BRCA1 and *BRCA2* Mutations Account for a Large Proportion of Ovarian Carcinoma Cases

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BACKGROUND. It is believed that *BRCA1* and *BRCA2* germline mutations account for the majority of hereditary ovarian carcinomas; however, to the authors' knowledge, there are scant data on the prevalence and spectrum of mutations, genotype/phenotype correlations, tumor histology, and family history characteristics. To address this gap, the authors conducted a population-based study of 232 incident epithelial ovarian carcinomas in the Tampa Bay area.

METHODS. Genetic testing for the *BRCA1* and *BRCA2* genes was performed through full sequencing and *BRCA1* rearrangement testing.

RESULTS. Of 209 women with invasive ovarian carcinoma, 32 women (15.3%) had mutations in *BRCA1* or *BRCA2*, including 20 *BRCA1* mutations and 12 *BRCA2* mutations. Of the *BRCA2* mutations, 58% were outside the "ovarian cancer cluster region" (OCCR). Variants of uncertain significance were detected in 8.2% of women with invasive ovarian carcinoma. No mutations were identified in women with borderline or invasive mucinous tumors. Among the BRCA mutation-positive women, 63% had serous tumors. A family history of breast and/or ovarian carcinoma was reported in 65%, 75%, and 43.5% of relatives of *BRCA1* carriers, *BRCA2* carriers, and non-*BRCA1*/*BRCA2* carriers, respectively.

CONCLUSIONS. The data from this study suggested that 1) previous studies may have underestimated the frequency of *BRCA1* and *BRCA2* mutations in ovarian carcinomas, especially outside the OCCR; 2) it may be reasonable to offer genetic counseling to any woman with an invasive, nonmucinous epithelial ovarian tumor; and 3) among patients with invasive ovarian carcinoma, family history is not sufficiently accurate to predict mutation status. ***Cancer*** **2005;104:2807–16.**

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Ovarian carcinoma represents the seventh leading cancer among women in the U.S., accounting for 3% of all female cancers. In 2005, an estimated 22,220 new ovarian carcinomas will be diagnosed, and approximately 16,210 ovarian carcinoma-related deaths are expected.[1] Established risk factors for ovarian carcinoma include age;

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family history of ovarian and other associated malignancies, including breast, uterine, and/or colorectal carcinomas; and inherited mutations in carcinoma-predisposition genes.[2–4] The risk of ovarian carcinoma also may be increased by hormonal and reproductive factors, such as prolonged consumption of unopposed estrogen-replacement therapy, infertility and/or long-term use of fertility drugs, early age of menarche, late age at menopause, delayed childbirth, and low parity or nulliparity.[1]

Overall, it is believed that approximately 5–12% of invasive ovarian carcinomas are due to hereditary susceptibility.[5,6] Mutations in the *BRCA1* and *BRCA2* genes account for the majority of hereditary ovarian carcinomas,[2,3] followed by the *MLH1* and *MSH2* genes implicated in hereditary nonpolyposis colorectal carcinoma,[4] yet few population-based studies, including those from the U.S., have been performed specifically to investigate the role of hereditary susceptibility genes in this deadly disease. Estimates of the frequency of *BRCA1* and *BRCA2* germline mutations in ovarian carcinomas have ranged between 2–12% and 2–6% for *BRCA1* and *BRCA2* mutations, respectively.[6–21] The limitations of those studies included small sample sizes and/or case selection based on early age of diagnosis, a hospital-based series, prevalent cases, or self-referred patients. Furthermore, some of those studies were specific to certain ethnic groups with ethnicity-specific founder mutations.

Determining the proportion and characteristics of women who have epithelial ovarian carcinomas associated with mutations of the *BRCA1* and *BRCA2* genes is important, because it enables the appropriate provision of genetic counseling and testing services to these women and their families to aid in medical management. Few studies have addressed whether women who have ovarian carcinomas associated with *BRCA1* and *BRCA2* differ from each other and/or from women who have sporadic tumors with regard to the following characteristics: age at diagnosis, histologic subtype, stage at presentation, and personal and family history of cancer. To address these questions, we studied 232 unselected, population-based, incident ovarian carcinomas in 2 counties within the Tampa Bay area and examined the occurrence of mutations with respect to these characteristics.

