Characteristics of Triple-Negative Breast Cancer in Patients With a *BRCA1* Mutation: Results From a Population-Based Study of Young Women

Eunjung Lee, Roberta McKean-Cowdin, Huiyan Ma, Darcy V. Spicer, David Van Den Berg, Leslie Bernstein, and Giske Ursin

Eunjung Lee, Roberta McKean-Cowdin, Darcy V. Spicer, David Van Den Berg, and Giske Ursin, Keck School of Medicine, University of Southern California, Los Angeles; Huiyan Ma and Leslie Bernstein, City of Hope Comprehensive Cancer Center and Beckman Research Institute, Duarte, CA; and Giske Ursin, University of Oslo; Cancer Registry of Norway, Oslo, Norway.

Submitted November 16, 2010; accepted July 18, 2011; published online ahead of print at www.jco.org on October 17, 2011.

Supported by Grants No. CA17054 and CA74847 from the National Cancer Institute, National Institutes of Health (NIH), No. 4PB-0092 from the California Breast Cancer Research Program of the University of California, and in part through Contracts No. N01-PC-35139 and T32 ES-013678 with the National Institute of Environmental Health Sciences, NIH. The collection of cancer incidence data used in this publication was supported by the California Department of Health Services as part of the statewide cancer reporting program mandated by California Health and Safety Code Section 103885.

The ideas and opinions expressed herein are those of the authors, and no endorsement by the State of California, Department of Health Services, is intended or should be inferred.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Corresponding author: Giske Ursin, MD, PhD, University of Southern California/Norris Comprehensive Cancer Center, 1441 Eastlake Ave, Room 4407, Los Angeles, CA 90089; e-mail: gursin@usc.edu.

© 2011 by American Society of Clinical Oncology

0732-183X/11/2933-4373/\$20.00

DOI: 10.1200/JCO.2010.33.6446

ABSTRACT

Purpose

Triple-negative breast cancers (TNBCs) are tumors with low or no expression of estrogen receptor, progesterone receptor, or human epidermal growth factor receptor 2. These tumors have a poor prognosis, remain a clinical challenge, and are more common among women with *BRCA1* mutations. We tested whether there are distinguishing features of TNBC after *BRCA1* mutation status has been taken into account.

Patients and Methods

We sequenced *BRCA1* and *BRCA2* genes in a population-based sample of 1,469 patients with incident breast cancer age 20 to 49 years from Los Angeles County (California). Information on tumor receptor status was available for 1,167 women. Clinical, pathologic, and hormone-related lifestyle characteristics were compared across patient subgroups defined by *BRCA1* mutation status and triple-negative receptor status.

Results

Forty-eight percent of *BRCA1* mutation carriers had TNBC compared with only 12% of noncarriers. Within *BRCA1* mutation carriers, as well as within noncarriers, triple-negative receptor status was associated with younger age at diagnosis and higher tumor grade. Among women without a *BRCA1* mutation, we observed that women with TNBC had higher premenopausal body mass index and earlier age at first full-term pregnancy than those with non-TNBC. Age at menarche and other reproductive factors were not associated with triple-negative status regardless of *BRCA1* mutation status. Within *BRCA1* mutation carriers, Ashkenazi Jewish women were about five times more likely to have TNBC than non-Ashkenazi Jewish women.

Conclusion

Our results suggest that among *BRCA1* mutation carriers, as among noncarriers, there are unique characteristics associated with the triple-negative subtype. The findings in Ashkenazi Jewish *BRCA1* mutation carriers should be confirmed.

J Clin Oncol 29:4373-4380. © 2011 by American Society of Clinical Oncology

INTRODUCTION

Modern breast cancer treatment includes targeted therapies for estrogen receptor (ER)-positive breast cancers (tamoxifen and aromatase inhibitors) and for tumors that overexpress human epidermal growth factor receptor 2 (HER2; trastuzumab).^{1,2} However, roughly 10% to 20% of breast cancers are triple-negative, that is, they have low or no expression of ER, progesterone receptor (PR), and HER2.^{3,4} Women diagnosed with triple-negative breast cancer (TNBC) have poorer prognosis than those diagnosed with other breast cancer subtypes.^{4,5} The lack of an established receptor target reduces the therapeutic op-

tions and remains a critical challenge in treating patients with TNBC.⁶

TNBCs are associated with distinct clinical and pathologic characteristics such as younger age at diagnosis and higher grade.⁷ Available evidence suggests that risk factors implicated in breast cancer etiology may differ between patients with TNBC and those with another subtype. For example, parity, age at first birth, and obesity in younger women were not associated with TNBC risk in a large collaborative study.⁸ The findings for parity and age at first birth were replicated in another collaborative study of women undergoing screening mammograms,⁹ as well as in a study of postmenopausal women enrolled in the Women's Health Initiative

Fig 1. Description of participants of the Women's Learning the Influence of Family and Environment (LIFE) study who were included in the current analyses.

(WHI),¹⁰ which also showed that age at menarche is not associated with TNBC risk. However, in the large collaborative study, earlier age at menarche was a TNBC risk factor.⁸ Similarly, studies of oral contraceptive use have mixed results, but the numbers in these studies were relatively small.¹⁰⁻¹³

Patients with breast cancer who have a germline mutation in *BRCA1* are more likely to have a family history of breast or ovarian cancer¹⁴ and an earlier age at diagnosis than those without *BRCA1* mutations.^{15,16} *BRCA1*-related breast cancers are more likely to be negative for ER, PR, and HER2,¹⁶⁻¹⁸ and to have high-grade tumor.¹⁹⁻²⁴

Nevertheless, it is notable that not all *BRCA1*-related breast cancers are triple-negative. In general, the characteristics of TNBC in patients with breast cancer are well known, but an interesting question remains: Do *BRCA1* mutation carriers with TNBC have pathologic and etiologic characteristics different from those of carriers with other subtypes? A study using cases from a genetic counseling program²⁵ reported that, among *BRCA1* mutation carriers, TNBC occurred in 57% of patients with breast cancer and was associated with higher nuclear grade and earlier age at menarche. To the best of our knowledge, no population-based studies have addressed this question. We studied the characteristics and etiologic risk factors for TNBC among *BRCA1* mutation carriers and noncarriers in our large population-based study of early-onset breast cancer.

