the counts will add up to more than the total, and percentages will add up to >100%.

at <50 years, and 6 mutations (3 *BRCA1* and 3 *BRCA2*) were identified among the remaining 108 patients diagnosed at ≥50 years (Table 4).

Testing History of Cohort

Sixty-two (31. 2%) patients had previous *BRCA* testing (Table 4), and of them, 10 (16.1%) had mutations. Among the 137 patients (68.8%) who did not have previous *BRCA* testing, 11 (8%) mutations were identified. Four of the 21 deleterious mutations (19%) were identified among the 91 patients who would not have met old NCCN guidelines, and 2 of the 21 deleterious mutations (10%) were identified in the 45 patients who would have not met the recent NCCN guidelines recommending testing for women who present with triple-negative BC at <60 years of age.

Age at First Diagnosis of BC Versus Probability of Having a Mutation

Logistic regression modeling identified age at diagnosis of first BC and significant family history as statistically significant predictors of mutation status. The probability of carrying a *BRCA* mutation decreases with age of diagnosis of BC and is well documented. The probability of carrying a *BRCA* mutation versus age at diagnosis of first BC stratified by significant family history was modeled (Fig. 1). For the 9 patients who had a prior diagnosis of BC before their current triple-negative diagnosis, their age at their first BC diagnosis was used. Among the patients without a significant family history, the probability of being a mutation carrier is 5% for a diagnosis at age 50 years.

Table 3. Estimates of *BRCA* Mutation Prevalence

Family History Type/Parameters	Yes	No	Total, N = 199
Significant family history			
Patients, No.	46	153	199
BRCA			
Patients with *BRCA* mutations, No.	13	8	21
Estimate of *BRCA* mutation prevalence	28.3%	5.2%	10.6%
90% CI for *BRCA* mutation prevalence[a]	17.6%-41.1%	2.6%-9.2%	7.2%-14.8%
BRCA1			
Patients with *BRCA1* mutations, No.	10	3	13
Estimate of *BRCA1* mutation prevalence	21.7%	2.0%	6.5%
90% CI for *BRCA1* mutation prevalence[a]	12.3%-34.1%	0.5%-5.0%	3.9%-10.2%
BRCA2			
Patients with *BRCA2* mutations, No.	3	5	8
Estimate of *BRCA2* mutation prevalence	6.5%	3.3%	4.0%
90% CI for *BRCA2* mutation prevalence[a]	1.8%-16.0%	1.3%-6.8%	2.0%-7.1%
Family history of any breast/ovarian cancer			
Patients, No.[b]	108	88	199
BRCA			
Patients with *BRCA* mutations, No.	16	5	21
Estimate of *BRCA* mutation prevalence	14.8%	5.7%	10.6%
90% CI for BRCA mutation prevalence[a]	9.5%-21.6%	2.3%-11.6%	7.2%-14.8%
BRCA1			
Patients with *BRCA1* mutations, No.	11	2	13
Estimate of *BRCA1* mutation prevalence	10.2%	2.3%	6.5%
90% CI for *BRCA1* mutation prevalence[a]	5.8%-16.3%	0.4%-7.0%	3.9%-10.2%
BRCA2			
Patients with *BRCA2* mutations, No.	5	3	8
Estimate of *BRCA2* mutation prevalence	4.6%	3.4%	4.0%
90% CI for *BRCA2* mutation prevalence[a]	1.8%-9.5%	0.9%-8.6%	2.0%-7.1%

Abbreviation: CI, confidence interval.
[a] Exact 2-sided 90% CIs are presented for the prevalence estimate because the subject counts in some categories are small.
[b] Three patients with unknown family history are not included as having family history of any breast/ovarian cancer. They are, however, included in the Total column. There were no mutations found among these 3 patients.

Observed Versus Expected Mutations Based on Myriad Prevalence Tables

Myriad prevalence tables were derived from >100,000 women and have been previously published.[15,16] They provide robust estimates for expected mutation rate in given population subgroups based on family and personal history characteristics (including age at diagnosis of first BC). On the basis of prevalence table estimates, a mutation rate of 15.9% would be expected in women diagnosed at age <50 years with a significant family history, but the study observed a rate of 36% (Table 5). Among women diagnosed at age <50 years but without a significant family history, the expected rate is 4.7%, but the observed rate is 9.1%. In women diagnosed at age ≥50 years with a significant family history, the expected rate is 6.4%, but the observed rate is 19.0%. The only cohort where expected rate was close to observed (2.2% vs 2.3%) was in women diagnosed at age ≥50 years but without a significant family history. A general linear model analysis shows that the observed mutation rate among patients with triple-negative BC is significantly higher ($P = .0005$) than the expected rate among patients with BC unselected for pathology. Therefore, the diagnosis of triple-negative BC increased the mutation rate 2-fold to 3-fold in all subgroups except 1. Although the study was not powered for such hypothesis testing, this is the largest cohort of triple-negative BC patients analyzed to date.