MATERIALS AND METHODS

This study was approved by the Institutional Review Board of the University of South Florida. Written informed consent was obtained from each patient specifically for genetic testing.

Patients

The Tampa Bay Ovarian Cancer Study (TBOCS) is a population-based study in a heavily populated, 2-county region of west central Florida with a population in excess of 2 million. Because of the rapidly fatal nature of ovarian carcinoma among women who are diagnosed at late stages, we developed a rapid case-ascertainment mechanism that included 7 regional gynecologic and medical oncologists who treat 85% of women with ovarian carcinoma in the area. According to the most current Florida Cancer Data System (FCDS) statistics, 362 incident epithelial ovarian carcinomas in women ages 18–80 years were diagnosed between December 13, 2000 and September 30, 2003, and were eligible for participation in the current study.

The diagnosis of ovarian carcinoma was confirmed for all patients in a review of pathology records and tumor slides by a single pathologist with expertise in ovarian carcinoma. Clinical stage was determined according to International Federation of Gynecologists and Obstetricians (FIGO) criteria,[22] and the histologic subtype was evaluated according to the World Health Organization (WHO) classification.[23] When ambiguities in pathologic interpretation were present, the patient records were analyzed by a second ovarian carcinoma expert for a consensus.

Procedure

The treating physicians introduced the study to eligible patients and informed the study team of patients who agreed to be contacted by a study member. A genetic counselor scheduled an in-person interview with the participant at the treating physician's office or at another location that was convenient for the patient. After obtaining informed consent, a questionnaire was completed, genetic counseling was provided, and a blood sample was collected. The questionnaire included information on multiple epidemiologic risk factors, including reproductive history. No TBOCS participant refused genetic testing, and all but 1 participant chose to obtain results of the *BRCA1* and *BRCA2* analysis.

Funding for the study covered all costs, including genetic counseling and genetic testing of the *BRCA1* and *BRCA2* genes. During the genetic counseling interview, family history details were obtained, including current age, age at diagnosis, and site (and, when possible, histologic type) of malignancy in three generations. Family history information was taken as that reported by the proband, because medical record verification from family members was not possible. Studies suggest that the diagnosis of carcinoma reported in

relatives is likely to be accurate at least for first-degree relatives, especially in families with hereditary breast carcinomas.[24–27]

BRCA1 and *BRCA2* Analysis

Lymphocyte DNA was extracted from whole blood by standard procedures. All samples were analyzed for *BRCA1* and *BRCA2* through full gene sequencing and rearrangement testing according to published protocol[28] through Myriad Genetics Laboratories, Inc. This analysis identifies gene mutations in all of the known protein coding sequences and additional adjacent areas in the genes and detects approximately 90–95% of mutations in *BRCA1* and *BRCA2*. The samples also were analyzed for the presence of 5 *BRCA1* genomic rearrangements by using recombination-specific polymerase chain reaction analysis with primers that were specific for the normal gene as well as for the rearrangement, including: a 3.8-kb deletion of exon 13 and a 510-base pair deletion of exon 22 described in individuals of Dutch ancestry,[29] a 6-kb duplication of exon 13 described in individuals of European (particularly British) ancestry,[30,31] a 7.1-kb deletion of exons 8 and 9 described in individuals of European ancestry,[32] and a 26-kb deletion of exons 14–20.[33]

Statistical Analysis

Confidence limits for mutation frequencies were calculated under the assumption of binomial distributions of the observed numbers of patients. To compare personal and family history characteristics between carriers and noncarriers, the contingency table chi-square test was performed, and the asymptotic *P* values were calculated. The Proc Freq procedure in SAS® statistical software (version 8.2) was used.

RESULTS

Study Population

Of the 362 women with epithelial ovarian carcinoma in the 2-county region who were identified through FCDS data, we were given contact information on 267 women through our 7 regional gynecologic and medical oncologists. Of these women, 232 agreed to participate. The reasons for nonparticipation included patients were too ill (20 women) and patients refused to participate (15 women). Reasons for refusal were unwillingness to make an extra trip to participate (10 women) and patients indicated they had no female children or siblings for whom they believed the genetic testing would be useful (5 women). Thus, the overall participation rate was 64%.