PATIENTS AND METHODS

Patients

This study used data from young patients with breast cancer collected in the Women's Learning the Influence of Family and Environment (LIFE)

Study.²⁶ We identified female patients with histologically confirmed first primary invasive breast cancer through the Los Angeles County Cancer Surveillance Program (LACCS), a population-based registry sponsored by the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) program. Eligibility criteria included the following: US-born and English speaking, white (including Hispanic) or African-American, age 20 to 49 years at diagnosis, no prior in situ or invasive breast cancer diagnosis, and resident of Los Angeles County at diagnosis. We identified 2,882 potentially eligible patients (2,534 whites and 348 African-Americans). Recruitment of African-Americans began after the initiation of the study. White patients were diagnosed between February 1998 and May 2003, and African-American patients were diagnosed between January 2000 and May 2003. Interviews were conducted with 62% (1,794: 1,585 white, 209 African-American) of the potentially eligible patients. Reasons for nonparticipation are shown in Figure 1.

Data and Blood Specimen Collection

All LIFE participants were interviewed in person by using a validated structured questionnaire that obtained information on family history and lifestyle factors (information up to the date of diagnosis of the patient's invasive breast cancer). All participants were interviewed within 18 months of diagnosis; 90% of the participants were interviewed within 1 year of diagnosis, with approximately 60% interviewed within 6 months. Blood specimens were collected from 1,519 patients (85%) around the time of the interview. The study was approved by the institutional review board of the University of Southern California (USC). All participants provided written informed consent.

Sequencing of *BRCA1* Gene

The detailed procedures for *BRCA1* sequencing were previously reported.²⁷ Briefly, we sequenced all *BRCA1* exons (except exon 1 located upstream of the translation start site and exon 4 not found in normal *BRCA1* mRNA transcript) and all exon-intron boundaries in the USC Genomics Core Laboratory. We successfully sequenced *BRCA1* genes for 1,469 of 1,519 blood specimens. We considered women with a truncating mutation in the *BRCA1* gene and women with *BRCA1* M1775R and A1708E as the mutation carriers,

as reported previously.²⁸⁻³¹ The detected *BRCA1* mutations are listed in Appendix Table A1 (online only).

Pathology Data Collection

We obtained pathology data, including ER, PR, and HER2 status, from surgical pathology reports collected through LACCSP. We defined TNBC as ER-negative, PR-negative, and HER2-negative as reported by the local laboratories. We did not have information on the definition of ER and PR positivity used in each laboratory. However, in the subset of patients (approximately 500) in whom percentage positive was additionally reported, the cutoff point for receptor positivity was 10% for the majority of patients (more than 95%). HER2 status was categorized as negative (low/weak/negative/1+), intermediate (moderate/borderline/equivocal/2+), or positive (high/strong/positive/3+). Intermediate HER2/neu was considered noninformative. We could classify the TNBC status of 1,196 of the 1,469 patients screened for *BRCA1*. Among the 1,196 patients, 29 had previously been identified as carrying mutations in *BRCA2* and were excluded from this study. Only one *BRCA2* mutation carrier had TNBC.²⁷ Inclusion of these *BRCA2* mutation carriers did not change the results.

Statistical Analyses

When performing a univariate comparison of demographic, clinical, and pathologic characteristics according to *BRCA1* mutation and/or triple-negative status, we used Fisher's exact test, exact Cochran-Armitage trend test, and Wilcoxon rank sum test for categorical, ordinal, and continuous variables, respectively. Analyses of body mass index (BMI) were limited to premenopausal women since our study participants are mostly premenopausal (80%) and the effect of BMI on breast cancer risk is known to differ according to menopausal status.³² Higher BMI in premenopausal women is associated with lower breast cancer risk whereas higher BMI in postmenopausal women is associated with higher breast cancer risk.³² Analysis of covariance models were used when comparing continuous variables adjusting for potential confounders. We used unconditional logistic regression to examine the association of clinical, pathologic, and lifestyle characteristics (exposures) with *BRCA1* mutation status or triple-negative status (outcomes). Potential confounders were included in the logistic models as needed (see footnotes for Tables 2, 3, and 4). Further adjustment for Ashkenazi Jewish origin did not change the results. For the TNM stage comparison, we fit an ordinal variable of stage (I, II, and III to IV) and estimated the relative risks (odds ratios) of one category increase in stage (ie, II v I, or III to IV v II) by *BRCA1* mutation status or triple-negative status. All reported *P* values are two-sided. The SAS 9.2 (SAS Institute, Cary, NC) package was used for all analyses.

RESULTS

Characteristics of Patients With Breast Cancer Who Carry *BRCA1* Germline Mutations

About half (48%) of the *BRCA1* mutation carriers had TNBC, whereas only 12% of the women without *BRCA1* mutation had TNBC (Table 1). *BRCA1* mutation carriers were younger, more likely to have a family history of breast or ovarian cancer, and be of Ashkenazi Jewish origin than noncarriers of *BRCA1* mutations, as we previously described.²⁸ The *BRCA1* mutation carriers had higher-grade tumors than noncarriers. A similar trend was observed for stage at diagnosis, but it was not statistically significant. Premenopausal women with *BRCA1* mutations had higher BMI than noncarriers. The *BRCA1* mutation carriers had a higher proportion of medullary carcinoma than mutation noncarriers (data not shown). The proportion of *BRCA1* mutation carriers was higher among patients with TNBC than among patients without TNBC, regardless of family history (Appendix Table A2, online only). Among patients with TNBC younger than age 40, 8% carried a *BRCA1* mutation in the absence of first- or second-degree family history of breast or ovarian cancer.