DISCUSSION

Limitations of previously published studies evaluating the prevalence of *BRCA* mutations in triple-negative BC cohorts have been discussed earlier. This study provides new insights into and estimates of the frequency of triple-negative BC under the new stricter definition. Both *BRCA1* and *BRCA2* mutation prevalence are estimated along with stratification by family history status over an unselected triple-negative BC cohort.

Table 4. Mutation Prevalence by Diagnosis Age, NCCN Guideline Criteria, and Previous Testing

Subgroup Classification	Patients, N = 199, No. (%)	Number of Mutation Carriers, No.	Mutation Prevalence	
			Estimate	90% CI[a]
Age at TNBC diagnosis, years				
<50	86 (43.2%)	13	15.1%	9.2%-23.0%
≥50	113 (56.8%)	8	7.1%	3.6%-12.4%
Age at 1st BC diagnosis, years				
<50	91 (45.7%)	15	16.5%	10.4%-24.2%
≥50	108 (54.3%)	6	5.6%	2.5%-10.7%
Met old NCCN guidelines for HBOC testing				
Yes	108 (54.3%)	17	15.7%	10.3%-22.7%
No	91 (45.7%)	4	4.4%	1.5%-9.8%
Met new NCCN guidelines for HBOC testing				
Yes	154 (77.4%)	19	12.3%	8.2%-17.6%
No	45 (22.6%)	2	4.4%	0.8%-13.3%
Previously tested for sequence and/or rearrangement mutations[b]				
Yes[c]	62 (31.2%)	10	16.1%	9.0%-25.8%
No	137 (68.8%)	11	8.0%	4.6%-12.9%

Abbreviations: BC, breast cancer; CI, confidence interval; HBOC, hereditary breast and ovarian cancer; NCCN, National Comprehensive Cancer Network; TNBC, triple-negative breast cancer.

[a] Exact 2-sided 90% CIs are presented for the mutation prevalence estimate because the subject counts in some categories are small.

[b] Of the 10 mutations identified among the previously tested 62 patients, 8 were in *BRCA1* and 2 were in *BRCA2*. Of the 11 mutations in the previously untested 137 patients, 5 were in *BRCA1* and 6 were in *BRCA2*.

[c] Of the previously tested 62 patients, only 1 was previously tested for both sequence and rearrangement mutations. The remaining 61 patients were tested only for sequence mutations previously, and rearrangement mutation testing was conducted later as part of the study. Of the 10 mutation carriers identified, 1 was previously tested for sequence mutation only. When tested for rearrangement mutation later as part of the study, a rearrangement mutation in *BRCA1* was identified. Therefore, this rearrangement mutation would have actually been missed even with the subject's prior testing.