The distribution of women in the TBOCS by race and ethnicity was similar to the distribution of women with ovarian carcinoma in the catchment area. The state registry data revealed that the ethnic distribution in the catchment area was 91% white and 9% nonwhite, similar to the 90.9% and 9.1% distribution observed in our study among white patients and nonwhite patients, respectively. The distribution according to histologic subtype and stage also was similar to that seen in the general population. Briefly, the distribution of histologic features expected in the general population is approximately 75% serous ovarian carcinomas and approximately 15% endometrioid ovarian carcinomas. Among women in the TBOCS, the distribution is similar, with 58.2% serous ovarian carcinomas and 12.9% endometrioid ovarian carcinomas. The distribution by stage was similar to that seen in the general population, in which approximately 30% of patients present with Stage I or II disease, and 70% present with Stages III or IV disease. A summary of data from the TBOCS is included in Table 1.

TABLE 1
Summary of Data on Patients in the Tampa Bay Ovarian Cancer Study

Variable	No. of patients (%)		
	Total (*n* = 232)	Invasive carcinoma (*n* = 209)	Borderline tumors (*n* = 23)
Age at diagnosis			
Mean age (yrs)	56.8	56.6	49.3
Range (yrs)	19–79	19–79	25–79
≤ 40 yrs	16 (6.9)	11 (5.3)	5 (21.7)
41–50 yrs	57 (24.6)	49 (23.4)	8 (34.8)
51–60 yrs	69 (29.7)	62 (29.7)	7 (30.4)
≥ 60 yrs	90 (38.8)	87 (41.6)	3 (13.0)
Race			
White	211 (90.9)	191 (91.4)	20 (87.0)
Nonwhite	21 (9.1)	18 (8.6)	3 (13.0)
Prevalence of Ashkenazi Jewish ancestry	9 (3.9)	9 (4.3)	0 (0)
Histology			
Serous	135 (58.2)	121 (57.9)	14 (60.9)
Mucinous	19 (18.2)	13 (6.2)	6 (26.1)
Endometrioid	30 (12.9)	29 (13.9)	1 (4.3)
Clear cell	9 (3.9)	9 (4.3)	0 (0.0)
Other	39 (16.8)	37 (17.7)	2 (8.7)
Prevalence of deleterious mutation			
BRCA1	20 (8.6)	20 (9.6)	0 (0.0)
BRCA2	12 (5.2)	12 (5.7)	0 (0.0)

The Prevalence of *BRCA1* and *BRCA2* Mutations

Of the 232 women enrolled in the study, 32 women (13.8%) had mutations in *BRCA1* or *BRCA2*, including 20 *BRCA1* mutations (8.6% of women) and 12 *BRCA2* mutations (5.2% of women). Two of 32 women with *BRCA1* mutations were related, and the remaining 30 women were unrelated. No mutations were identified

TABLE 2
Characteristics of Women who had Ovarian Carcinoma with *BRCA1* and *BRCA2* Mutations (n = 32)

Gene/mutation	Ethnicity[a]	Age at diagnosis in yrs	Histology	Stage	Prior breast cancer diagnosis with age(s)	Family history of breast and/or ovarian carcinoma
BRCA1						
187delAG	Mixed European	52	S	IIIC	—	Yes
187delAG	Ashkenazi Jewish	56	E, S	IIIC	—	Yes
187delAg	Indian	43	E, S	IIIA	—	No
187delAG	Mixed European	76	S	IIIC	48, 58	Yes
187delAG	Hispanic	46	S	IIIC	—	Yes
C61G	Hungarian	56	S	IIIA	31	Yes
C944X[b]	Hispanic	54	TC	IIIC	—	Yes
1294del40	Mixed European	52	S	IA	—	Yes
2576delC	Mixed European	60	S	IIIC	49	No
2576delC	Mixed European	43	S	IV	—	No
2530delAG	Mixed European	53	S	IV	42	No
2800delAA	Mixed European	49	S	IIB	—	Yes
2800delAA	Mixed European	43	S, C	IIIC	—	Yes
3790ins[c]	British	46	S	IIIC	—	Yes
3875del[c]	English	52	S	IVC	—	No
4154delA	Mixed European	55	S	IIIC	—	Yes
E1134X	Mixed European	42	S	IIIC	41	No
K679X	Mixed European	45	E, C	IIIC	—	Yes
4440insG	Mixed European	77	S	II	—	Yes
5385insC	Mixed European	52	E	IIA	50	No
BRCA2						
E49X	Mixed European	49	S	IV	26, 41	Yes
2041insA	Mixed European	63	E	IC	—	Yes
1983del5	Mixed European	46	S	IB	—	Yes
Q1931X[b,c]	Greece	63	S, C	III	56	Yes
4075delGT[c]	Mixed European	59	S	IIIC	—	No
4706del[c]	Mixed European	61	U	IA	—	Yes
6174delT[c]	Ashkenazi Jewish	60	PP	IIIC	—	Yes
6174delT[c]	Ashkenazi Jewish	62	E	IC	48	No
4512insT[c]	French	73	S	IIIC	—	No
Q2009X[c]	Ashkenazi Jewish	59	S	IIIC	—	Yes
R2520X	Irish	64	B	IIB	—	Yes
R2520X	Mixed European	34	S	IIIC	—	Yes