Table 1. Demographic and Pathology Characteristics Based on *BRCA1* Mutation Status Among Women With Known Triple-Negative Receptor Status

Characteristic	BRCA1 Mutation				P
	Carriers (n = 46)		Noncarriers (n = 1,121)		
	No.	%	No.	%	
Receptor status					< .001*
Triple negative	22	47.8	134	12.0	
Not triple negative	24	52.2	987	88.1	
Family history of breast or ovarian cancer					< .001*
None	9	20.5	603	55.6	
First degree	26	59.1	190	17.5	
Second degree	9	20.5	292	26.9	
Age at diagnosis, years					< .001†
Mean ± SD	39.3 ± 7.0		43.0 ± 5.1		
Median	39.5		44		
Range	24-49		23-49		
Race					.42*
White	44	95.7	1025	91.4	
African-American	2	4.4	96	8.6	
Hispanic‡					.83*
Non-Hispanic	39	84.8	965	86.1	
Hispanic	7	15.2	156	13.9	
Ashkenazi Jewish					.002*
Yes	13	28.3	131	11.7	
No	33	71.7	990	88.3	
Grade					< .001*
1-2	9	20.5	596	56.1	
3	35	79.6	467	43.9	
Stage					.11§
I	14	32.6	428	40.2	
II	21	48.8	536	50.4	
III-IV	8	18.6	100	9.4	
BMI (premenopausal women)	n = 40		n = 897		.037¶
Mean ± SD	27.1 ± 6.6		25.6 ± 5.9		
Median	25.4		24.1		
Range	18.9-42.5		16.1-71.7		

Abbreviations: BMI, body mass index; SD, standard deviation.

*Based on Fisher's exact test.

†Based on Wilcoxon rank sum test.

‡All Hispanic women were white except for one African-American woman who was of Hispanic origin. This patient did not carry *BRCA1* mutation. When restricting the analysis to white women, the proportion of Hispanic women remained similar in *BRCA1* mutation carriers (16%) and noncarriers (15%).

§Based on exact Cochran-Armitage trend test.

¶Based on *F* test using analysis of covariance model adjusted for age (< 35, 35 to < 40, 40 to < 45, 45 to < 50 years) and race (white, African-American); exclusion of two patients with BMI > 54.9 strengthened the difference by *BRCA1* mutation status.

Characteristics of Patients With TNBC

Patients with TNBC were younger at diagnosis than patients with non-TNBC (Table 2). TNBC tumors were about 10 times more likely to be high grade than non-TNBC tumors. Women with TNBC tended to be diagnosed at higher stage than those with non-TNBC. These associations remained similar when we further adjusted for *BRCA1* mutation status (data not shown).

Clinical and Pathologic Characteristics of TNBC Within Subgroups Defined by *BRCA1* Mutation Status

Within *BRCA1* mutation carriers, patients with TNBC were younger at diagnosis and had higher-grade and higher-stage tumors

Table 2. Clinical and Pathology Characteristics by TNBC Status

Characteristic	Non-TNBC (n = 1,011)			TNBC (n = 156)			95% CI	<i>P</i> ^b
	No.	%	OR ^a	No.	%	OR ^a		
Age, years								
Median	44			42				< .001
Range	23-49			24-49				
Mean ± SE, adjusted ^c	43.4 ± 0.34			41.1 ± 0.49				< .001
First-degree family history			1 (Ref)			1.14 ^d	0.74 to 1.77	.74
No	794	81		119	80			
Yes	186	19		30	20			
Race								.014
White	934	92		135	86			
African-American	77	8		21	13			
Ashkenazi (among whites) ^e								.96
No	808	87		117	87			
Yes	126	13		18	13			
Grade			1 (Ref)			10.6 ^f	6.36 to 17.8	< .001
1	161	17		2	1			
2	426	44		16	11			
3	370	39		132	88			
TNM stage			1 (Ref)			1.53 ^g	1.08 to 2.16	.008
I	400	42		42	29			
II	473	49		84	58			
III-IV	90	9		18	13			

NOTE. Totals differ because of missing values. The results remained similar when additionally adjusted for *BRCA1* mutation status.

Abbreviations: OR, odds ratio; Ref, reference; TNBC, triple-negative breast cancer.

^aOR of having each clinical/pathologic characteristic associated with TNBC status.

^bFor comparison of continuous variables (age), *P* values are based on Wilcoxon rank sum test for crude analyses and analysis of covariance model for adjusted comparisons. For categorical variables, *P* values are based on χ^2 tests, except for race comparison among the mutation carriers for whom Fisher's exact test was used. For comparison of grade and TNM stage, *P* values are based on exact Cochran-Armitage trend test.

^cAdjusted for race (white, African-American) and family history of breast or ovarian cancer (none, first degree, second degree, unknown).

^dORs and 95% CIs of having a first-degree family history of breast or ovarian cancer (v not having first-degree family history) were estimated using logistic regression, adjusted for age (< 35, 35 to < 40, 40 to < 45, and 45 to < 50 years).

^eThe results did not change when including African-Americans (and adjusted for race).

^fORs and 95% CIs of being high grade (grade 3 compared with grade 1 or 2) were estimated using logistic regression, adjusted for age (< 35, 35 to < 40, 40 to < 45, and 45 to < 50 years).

^gORs and 95% CIs of one category increase in stage (ie, II v I, or III-IV v II) were estimated using proportional odds model, adjusted for age (< 35, 35 to < 40, 40 to < 45, and 45 to < 50 years) and race (white, African-American). Further adjustment for family history of breast or ovarian cancer and education did not change the results.

than patients with non-TNBC (Table 3). Among *BRCA1* mutation carriers, 43% of white patients with TNBC were Ashkenazi Jewish women, but only 17% of white patients with non-TNBC were Ashkenazi Jewish women. In other words, among *BRCA1* mutation carriers, Ashkenazi Jewish women were more likely to have TNBC (nine of 13; 69%) compared with women in other ethnic or religious groups (12 of 31; 39%; age-adjusted odds ratio, 6.38; *P* = .044).