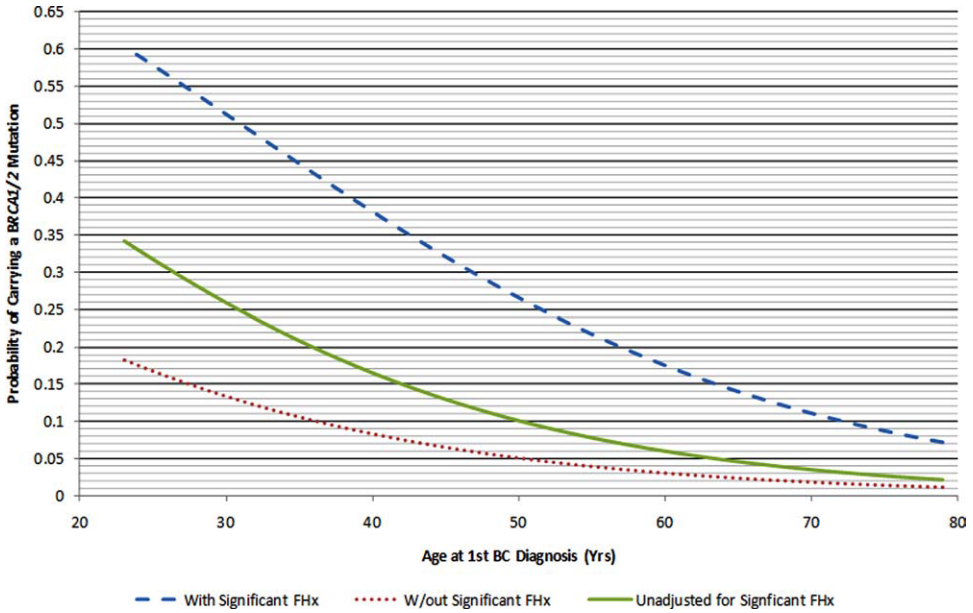

Figure 1. Probability of carrying a *BRCA* mutation by age at first breast cancer (BC) diagnosis is shown. FHx, family history.

Compared with other published studies, we observed a lower prevalence of *BRCA1* mutations in triple-negative BC. Several previous studies were done in predominantly Ashkenazi populations, demonstrating a range of prevalence from 23% to 34% for *BRCA1* and 0% to 9% for *BRCA2*. Two recent studies with Ashkenazi-

Table 5. Observed Versus Expected Mutation Prevalence

Subject History Classification	Family History Classification	Expected Mutation Prevalence per Myriad Prevalence Tables[a]	Observed Proportion of Mutation Carriers	Observed Mutation Prevalence		Test for Differences in Mutation Prevalence, Observed vs Expected, *P*[c]
				Estimate	90% CI[b]	
Age at 1st BC diagnosis, years	**Significant family history**					
≥50	No	2.2%	2/87	2.3%	0.4%-7.1%	.0005
	Yes	6.4%	4/21	19.0%	6.8%-38.4%	
<50	No	4.7%	6/66	9.1%	4.0%-17.2%	
	Yes	15.9%	9/25	36.0%	20.2%-54.4%	

Abbreviation: BC, breast cancer; CI, confidence interval.
[a] Percentages in this column are mutation prevalence estimates that would be expected per current Myriad prevalence tables based on Myriad's *BRCA* commercial test database.
[b] Exact 2-sided 90% CIs are presented for the mutation prevalence estimate because the subject counts in some categories are small.
[c] The *P* value is from a generalized linear model to compare observed prevalence among triple-negative BC patients with expected prevalence over unselected BC patients per Myriad prevalence tables.

predominant cohorts reported a *BRCA1* mutation prevalence range from 24% to 30%.[2,7] These data suggest that Ashkenazi patients with triple-negative BC are more enriched for *BRCA1* mutations than unselected cohorts. In addition, other published studies reporting a high *BRCA1* prevalence rate ascertained patients with triple-negative BC either presenting to a cancer genetics clinic or in an age-selected cohort. Atchley et al established their cohort from individuals presenting to a cancer genetics clinic for genetic testing and identified a 34% *BRCA1* prevalence rate.[5] Young et al ascertained 54 women with triple-negative BC at or before age 40 years with little or no family history of BC or ovarian cancer.[14] A mutation rate of 11% was observed with 5 deleterious *BRCA1* mutations.[14] A recent study ascertaining 77 patients with triple-negative BC for whom ethnicity and family history were unknown and who were referred to a tertiary center identified 11 *BRCA1* mutation, giving a prevalence of 14%.[17] Taking into account the low occurrence of significant family history and Ashkenazi heritage, and a higher than expected mean, our observed mutation rate of 10.6% is not inconsistent with the published literature.

Compared with previous studies, we identified a relatively high rate of *BRCA2* mutations (4%) in our cohort given the low incidence of significant family history and Ashkenazi prevalence. Comen et al observed a 9.2% prevalence of *BRCA2* carriers in a cohort of 65 Ashkenazi women with triple-negative BC.[7] Atchley et al identified 7 *BRCA2* carriers of 93 (7.5%) triple-negative BC patients ascertained through a high-risk clinic. The median age at diagnosis was 52 for *BRCA2* carriers versus 41.5 for *BRCA1* carriers.[5] Young et al identified 1 *BRCA2* mutation in their cohort of 54 women aged <40 years with triple-negative BC without family history.[14] The observation of only 1 *BRCA2* mutation carrier in this cohort fits with the hypothesis and described data that *BRCA2* carriers who develop triple-negative BC do so later in life compared with *BRCA1* carriers. *BRCA2* prevalence in triple-negative BC might be higher than expected in unselected cohorts and therefore testing, particularly in older patients, may be informative.