S: serous; E: endometrioid; C: clear cell; PP: primary peritoneal; TC: transitional cell; U: unknown; B: Brenner cell.
[a] All ethnicities were non-Ashkenazi Jewish, except as indicated.
[b] Novel mutation.
[c] Ovarian carcinoma cluster region.

in the 23 women who had borderline tumors. When considering only the women with invasive ovarian tumors (n = 209 women), 15.3% had mutations in *BRCA1* or *BRCA2*. Among the 209 women with invasive tumors, no mutations were identified among the 13 women with mucinous tumors. Thus, the percentage of *BRCA1* and *BRCA2* mutations among the 196 women with invasive, nonmucinous tumors was 16.3%.

Table 2 shows that the *BRCA1* mutations detected in this study were distributed across the length of the gene. Of the 12 *BRCA2* mutations, 5 mutations (42%) were outside the OCCR, as shown in Table 2.

Of the nine women in the study (3.9%) who reported Ashkenazi Jewish ancestry, 4 women tested positive for a BRCA mutation, including 1 woman who had the *BRCA1* 187delAG mutation, 2 women who had the 6174 delT *BRCA2* mutation, and 1 woman who had a nonfounder mutation, Q2009X.

The Prevalence of *BRCA1* and *BRCA2* Variants of Uncertain Significance

Variants of uncertain significance (VUS) were detected in 21 of the women enrolled in the study (9.1%), including 2 *BRCA1* variants (0.86% of women) and 19 *BRCA2* variants (8.2% of women), as shown in Table 3.

TABLE 3
Variants of Uncertain Significance Detected in 23 Women from the Tampa Bay Ovarian Cancer Study

Gene/mutation[a]	No. of previous observations of the variant[b]	Observations with > 1 deleterious mutation in the same gene	Predominant ancestry
BRCA1			
V1833M	2–10	No	Central/Eastern European
R1645S	First	—	—
BRCA2			
D596H[c]	26–50	No	Western European
G1529R	26–50	Yes (multiple mutations)	Western European
S384F	> 50	Yes (multiple mutations)	Western European
N3124I	2–10	No	Western European
E2856A	> 50	No	Western European
I2285V	26–50	No	Ashkenazi Jewish
D2110A	One additional family	No	—
A2889S	First	—	—
S2148Y	2–10	No	Western European
S2835P	2–10	No	Western European, Latin American, and African
S384F	50	Yes (multiple mutations)	Western European
R2973H[c]	One additional family	No	Western European
E1046G	First	—	—
E2586A	> 50	No	Western European
P655R	11–25	Yes (1 mutation)	Ashkenazi Jewish
V1048I	First	—	—
S326R	> 50	Yes (multiple mutations)	Western European
A75P	11–25	Yes (1 mutation)	Western European
I2944F	> 50	Yes (multiple mutations)	African

[a] All mutations were missense. None of the *BRCA2* variants were located within the ovarian carcinoma cluster region.
[b] Data provided by Myriad Laboratories are in ranges rather than exact numbers of previous observations of the variant in unrelated families.
[c] Tumors with low malignant potential.

Nineteen of 21 variants were detected in women who had invasive ovarian carcinoma, and 2 of 21 variants were detected in women who had borderline ovarian tumors. Table 3 shows that all of these variants were missense mutations. Each of the 19 detected *BRCA2* variants was located outside of the OCCR. Information regarding the classification of these variants was provided by Myriad Genetic Laboratories (see Table 3). Seven of 19 *BRCA2* variants (36.8%) had previously been observed with ≥ 1 deleterious mutations.