We calculated TNBC proportion in 14 women who carried 185delAG (in exon 2), the most frequently observed mutation and a founder mutation in the Ashkenazi Jewish population. Fifty-seven percent (*n* = 8; seven were Ashkenazi Jewish) of the 14 women with 185delAG had TNBC, whereas 44% (*n* = 14) of the 32 women with other *BRCA1* mutations had TNBC (data not shown). Three of seven women (43%) with 5382InsC (in exon 20), another Ashkenazi Jewish founder mutation, had TNBC.

Among noncarriers of *BRCA1* mutation, similar characteristics of TNBC were observed: patients with TNBC were younger at diagnosis and had high-grade and high-stage tumors. However, among noncarriers of *BRCA1* mutation, Ashkenazi Jewish women had a proportion of TNBC (8%) similar to that of other ethnic or religious groups (13%).

Within noncarriers of *BRCA1* mutation, patients with TNBC were more likely to be African-American. The small number of African-American women carrying a *BRCA1* mutation prohibited the same comparison within *BRCA1* mutation carriers.

Etiologic Reproductive and Hormone-Related Factors Associated With TNBC Within Subgroups Defined by BRCA1 Mutation Status

Within noncarriers of *BRCA1* mutation, TNBC patients had earlier age at first full-term pregnancy and higher premenopausal BMI than those with other cancer subtypes (Table 4). We did not find any differences in age at menarche, oral contraceptive use, parity, or breastfeeding duration (data not shown) by triple-negative status within noncarriers. No association was observed among *BRCA1* mutation carriers for any reproductive or hormone-related factors, although we had limited sample sizes for many of these comparisons.

DISCUSSION

This study is based on a population-based sample of patients with breast cancer recruited for a case-control study who were extensively

Table 3. Clinical and Pathology Characteristics According to TNBC and *BRCA1* Mutation Status

Characteristic	BRCA1 Mutation																	
	Carriers								Noncarriers									
	Non-TNBC (n = 24)				TNBC (n = 22)				Non-TNBC (n = 987)				TNBC (n = 134)					
	No.	%	OR		No.	%	OR ^a	95% CI	P ^b	No.	%	OR		No.	%	OR ^a	95% CI	P ^b
Age, years																		
Median		44.5				38			.053		44				42			.001
Range		26-49				24-48					23 to 49				26-49			
Adjusted mean ± SE ^c		39.0 ± 2.95				36.5 ± 3.14			.25		43.5 ± 0.34				41.8 ± 0.51			< .001
First-degree family history				1 (Ref)			0.69	0.16 to 2.92 ^d	> .99			1 (Ref)				0.75 ^d	0.44 to 1.29	.19
No	9	41			9	41				785	82		110	87				
Yes	13	59			13	59				173	18		17	13				
Race									> .99								.005	
White	23	96			21	95				911	92		114		85			
African-American	1	4			1	5				76	8		20		15			
Ashkenazi (among whites) ^e				1 (Ref)			6.28 ^f	1.05 to 37.6	.044			1 (Ref)				0.59 ^f	0.29 to 1.20	.10
No	19	83			12	57				789	87		105	92				
Yes	4	17			9	43			.064	122	13		9	8				
Grade				1 (Ref)			6.31	0.62 to 63.9 ^g	.018			1 (Ref)				10.2 ^g	6.00 to 17.3	< .001
1	2	9			0	0				159	17		2	2				
2	6	26			1	5				420	45		15	12				
3	15	65			20	95				355	38		112	87				
TNM stage				1 (Ref)			1.21	0.33 to 4.45 ^h	.52			1 (Ref)				1.47 ^h	1.02 to 2.13	.028
I	9	41			5	24				391	42		37	30				
II	9	41			12	57				464	49		72	59				
III-IV	4	18			4	19				86	9		14	11				

NOTE. Totals differ because of missing values.

Abbreviations: OR, odds ratio; Ref, reference; TNBC, triple-negative breast cancer.

^aOR of having each clinical/pathologic characteristic associated with TNBC status.^bFor comparison of continuous variables (age), *P* values are based on Wilcoxon rank sum test for crude analyses and analysis of covariance model for adjusted comparisons. For categorical variables, *P* value are based on χ^2 tests, except for race comparison among the mutation carriers for whom Fisher's exact test was used. For comparison of grade and TNM stage, *P* values are based on exact Cochran-Armitage trend test.^cAdjusted for race (white, African-American) and family history of breast or ovarian cancer (none, first degree, second degree, unknown).^dORs and 95% CIs of having a first-degree family history of breast or ovarian cancer (v not having first-degree family history) were estimated by using logistic regression, adjusted for age (< 35, 35 to < 40, 40 to < 45, and 45 to < 50 years).^eThe results did not change when including African-Americans (and further adjusted for race).^fORs and 95% CIs of being Ashkenazi Jewish were estimated using logistic regression, adjusted for age (< 35, 35 to < 40, 40 to < 45, and 45 to < 50 years).^gORs and 95% CIs of being high grade (grade 3 compared with grade 1 or 2) were estimated using logistic regression, adjusted for age (< 35, 35 to < 40, 40 to < 45, and 45 to < 50 years).^hORs and 95% CIs of one category increase in stage (ie, II v I, or III-IV v II) were estimated using a proportional odds model, adjusted for age (< 35, 35 to < 40, 40 to < 45, and 45 to < 50 years) and race (white, African-American). Further adjustment for family history of breast or ovarian cancer and education did not change the results.

screened for *BRCA1* mutations through direct screening and who provided breast cancer risk factor information at recruitment.