The American Society of Clinical Oncology and the College of American Pathologists published guidelines in 2010 for IHC testing of ER and PR in BC, recommending that ER and PR assays be considered positive if there are at least 1% positive tumor nuclei in the sample.[18] The adoption of this stricter criterion in our study likely decreased the number of patients who previously fell into the triple-negative phenotype when the previous studies using more liberal ER staining criteria (<5% or 10%) were conducted. As approximately 3280 patients were screened to identify 199 triple-negative cases, and 30 patients declined to participate, our observed incidence of triple-negative BC is 7.0%. Given that some of the enrolled patients were identified from an electronic database, we cannot report a true triple-negative prevalence rate, but estimate it to be closer to 10% with stricter ER and PR criteria, rather than the 15% to 20% previously reported in selected cohorts. A Swedish population-based cohort observed a 9% prevalence of basal BCs—a phenotype overlapping heavily with triple-negative BC—although mutation testing was not done, supporting the

idea that triple-negative BC incidence may be closer to 10% in unselected populations.[6]

The majority of the cohort was neither previously tested for *BRCA* mutations, nor would have met previous NCCN guidelines for testing. This study was designed, conducted, and analyzed in accordance with NCCN guidelines as recent as March 2011 (women with BC who were diagnosed up to age 45 years with or without triple-negative BC, or those diagnosed up to age 50 years with 1 close relative diagnosed with BC before age 50 years[19]). With these guidelines, 4 of the 21 mutations (19%) would not have been identified. New April 2011 guidelines state that patients with triple-negative BC <60 years of age should be considered for testing. With these guidelines, 2 of the 21 (10%) mutations would still not be identified. However, if the age limit was raised to <65 years, all of the 21 patients with mutations identified in this study would be eligible for testing.

This study had a relatively low enrollment of Ashkenazi patients (0.5% vs 2.2% US incidence) and a comparatively low number of patients with significant family history (23% vs 50% assumed for power). As such, lower prevalence of *BRCA* mutations was observed in terms of percentages.

The *BRCA* mutation prevalence observed among these triple-negative BC patients was almost twice of what would be expected based on a weighted analysis from Myriad's prevalence tables. These data suggest that the diagnosis of triple-negative BC increases the likelihood of a woman carrying a mutation irrespective of family history in women diagnosed at age <50 years and in women diagnosed at age ≥50 years with a family history.

A recent study demonstrated the cost-effectiveness of *BRCA* testing in patients with triple-negative BC in women <50 years of age.[20] The authors demonstrated that *BRCA* mutation testing for women with triple-negative BC who were younger than 50 years was cost-effective and could reduce subsequent BC and ovarian cancer risks by up to 40%.

Strengths of our study include the large sample size, the unselected and therefore unbiased cohort of patients with triple-negative BC, and enforced stringent criteria for ER and PR negativity. Furthermore, we conducted an independent pathological review for 50 cases to confirm triple-negative status, thus limiting a potential bias for underestimation for *BRCA* prevalence. In addition, this cohort included ethnically diverse patients, including African American and Hispanic patients, thereby allowing the data to be applied to various populations. Potential limitations of our study include an under-representation of Ashkenazi ethnicity, which may have caused a modest underestimation of the number of identified mutations, as well as the lack of information on basal subtype representation in the cohort. An additional limitation is the lack of information regarding the number of relatives the women without family history had in their family. There are data to suggest that predictive models break down when there are too few women in the family.

The results were surprising because of an overall lower prevalence of *BRCA* mutations than expected based on previous studies, including a lower *BRCA1* prevalence, but higher than expected *BRCA2* prevalence. The majority of our cohort was comprised of patients who did not have a significant family history of BC or ovarian cancer, providing us with sound data regarding the true prevalence of *BRCA* mutations in patients with triple-negative BC and no family history of BC or ovarian cancer. Our data point to a roughly 4% to 5% risk of carrying a mutation if an individual is diagnosed with triple-negative BC and does not have any family history of BC or ovarian cancer.

Further planned studies include assessing the prevalence of other mutations in double-strand break repair pathways, including *PALB2* and somatic *BRCA* mutations in triple-negative BC. These additional analyses will offer more insight into the underlying genetic defects that may drive the development of triple-negative BC, and may allow these patients to receive more personalized therapy for their disease and prevention of potential new cancers they may develop in the future.

FUNDING SOURCES

This study was funded by Myriad Genetic Laboratories, Inc.

CONFLICT OF INTEREST DISCLOSURES

Financial Disclosure: A.-R.H., R.R.K., C.E.G., S.A.H., C.A.F., and R.J.W. are employees of, and receive stock options from, Myriad Genetic Laboratories, Inc.

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