Personal and Family History Characteristics: Invasive Tumors (n = 209)

The average age at diagnosis was 53 years among *BRCA1* carriers (n = 20 women), 58 years among *BRCA2* carriers (n = 12 women), and 57 years among women with sporadic tumors (n = 177 women) (Table 4). Similarly, the age distribution for ovarian carcinoma diagnosis varied between *BRCA1* carriers, *BRCA2* carriers, and women with sporadic tumors, with 90%, 50%, and 56% of ovarian carcinomas, respectively, diagnosed in women younger than age 60 years. Women who were diagnosed at ages 51–60 years had the highest frequency of mutations (40.6%), and women who had ovarian carcinoma diagnosed when they were younger than age 40 years had a lower prevalence of mutations (3.1%).

Almost 30% (n = 9 patients) of the women with *BRCA1* and *BRCA2* mutation-positive ovarian carcinoma had a personal history of breast carcinoma, with an average age at breast carcinoma diagnosis of approximately 43 years. This compared with 5% (n = 8 patients) of the 156 women with sporadic tumors who had a personal history of breast carcinoma, with an average age at diagnosis of 52.8 years.

The proportion of women with invasive carcinoma who had a positive family history of breast and/or ovarian carcinoma in a first-degree or second-degree relative was 65.0% among *BRCA1* carriers (n = 20 patients), 75.0% among *BRCA2* carriers (n = 12 patients), and 43.5% among women with sporadic tumors (n = 177 patients) (Table 4). Thirty-one percent of mutation carriers had no family history of breast or ovarian carcinoma in any first-degree or second-degree relative.

Reproductive and Other Factors and Ovarian Cancer Risk

Comparisons of reproductive and other factors (such as age at menarche, age at first live birth, parity, history of infertility, duration of hormone replacement therapy, and body mass index) among carriers and noncarriers (with invasive or borderline tumors) showed no statistically significant differences between the groups.

Tumor Characteristics

The majority (63%) of the *BRCA1* and *BRCA2*-associated tumors were of serous histology, and none were mucinous or borderline tumors (Table 5). Three histologic subtypes seen among the *BRCA2* carriers were not observed among *BRCA1* carriers: primary peritoneal, Brenner, and clear cell. The distribution by stage

TABLE 4
Personal and Family History of Cancer among Patients with Invasive Ovarian Carcinoma (n = 209)

Personal and family history Characteristics	*BRCA1* positive (n = 20)	*BRCA2* positive (n = 12)	Total *BRCA* positive (n = 32)	*BRCA* negative (n = 156)	*BRCA* VUS (n = 21)	Total sporadics (n = 177)[a]	OR	P value[b]
Proband								
Age at diagnosis								
≤ 40 yrs	0 (0.0)	1 (8.3)	1 (3.1)	9 (5.8)	1 (4.8)	10 (5.6)	—	—
41-50 yrs	8 (40.0)	2 (16.7)	10 (31.3)	35 (22.4)	4 (19.0)	39 (22.0)	—	—
51-60 yrs	10 (50.0)	3 (25.0)	13 (40.6)	40 (25.6)	9 (42.9)	49 (27.7)	—	—
> 60 yrs	2 (10.0)	6 (5f0.0)	8 (25.0)	72 (46.2)	7 (33.3)	79 (44.6)	—	—
Mean age (yrs)	53	58	55	58	56	57	—	—
Range (yrs)	42–77	34–73	34–77	19–79	35–73	19–79	—	—
Prior breast carcinoma	6 (30.0)	3 (25.0)	9 (28.0)	8 (5.1)	1 (4.8)	9 (5.1)	7.2	< 0.0001
Family history								
FDR or SDR with breast or ovarian carcinoma	13 (65.0)	9 (75.0)	22 (69.0)	65 (41.7)	12 (57.1)	77 (43.5)	2.9	0.0085
≥ 1 FDR with breast carcinoma	4 (20.0)	5 (42.0)	9 (28.0)	30 (19.2)	3 (14.3)	33 (18.7)	1.2	0.2181
≥ 1 FDR with ovarian carcinoma	6 (30.0)	2 (17.0)	8 (25.0)	8 (5.1)	1 (4.8)	9 (5.1)	6.2	0.0001
≥ 1 SDR with breast carcinoma[c]	8 (40.0)	4 (44.0)	12 (38.0)	38 (24.4)	8 (38.1)	46 (26.0)	1.7	0.1808
≥ 1 SDR with ovarian carcinoma[c]	3 (15.0)	1 (8.0)	4 (13.0)	7 (4.5)	1 (4.8)	8 (4.5)	3.0	0.0741
No FDR or SDR with breast or ovarian carcinoma	7 (35.0)	3 (25.0)	10 (31.0)	91 (58.3%)	9 (42.9)	100 (56.5)	2.9	0.0085