As expected, we found that *BRCA1* mutation carriers were more likely to have TNBC than noncarriers. Furthermore, TNBC was diagnosed at a younger age and with higher tumor grade and stage than non-TNBCs among both *BRCA1* mutation carriers and noncarriers. However, several TNBC characteristics differed between *BRCA1* mutation carriers and noncarriers. Among *BRCA1* mutation carriers, women with TNBC were more likely to be Ashkenazi Jewish than women with non-TNBC. Among the noncarriers, women with TNBC tended to have younger age at first full-term pregnancy and higher premenopausal BMI.

Recently, a genetic counseling center–based study of women of all ages reported that 57% of patients with breast cancer who had *BRCA1* mutations had TNBC.²⁵ Our population-based study of early-onset breast cancer confirms a high proportion (approximately 50%) of TNBC among *BRCA1* carriers. This proportion is consistent with

the proportions in previous studies. Studies of *BRCA1* mutation carriers in diverse ethnic groups,^{18,33-38} including studies in Ashkenazi Jewish women,^{36,39,40} have found that tumors from *BRCA1* mutation carriers are more likely to be negative for ER, PR, and HER2 (although not examined as a combined triple-negative category) than tumors among noncarriers of *BRCA1* mutation. In addition, we observed that TNBC status and younger age at diagnosis appeared to predict *BRCA1* mutation status independently of family history (Appendix Table A2). Basal-like breast cancer makes up 66% to 91% of TNBC.⁴¹⁻⁴³ Therefore, our results are consistent with those of another population-based sample of 29 patients with breast cancer who had a *BRCA1* mutation, which found that morphologic characteristics associated with basal-like breast cancers²² were predictors of *BRCA1* mutation independently of family history.⁴⁴

Our data confirm that TNBCs are diagnosed at a younger age and at a higher tumor grade and stage than non-TNBCs, as is widely

Table 4. Lifestyle Characteristics According to TNBC and *BRCA1* Mutation Status

Characteristic	<i>BRCA1</i> Mutation													
	Carriers							Noncarriers						
	Non-TNBC (n = 24)			TNBC (n = 22)			<i>P</i> ^b	Non-TNBC (n = 987)			TNBC (n = 134)			<i>P</i> ^b
	No.	%	OR	No.	%	OR ^a		No.	%	OR	No.	%	OR ^a	95% CI
Menarche														
Median	12			12			.21	12			12			.70
Range	9-16			10-15				8-18			9-17			
Adjusted mean ± SE ^c	12.1 ± 0.61			12.3 ± 0.63			.70	12.3 ± 0.088			12.2 ± 0.14			.56
Oral contraceptive use						N/A	.89			1 (Ref)			1.09 ^d	0.64 to 1.85
Never	7	29		6	27			140	14		18	13		
Ever	17	71		16	73			847	86		116	87		
Parity ^e						N/A	.63			1 (Ref)			1.36 ^f	0.81 to 2.28
Never pregnant	8	35		8	42			172	21		21	18		
Ever had a full-term pregnancy	15	65		11	58			656	79		98	82		
Limited to women who had a full-term pregnancy														
Number of full-term pregnancies														
Median	2			2			.93	2			2			.66
Range	1-4			1-4				1-7			1-5			
Adjusted mean ± SE ^c	3.2 ± 0.8			2.9 ± 0.7			.51	1.8 ± 0.08			1.9 ± 0.1			.41
Age at first full-term pregnancy, years														
Median	28			24			.57	27			25			.002
Range	19-34			16-36				14-44			16-41			
Adjusted mean ± SE ^g	21.4 ± 2.61			21.3 ± 2.39			.94	23.9 ± 0.42			22.3 ± 0.63			.011
Limited to premenopausal women														
BMI	n = 19			n = 21			.11	n = 794			n = 104			.008
Median	28.3			23.4				24.0			25.9			
Range	18.9-41.6			19.1-42.5				16.1-71.7			17.8-50.5			
Adjusted mean ± SE ^c	29.3 ± 2.4			28.4 ± 2.4			.64	26.3 ± 0.4			28.3 ± 0.6			.002

NOTE. Numbers do not add up to total because of missing values.

Abbreviations: BMI, body mass index; N/A, not applicable; OR, odds ratio; TNBC, triple-negative breast cancer.

^aOR of having each clinical/pathologic characteristic associated with TNBC status.

^bFor continuous or ordinal variables, *P* values are based on Wilcoxon rank sum test for crude analyses and analysis of covariance for adjusted comparisons. For categorical variables, *P* values are based on χ^2 tests.

^cAdjusted for age (< 35, 35 to < 40, 40 to < 45, and 45 to < 50 years) and race (white, African-American).

^dORs and 95% CIs were estimated using logistic regression, adjusted for age (< 35, 35 to < 40, 40 to < 45, and 45 to < 50 years) and race (white, African-American). Further adjustment of family history of breast or ovarian cancer (none, first degree, second degree, unknown) and full-term pregnancy (ever, never) did not change the results.

^eExcluding women who had a non-full-term pregnancy.

^fORs and 95% CIs were estimated by using polytomous logistic regression, adjusted for age (< 35, 35 to < 40, 40 to < 45, and 45 to < 50 years) and race (white, African-American). Reference category of dependent variable (parity) is "never full-term pregnancy."

^gAdjusted for age (< 35, 35 to < 40, 40 to < 45, and 45 to < 50 years), race (white, African-American), and number of full-term pregnancies.

recognized.⁴ Interestingly, we observed this among noncarriers of *BRCA1* mutation as well as among carriers of *BRCA1* mutation, suggesting that the characteristics of TNBCs are unlikely to be a mere consequence of a higher proportion of *BRCA1* mutation carriers in TNBC. The differences in age at diagnosis by TNBC and *BRCA1* mutation status may have been underestimated, since our study was limited to women younger than age 50.