VUS: variant of uncertain significance; OR: odds ratio; FDR: first-degree relative; SDR: second-degree relative.

[a] Total sporadics include probands who tested BRCA negative and probands who had a BRCA variant of uncertain significance.

[b] P values are for comparisons of carriers with noncarriers and are based on chi-square contingency tables. Values were significant at $P < 0.05$.

[c] Data corresponding to second-degree relatives did not include first-degree relatives.

of BRCA carriers overall was similar to that seen in the general population, in which approximately 30% present with Stage I or II disease, and 70% present with Stage III or IV disease (Table 5). When stratifying *BRCA1* and *BRCA2* carriers by stage, 20% (4 of 20 women) of *BRCA1* carriers and 42% (5 of 12 women) of *BRCA2* carriers presented with early-stage disease (i.e., Stage I or II). The majority of BRCA carriers had poorly differentiated tumors.

DISCUSSION

Although previous estimates have suggested that at least 10% of invasive ovarian carcinomas are due to mutations in *BRCA1* and *BRCA2*, results from the current study indicate that the proportion actually may be as high as 15% when a comprehensive mutation-detection strategy is employed. The largest population-based study to date was performed in Ontario, Canada,[6] and was based on 649 unselected women with ovarian carcinoma, including 515 women with invasive ovarian carcinoma. The methodology for mutation detection in that study included screening of exon 11 of *BRCA1*, screening of exons 10 and 11 of *BRCA2* through protein truncation testing, and screening for 11 common mutations by rapid multiplex methods (including 3 Jewish founder mutations and 6 French-Canadian mutations). Using this limited mutation-detection strategy, among the 515 women with invasive ovarian carcinoma, 60 mutations (11.7%; 95% confidence interval [95% CI], 9.2–14.8%) were identified, including 39 mutations that were identified in *BRCA1* and 21 mutations that were identified in *BRCA2*. More recently, in a Swedish population-based study, 161 women with invasive epithelial ovarian carcinoma were investigated, and 13 patients (8%) were identified with deleterious mutations.[13] Although the strategy for mutation detection in that study included several molecular techniques, full gene sequencing

TABLE 5
Tumor Histology: Subtype, Stage, and Grade among BRCA Mutation Carriers and Noncarriers

	No. of patients (%)						
Variable	Total sample (n = 232)	Invasive carcinoma (n = 209)	Borderline tumors (n = 23)	BRCA positive (n = 20)	*BRCA2* positive (n = 12)	Total *BRCA* positive[a] (n = 32)	Total noncarriers (n = 200)[a]
Tumor type							
Serous	135 (58.2)	121 (57.9)	14 (60.9)	14 (70)	6 (50)	20 (63)	115 (57.5)
Endometrioid	30 (12.9)	29 (13.9)	1 (4.3)	1 (5.0)	2 (16.8)	3 (9.0)	27 (13.5)
Transitional	3 (1.3)	3 (14.4)	0 (0.0)	1 (5.0)	0 (0.0)	1 (3.0)	2 (0.1)
Mucinous	19 (8.2)	13 (6.2)	6 (26.1)	0 (0.0)	0 (0.0)	0 (0.0)	19 (9.5)
Mixed	20 (8.6)	18 (8.6)	2 (8.7)	4 (20.0)	1 (8.3)	5 (16)	15 (7.5)
Peritoneal	6 (2.6)	6 (2.9)	0 (0.0)	0 (0.0)	1 (8.3)	1 (3.0)	5 (2.5)
Brenner	3 (1.3)	3 (1.4)	0 (0.0)	0 (0.0)	1 (8.3)	1 (3.0)	1 (1.0)
Clear cell	9 (3.9)	9 (4.3)	0 (0.0)	0 (0.0)	1 (0.0)	0 (0.0)	9 (4.5)
Unknown	7 (3.0)	7 (3.3)	0 (0.0)	0 (0.0)	1 (8.3)	1 (3.0)	6 (3.0)
Stage							
I	47 (20.3)	28 (13.4)	19 (82.6)	1 (5)	4 (33.4)	5 (15.6)	42 (21.0)
II	20 (8.6)	20 (9.6)	0 (0.0)	3 (15)	1 (8.3)	4 (12.5)	16 (8.0)
III	136 (58.6)	132 (63.15)	4 (17.4)	13 (65)	6 (50)	19 (59.4)	117 (58.5)
IV	28 (12.1)	28 (13.4)	0 (0.0)	3 (15)	1 (8.3)	4 (12.5)	24 (12.0)
Unknown	1 (0.4)	1 (0.45)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)
Grade							
1	46 (20.4)	27 (12.9)	19 (82.6)	3 (15)	3 (25)	6 (18.75)	40 (20.0)
2	43 (19.0)	42 (20.1)	2 (8.7)	3 (15)	1 (8.3)	4 (12.5)	39 (19.5)
3	137 (60.6)	136 (65.1)	1 (4.35)	14 (70)	8 (66.7)	22 (68.75)	115 (57.5)
Unknown	6 (2.6)	4 (1.9)	1 (4.35)	0 (0.0)	0 (0.0)	0 (0.0)	6 (3.0)

aTotal noncarriers include probands who tested BRCA negative and probands who had BRCA variants of uncertain significance.

was not performed; thus, mutations may have been missed. In a Polish study, 364 unselected women with ovarian carcinoma were investigated for 3 common founder *BRCA1* mutations, and 49 deleterious mutations were detected (13.5%).[14]

In the current study, among the 209 women with invasive ovarian carcinoma, 32 mutations were identified (15.3%; 95% CI, 11–21%), including 20 mutations in *BRCA1* and 12 mutations in *BRCA2*. The higher rate of mutation detection compared with the Ontario study[6] was due in part to the more comprehensive analysis performed in the current study, including regions outside the OCCR. Specifically, only 42% of *BRCA2* mutations were in the OCCR region in our study, compared with 62% in the Ontario study.

BRCA VUS were detected in almost 10% of TBOCS participants, which is higher than previously reported.[13,20] This likely is attributable to the more comprehensive mutation-detection strategy we employed. The high proportion of women with VUS in this study highlights the importance of presenting this possible testing outcome during the pretest counseling session. The majority of VUS (90.5%) detected in the current study were in *BRCA2*. Data from Myriad Genetic Laboratories suggest that almost 40% of the *BRCA2* missense variants detected in this study are likely to be of limited clinical significance, because they have been observed previously with $\geq$ 1 deleterious mutations, which is consistent with other reports.[34] Results from a survey of cancer genetic counselors suggest that the clinical interpretation and recommendations for individuals with a VUS in *BRCA1* and *BRCA2* continues to be challenging.[35] Improved understanding of the functional significance of missense and other types of VUS would allow more informed recommendations regarding medical management for women with these variants and their families.

Hereditary carcinoma syndromes often are characterized by a younger age of onset, multiple cancer-affected family members, and a characteristic histopathologic profile. For ovarian carcinoma, previous studies have shown that, although *BRCA1*-associated ovarian carcinomas occur an average of 5–10 years prior to sporadic ovarian carcinomas, *BRCA2*-associated ovarian carcinomas occur, on average, at the same age as sporadic ovarian cacinomars.[6] Findings in the current study were similar to those reported previously and showed that the mean age of onset of ovarian carcinoma in *BRCA1* carriers was approximately 5 years younger than the mean age of onset in

BRCA2 carriers and in women with sporadic ovarian carcinoma.