Our result that Ashkenazi Jewish women with *BRCA1* mutations were more likely to have TNBC (69%) than *BRCA1* mutation carriers in other ethnic and religious groups (39%) is unique; this association was statistically significant after adjusting for age at diagnosis (*P* = .044). Conversely, among noncarriers of *BRCA1* mutation, Ashkenazi Jewish women were, if anything, less likely to have TNBC. Although our findings may be due to chance, given the small number

of *BRCA1* mutation carriers of Ashkenazi Jewish origin (total *n* = 13), alternative explanations are possible. The *BRCA1* mutations in Ashkenazi Jewish women were predominantly the founder mutations: 185delAG (*n* = 10) and 5382InsC (*n* = 2). These two mutations are located at opposite ends of the gene, exon 2 and exon 20, respectively. In contrast, *BRCA1* mutations in women who were not Ashkenazi Jewish were spread throughout the gene (Appendix Table A1). It has been speculated that each specific *BRCA1* mutation might be associated with different cancer risks.⁴⁵ Although data demonstrating allelic heterogeneity in risk are scarce for *BRCA1*, some evidence suggests that mutations in the ovarian cancer cluster region of *BRCA2* are associated with a greater risk increase for ovarian cancer than for breast cancer.⁴⁶ The risk increase for breast cancer among families carrying 185delAG was lower than that for families carrying other

BRCA1 mutations among women in the age range included in our study (mostly age 30 to 50 years).⁴⁷ Similarly, the founder *BRCA1* mutations in Ashkenazi Jewish women might be associated with greater risk for TNBCs than other *BRCA1* mutations. Alternatively, unique lifestyle characteristics of Ashkenazi Jewish women might have interacted with *BRCA1* mutations to increase risk of TNBC. According to recent data from a hospital-based prevalent cohort of Ashkenazi Jewish patients with breast cancer unselected for family history,⁴⁸ 70% of Ashkenazi Jewish patients who were carrying *BRCA1* founder mutations had TNBC. In another study of *BRCA1* mutation carriers identified from high-risk genetic programs,⁴⁹ the proportion of ER-negative breast cancer was similar between patients of Ashkenazi Jewish origin and patients in other ethnic groups. However, this study of a select patient group may not be directly comparable with our population-based study of *BRCA1*-associated patients with TNBC.

We observed no difference in age at menarche by TNBC status among *BRCA1* mutation carriers and noncarriers, findings consistent with some,^{8,11,50} but not all,^{10,25,51-53} previous studies.

High BMI has been associated with reduced risk of breast cancer in premenopausal women.³² However, our observation (among non-carriers of *BRCA1*) that women with TNBC had higher BMI than other patients, suggests that high BMI is not as protective against TNBC as has been observed in other subtypes of breast cancer. This observation is consistent with results from a pooled analysis⁸ and most studies in the general population.^{11,51-53} In contrast, among *BRCA1* mutation carriers, women with TNBC appeared to have lower BMI than other patients. This nonstatistically significant finding needs to be confirmed in larger studies.

Patients with TNBC have a poorer prognosis than patients with other breast cancer subtypes.^{4,5} Several novel therapeutic agents are under investigation to treat TNBC, including inhibitors of poly-ADP-ribose-polymerase-1 (PARP), epidermal growth factor receptor, multityrosine kinase, or angiogenesis (reviewed in Gluz et al⁵⁴). PARP inhibitors impair DNA single-strand break repair and would particularly benefit patients with breast cancer who have mutations (or other deficits) in *BRCA1* or *BRCA2*.⁵⁵ These novel directions in breast cancer treatment highlight the importance of our finding that *BRCA1* mutation status and TNBC status jointly define a smaller subgroup of patients with breast cancer and may implicate more optimized therapeutic options in the future.

One limitation of our study is that receptor status was obtained through reports from outside pathology laboratories. HER2 status was typically derived from immunohistochemistry rather than from the more accurate fluorescent in situ hybridization method.⁵⁶ Nevertheless, immunohistochemistry and fluorescent in situ hybridization

results are highly concordant.^{56,57} High concordance has been also demonstrated between ER-negative/PR-negative and ER-positive/PR-positive status data obtained from outside pathology laboratories and that measured in an expert laboratory.⁵⁸ In addition, any error in receptor status classification is unlikely to differ by *BRCA1* status or demographic or clinical characteristics; any classification error would most likely bias results toward the null. Therefore, our findings that age at diagnosis and grade differed by triple-negative status are unlikely to be due to misclassification. Another limitation is that we were unable to perform breast cancer subtyping (ie, assessing basal-like phenotype), which may provide further insight on our observations.

The strengths of our study are that we sampled population-based patients with breast cancer and sequenced all translated exons of the *BRCA1* gene to search for mutations, which provides a unique opportunity to avoid referral bias. We interviewed participants within 18 months of their breast cancer diagnosis (90% interviewed within 1 year of diagnosis), minimizing the possibility of survival bias that would occur if patient prognosis differs according to tumor or lifestyle characteristics and *BRCA1* mutation status or TNBC status.

In conclusion, in a population-based sample, we confirmed that *BRCA1* mutation carriers have a high (48%) proportion of TNBC. We found among *BRCA1* mutation carriers that TNBCs were diagnosed at an earlier age and had higher-grade tumor, similar to patterns observed in the general population of patients with breast cancer. Among women without *BRCA1* mutations, those with TNBC had higher premenopausal BMI and earlier age at first full-term pregnancy than those with non-TNBC. Our observation that Ashkenazi Jewish women who carry the *BRCA1* mutation were more likely to have TNBC than other women warrants confirmation in larger studies.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Conception and design: Leslie Bernstein, Giske Ursin

Collection and assembly of data: Huiyan Ma, David Van Den Berg, Leslie Bernstein, Giske Ursin

Data analysis and interpretation: Eunjung Lee, Roberta McKean-Cowdin, Huiyan Ma, Darcy V. Spicer, David Van Den Berg, Leslie Bernstein, Giske Ursin

Manuscript writing: All authors

Final approval of manuscript: All authors

REFERENCES

1. Hudis CA: Trastuzumab: Mechanism of action and use in clinical practice. *N Engl J Med* 357:39-51, 2007
2. Riggs BL, Hartmann LC: Selective estrogen-receptor modulators: Mechanisms of action and application to clinical practice. *N Engl J Med* 348: 618-629, 2003
3. Reis-Filho JS, Tutt AN: Triple negative tumours: A critical review. *Histopathology* 52:108-118, 2008
4. Bauer KR, Brown M, Cress RD, et al: Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-

negative invasive breast cancer, the so-called triple-negative phenotype: A population-based study from the California Cancer Registry. *Cancer* 109:1721-1728, 2007

5. Carey LA, Dees EC, Sawyer L, et al: The triple negative paradox: Primary tumor chemosensitivity of breast cancer subtypes. *Clin Cancer Res* 13:2329-2334, 2007

6. Irvin WJ Jr, Carey LA: What is triple-negative breast cancer? *Eur J Cancer* 44:2799-2805, 2008

7. Anders C, Carey LA: Understanding and treating triple-negative breast cancer. *Oncology (Williston Park)* 22:1233-1239, 2008; discussion 1239-1240, 1243

8. Yang XR, Chang-Claude J, Goode EL, et al: Associations of breast cancer risk factors with tu-

mor subtypes: A pooled analysis from the Breast Cancer Association Consortium studies. *J Natl Cancer Inst* 103:250-263, 2011

9. Phipps AI, Buist DS, Malone KE, et al: Reproductive history and risk of three breast cancer subtypes defined by three biomarkers. *Cancer Causes Control* 22:399-405, 2011

10. Phipps AI, Chlebowski RT, Prentice R, et al: Reproductive history and oral contraceptive use in relation to risk of triple-negative breast cancer. *J Natl Cancer Inst* 103:470-477, 2011

11. Dolle JM, Daling JR, White E, et al: Risk factors for triple-negative breast cancer in women under the age of 45 years. *Cancer Epidemiol Biomarkers Prev* 18:1157-1166, 2009