In clinical practice, family history of cancer is used as a key factor in estimating the likelihood of a *BRCA1* or *BRCA2* mutation and in guiding recommendations for patient testing. However, there have been conflicting reports regarding the sensitivity of family history as a reliable predictor of mutation carrier status. Our results suggest that relying on a family history of breast or ovarian carcinoma to indicate the need for genetic counseling and testing would result in missing $>$ 30% of BRCA-associated ovarian carcinomas. In comparison, a recent Polish study reported a positive family history of breast and/or ovarian carcinoma in only 41% of mutation carriers and in only 9% of noncarriers.[14] In contrast, a Swedish population-based study of ovarian carcinomas reported the presence of a family history of breast and/or ovarian carcinoma in $>$ 90% of mutation carriers, compared with only 24% of noncarriers.[13] Thus, similar to recommendations made by Risch et al.,[6] these results suggest that it may be appropriate to offer genetic counseling and testing of *BRCA1* and *BRCA2* to all patients with invasive, nonmucinous epithelial ovarian carcinomas, regardless of the presence or absence of a family history of breast or ovarian carcinoma.

Histologic findings in the current study are consistent with previous reports, which have suggested that BRCA-associated ovarian carcinomas are predominantly of serous histology and that mutations are seen rarely in mucinous or borderline tumors.[6,36–42] In the Ontario study,[6] pathologic analysis showed that 56 of 60 mutation carriers (93%) had invasive serous tumors, and the remaining 4 women had endometrioid tumors. The Swedish population-based study showed similar results, with 62% serous carcinomas, 23% endometrioid carcinomas, 8% clear cell carcinomas, 8% serous surface papillary carcinomas, and no mucinous carcinomas.[13] In the current study, the results showed that 20 of 32 mutation carriers (63%) had invasive serous carcinomas, 5 women (16%) had mixed carcinomas, and 3 women (9%) had endometrioid-type carcinomas. No mutation carriers in our study had borderline or mucinous tumors. In addition, most mutation carriers (almost 70%) had poorly differentiated tumors, consistent with previous reports.[43]

To our knowledge, the current study is the first to provide detailed information regarding disease stage at presentation in a population-based sample of *BRCA1*-associated and *BRCA2*-associated ovarian carcinomas.[22] Our sample was representative of the stage at presentation of women with ovarian carcinoma in the general population, with 71% presenting with Stages III and IV disease. The stage at presentation of the BRCA carriers was similar to that seen in the general population.

Limitations of this study included a lower than desired participation rate (64%). Given the low survival rates for women with ovarian carcinoma, we decided to bypass the state cancer registry for patient ascertainment. Instead, we developed a rapid case-ascertainment system through gynecologic oncologists. However, we contend that the rapid ascertainment among clinics that diagnose $>$ 85% of the cases in the catchment area generated a more representative sample than is obtainable through typical population-based studies of rapidly fatal carcinomas.[6] Another limitation was the limited access to minority participants. However, our population was representative of the demographics of our catchment area (see Table 1).

Our mutation-detection strategy involved full gene sequencing and testing for common rearrangements, which is supposed to detect approximately 90% of mutations. It is possible, however, that some mutations may have been missed with this strategy, which is the most comprehensive testing currently available on a clinical basis. Thus, the hereditary fraction may be higher than what we reported.

Another possible weakness in this study is that family history information was obtained by personal interview and was not confirmed by either pathology report or other medical records. Studies suggest that the diagnosis of cancer reported in relatives is likely to be accurate, at least for first-degree relatives.[24–26] However, we acknowledge that reports of certain types of malignancies, such as ovarian carcinoma, may not be as accurate.[27] Self-reported family history should not affect our conclusions about the frequency of BRCA mutations in this population, because the patients were not selected on the basis of family history. Rather, because the study was population based, the reported family histories are likely representative of those of all women with ovarian carcinoma, and not of families that were selected for their high incidence of carcinoma.

Epithelial ovarian carcinoma results in the death of more American women than all other gynecologic malignancies combined. To our knowledge, the current report is the first U.S. population-based ovarian carcinoma study that comprehensively evaluates the role of the *BRCA1* and *BRCA2* genes in the etiology and pathology of this deadly disease. Findings from the current study suggest that 1) the incidence of hereditary ovarian carcinoma attributed to *BRCA1* and *BRCA2* mutations may be greater than reported previously; 2) *BRCA2* mutations account for a higher percentage of hereditary ovarian carcinomas than re-

ported previously, and previous studies may have underestimated the contribution of *BRCA2* to ovarian carcinoma, especially mutations outside the OCCR; 3) approximately 30% of patients with hereditary ovarian carcinoma have no obvious family history to suggest hereditary carcinoma susceptibility, hence, it appears reasonable to offer genetic counseling to all women who have invasive, nonmucinous epithelial ovarian carcinoma.

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