12. Phipps AI, Li CI: Reply to reproductive and hormonal risk factors for postmenopausal luminal, HER-2-overexpressing, and triple-negative breast cancer. *Cancer* 115:1802-1803, 2009
13. Aksoy S, Dizdar O, Harputluoglu H, et al: Demographic, clinical, and pathological characteristics of Turkish triple-negative breast cancer patients: Single center experience. *Ann Oncol* 18:1904-1906, 2007
14. Berry DA, Iversen ES Jr, Gudbjartsson DF, et al: BRCAPRO validation, sensitivity of genetic testing of BRCA1/BRCA2, and prevalence of other breast cancer susceptibility genes. *J Clin Oncol* 20:2701-2712, 2002
15. Shattuck-Eidens D, Oliphant A, McClure M, et al: BRCA1 sequence analysis in women at high risk for susceptibility mutations: Risk factor analysis and implications for genetic testing. *JAMA* 278:1242-1250, 1997
16. Eerola H, Heikkilä P, Tamminen A, et al: Histopathological features of breast tumours in BRCA1, BRCA2 and mutation-negative breast cancer families. *Breast Cancer Res* 7:R93-R100, 2005
17. Honrado E, Osorio A, Palacios J, et al: Pathology and gene expression of hereditary breast tumors associated with BRCA1, BRCA2 and CHEK2 gene mutations. *Oncogene* 25:5837-5845, 2006
18. Lakhani SR, Van De Vijver MJ, Jacquemier J, et al: The pathology of familial breast cancer: Predictive value of immunohistochemical markers estrogen receptor, progesterone receptor, HER-2, and p53 in patients with mutations in BRCA1 and BRCA2. *J Clin Oncol* 20:2310-2318, 2002
19. Rakha EA, Reis-Filho JS, Ellis IO: Basal-like breast cancer: A critical review. *J Clin Oncol* 26:2568-2581, 2008
20. Breast Cancer Linkage Consortium: Pathology of familial breast cancer: Differences between breast cancers in carriers of BRCA1 or BRCA2 mutations and sporadic cases. *Lancet* 349:1505-1510, 1997
21. Foulkes WD, Stefansson IM, Chappuis PO, et al: Germline BRCA1 mutations and a basal epithelial phenotype in breast cancer. *J Natl Cancer Inst* 95:1482-1485, 2003
22. Turner NC, Reis-Filho JS: Basal-like breast cancer and the BRCA1 phenotype. *Oncogene* 25:5846-5853, 2006
23. Lakhani SR: The pathology of familial breast cancer: Morphological aspects. *Breast Cancer Res* 1:31-35, 1999
24. Lakhani SR, Jacquemier J, Sloane JP, et al: Multifactorial analysis of differences between sporadic breast cancers and cancers involving BRCA1 and BRCA2 mutations. *J Natl Cancer Inst* 90:1138-1145, 1998
25. Atchley DP, Albarracin CT, Lopez A, et al: Clinical and pathologic characteristics of patients with BRCA-positive and BRCA-negative breast cancer. *J Clin Oncol* 26:4282-4288, 2008
26. Ma H, Bernstein L, Ross RK, et al: Hormone-related risk factors for breast cancer in women under age 50 years by estrogen and progesterone receptor status: Results from a case-control and a case-case comparison. *Breast Cancer Res* 8:R39, 2006
27. Lee E, McKean-Cowdin R, Ma H, et al: Evaluation of unclassified variants in the breast cancer susceptibility genes BRCA1 and BRCA2 using five methods: Results from a population-based study of young breast cancer patients. *Breast Cancer Res* 10:R19, 2008
28. Lee E, Ma H, McKean-Cowdin R, et al: Effect of reproductive factors and oral contraceptives on breast cancer risk in BRCA1/2 mutation carriers and noncarriers: Results from a population-based study. *Cancer Epidemiol Biomarkers Prev* 17:3170-3178, 2008
29. Chapman MS, Verma IM: Transcriptional activation by BRCA1. *Nature* 382:678-679, 1996
30. Carvalho MA, Marsillac SM, Karchin R, et al: Determination of cancer risk associated with germ line BRCA1 missense variants by functional analysis. *Cancer Res* 67:1494-1501, 2007
31. Vallon-Christersson J, Cayan C, Haraldsson K, et al: Functional analysis of BRCA1 C-terminal missense mutations identified in breast and ovarian cancer families. *Hum Mol Genet* 10:353-360, 2001
32. Renehan AG, Tyson M, Egger M, et al: Body-mass index and incidence of cancer: A systematic review and meta-analysis of prospective observational studies. *Lancet* 371:569-578, 2008
33. Jóhannsson OT, Idvall I, Anderson C, et al: Tumour biological features of BRCA1-induced breast and ovarian cancer. *Eur J Cancer* 33:362-371, 1997
34. Loman N, Jóhannsson O, Bendahl PO, et al: Steroid receptors in hereditary breast carcinomas associated with BRCA1 or BRCA2 mutations or unknown susceptibility genes. *Cancer* 83:310-319, 1998
35. Noguchi S, Kasugai T, Miki Y, et al: Clinicopathologic analysis of BRCA1- or BRCA2-associated hereditary breast carcinoma in Japanese women. *Cancer* 85:2200-2205, 1999
36. Karp SE, Tonin PN, Bégin LR, et al: Influence of BRCA1 mutations on nuclear grade and estrogen receptor status of breast carcinoma in Ashkenazi Jewish women. *Cancer* 80:435-441, 1997
37. Foulkes WD, Metcalfe K, Sun P, et al: Estrogen receptor status in BRCA1- and BRCA2-related breast cancer: The influence of age, grade, and histological type. *Clin Cancer Res* 10:2029-2034, 2004
38. Musolino A, Michiara M, Bella MA, et al: Molecular profile and clinical variables in BRCA1-positive breast cancers: A population-based study. *Tumori* 91:505-512, 2005
39. Quenneville LA, Phillips KA, Ozcelik H, et al: HER-2/neu status and tumor morphology of invasive breast carcinomas in Ashkenazi women with known BRCA1 mutation status in the Ontario Familial Breast Cancer Registry. *Cancer* 95:2068-2075, 2002
40. Robson M, Rajan P, Rosen PP, et al: BRCA-associated breast cancer: Absence of a characteristic immunophenotype. *Cancer Res* 58:1839-1842, 1998
41. Bidard FC, Conforti R, Boulet T, et al: Does triple-negative phenotype accurately identify basal-like tumour? An immunohistochemical analysis based on 143 'triple-negative' breast cancers. *Ann Oncol* 18:1285-1286, 2007
42. Bertucci F, Finetti P, Cervera N, et al: How basal are triple-negative breast cancers? *Int J Cancer* 123:236-240, 2008
43. Livasy CA, Karaca G, Nanda R, et al: Phenotypic evaluation of the basal-like subtype of invasive breast carcinoma. *Mod Pathol* 19:264-271, 2006
44. Southey MC, Ramus SJ, Dowty JG, et al: Morphological predictors of BRCA1 germline mutations in young women with breast cancer. *Br J Cancer* 104:903-909, 2011
45. Easton DF, Bishop DT, Ford D, et al: Genetic linkage analysis in familial breast and ovarian cancer: Results from 214 families—The Breast Cancer Linkage Consortium. *Am J Hum Genet* 52:678-701, 1993
46. Gayther SA, Mangion J, Russell P, et al: Variation of risks of breast and ovarian cancer associated with different germline mutations of the BRCA2 gene. *Nat Genet* 15:103-105, 1997
47. Milne RL, Osorio A, Cajal TR, et al: The average cumulative risks of breast and ovarian cancer for carriers of mutations in BRCA1 and BRCA2 attending genetic counseling units in Spain. *Clin Cancer Res* 14:2861-2869, 2008
48. Comen E, Davids M, Kirchoff T, et al: Relative contributions of BRCA1 and BRCA2 mutations to "triple-negative" breast cancer in Ashkenazi women. *Breast Cancer Res Treat* 129:185-190, 2011
49. Tung N, Wang Y, Collins LC, et al: Estrogen receptor positive breast cancers in BRCA1 mutation carriers: Clinical risk factors and pathologic features. *Breast Cancer Res* 12:R12, 2010
50. Phipps AI, Malone KE, Porter PL, et al: Reproductive and hormonal risk factors for postmenopausal luminal, HER-2-overexpressing, and triple-negative breast cancer. *Cancer* 113:1521-1526, 2008
51. Yang XR, Sherman ME, Rimm DL, et al: Differences in risk factors for breast cancer molecular subtypes in a population-based study. *Cancer Epidemiol Biomarkers Prev* 16:439-443, 2007
52. Millikan RC, Newman B, Tse CK, et al: Epidemiology of basal-like breast cancer. *Breast Cancer Res Treat* 109:123-139, 2008
53. Trivers KF, Lund MJ, Porter PL, et al: The epidemiology of triple-negative breast cancer, including race. *Cancer Causes Control* 20:1071-1082, 2009
54. Gluz O, Liedtke C, Gottschalk N, et al: Triple-negative breast cancer: Current status and future directions. *Ann Oncol* 20:1913-1927, 2009
55. Fong PC, Boss DS, Yap TA, et al: Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. *N Engl J Med* 361:123-134, 2009
56. Sauter G, Lee J, Bartlett JM, et al: Guidelines for human epidermal growth factor receptor 2 testing: Biologic and methodologic considerations. *J Clin Oncol* 27:1323-1333, 2009
57. Dybdal N, Leiberman G, Anderson S, et al: Determination of HER2 gene amplification by fluorescence in situ hybridization and concordance with the clinical trials immunohistochemical assay in women with metastatic breast cancer evaluated for treatment with trastuzumab. *Breast Cancer Res Treat* 93:3-11, 2005
58. Ma H, Wang Y, Sullivan-Halley J, et al: Breast cancer receptor status: Do results from a centralized pathology laboratory agree with SEER registry reports? *Cancer Epidemiol Biomarkers Prev* 18:2214-2220, 2009