

# Breast and Ovarian Cancer in Relatives of Cancer Patients, with and without BRCA Mutations

Jennifer S. Lee,<sup>1,2</sup> Esther M. John,<sup>3</sup> Valerie McGuire,<sup>1</sup> Anna Felberg,<sup>1</sup> Kimberly L. Ostrow,<sup>4</sup> Richard A. DiCioccio,<sup>4</sup> Frederick P. Li,<sup>5</sup> Alexander Miron,<sup>6</sup> Dee W. West,<sup>1,3</sup> and Alice S. Whittemore<sup>1</sup>

<sup>1</sup>Department of Health Research and Policy, Stanford University School of Medicine, Stanford, California; <sup>2</sup>Division of Endocrinology and Metabolism, University of California San Francisco, San Francisco, California; <sup>3</sup>Northern California Cancer Center, Fremont, California; <sup>4</sup>Department of Cancer Genetics, Roswell Park Cancer Institute, Buffalo, New York; and Departments of <sup>5</sup>Epidemiology and <sup>6</sup>Cancer Biology, Dana-Farber Cancer Institute, Boston, Massachusetts

## Abstract

**Background:** First-degree relatives of patients with breast or ovarian cancer have increased risks for these cancers. Little is known about how their risks vary with the patient's cancer site, carrier status for predisposing genetic mutations, or age at cancer diagnosis.

**Methods:** We evaluated breast and ovarian cancer incidence in 2,935 female first-degree relatives of non-Hispanic White female patients with incident invasive cancers of the breast ( $n = 669$ ) or ovary ( $n = 339$ ) who were recruited from a population-based cancer registry in northern California. Breast cancer patients were tested for BRCA1 and BRCA2 mutations. Ovarian cancer patients were tested for BRCA1 mutations. We estimated standardized incidence ratios (SIR) and 95% confidence intervals (95% CI) for breast and ovarian cancer among the relatives according to the patient's mutation status, cancer site, and age at cancer diagnosis.

**Results:** In families of patients who were negative or untested for BRCA1 or BRCA2 mutations, risks were elevated only for the patient's cancer site. The breast cancer SIR was 1.5 (95% CI, 1.2-1.8) for relatives of breast cancer patients, compared with 1.1 (95% CI, 0.8-1.6) for relatives of ovarian cancer patients ( $P = 0.12$  for difference by patient's cancer site). The ovarian cancer SIR was 0.9 (95% CI, 0.5-1.4) for relatives of breast cancer patients, compared with 1.9

(95% CI, 1.0-4.0) for relatives of ovarian cancer patients ( $P = 0.04$  for difference by site). In families of BRCA1-positive patients, relatives' risks also correlated with the patient's cancer site. The breast cancer SIR was 10.6 (95% CI, 5.2-21.6) for relatives of breast cancer patients, compared with 3.3 (95% CI, 1.4-7.3) for relatives of ovarian cancer patients (two-sided  $P = 0.02$  for difference by site). The ovarian cancer SIR was 7.9 (95% CI, 1.2-53.0) for relatives of breast cancer patients, compared with 11.3 (3.6-35.9) for relatives of ovarian cancer patients (two-sided  $P = 0.37$  for difference by site). Relatives' risks were independent of patients' ages at diagnosis, with one exception: In families ascertained through a breast cancer patient without BRCA mutations, breast cancer risks were higher if the patient had been diagnosed before age 40 years.

**Conclusion:** In families of patients with and without BRCA1 mutations, breast and ovarian cancer risks correlate with the patient's cancer site. Moreover, in families of breast cancer patients without BRCA mutations, breast cancer risk depends on the patient's age at diagnosis. These patterns support the presence of genes that modify risk specific to cancer site, in both carriers and noncarriers of BRCA1 and BRCA2 mutations. (Cancer Epidemiol Biomarkers Prev 2006;15(2):359-63)

## Introduction

There is extensive evidence indicating elevated risks for breast and ovarian cancer in relatives of patients with these cancers (1-8). Yet, relatively little is known about how the magnitudes of these risks vary with the patient's cancer site, BRCA1 and BRCA2 mutation status, and age at diagnosis. Such information is needed for preventive counseling. Breast and ovarian cancer risks in relatives of cancer patients with BRCA1 or BRCA2 mutations have been estimated in a combined analysis of 22 case series of patients with breast or ovarian cancer (9). However, this report did not describe risks in relatives of patients without these mutations. Risks in relatives of breast cancer patients with and without BRCA1 and BRCA2

mutations have been estimated from population-based case series in Australia (6), Sweden (7), and the United States (10). However, these studies did not include relatives of ovarian cancer patients.

Here, we analyze breast and ovarian cancer occurrence in first-degree relatives of non-Hispanic White patients who were diagnosed in northern California with incident cancers of the breast ( $n = 669$ ) or ovary ( $n = 339$ ). We estimated breast and ovarian cancer risks in the relatives according to patient characteristics: BRCA1 and BRCA2 mutation status, cancer site, and age at cancer diagnosis.

## Materials and Methods

**Patient Populations.** Patients diagnosed with incident breast or ovarian cancer were identified through the population-based cancer registry covering nine counties of the Greater San Francisco Bay area, operated by the Northern California Cancer Center as part of the Surveillance, Epidemiology, and End Results Program of the National Cancer Institute and the California Cancer Registry. Breast cancer patients were enrolled in the Northern California component of the Breast Cancer Family Registry, a consortium funded by the National Cancer Institute (11). Eligible patients were non-Hispanic White women diagnosed with invasive or *in situ*

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**Note:** F.P. Li is a Harry and Elsa Jiler American Cancer Society Clinical Research Professor.

**Requests for reprints:** Jennifer S. Lee, Division of Epidemiology, Department of Health Research and Policy, Stanford University School of Medicine, HRP Redwood Building, Stanford, CA 94305-5405. Phone: 650-867-3147; Fax: 650-725-6951. E-mail: catechols@gmail.com

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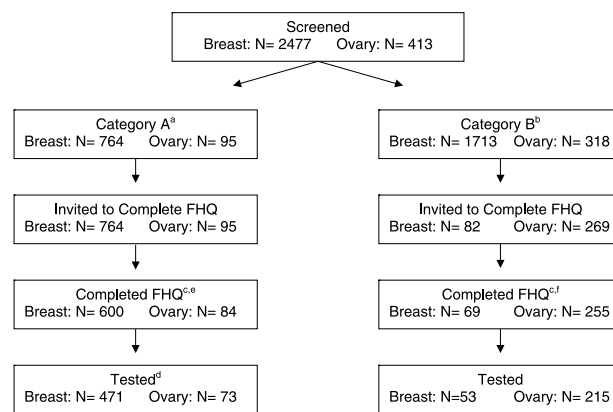
breast cancer at ages 35 to 64 years during the period January 1, 1995, to June 30, 1997. Whites diagnosed before age 35 were ascertained up to September 30, 1998. Participants were recruited using a two-stage sampling design that oversamples patients whose characteristics suggest an inherited basis for their cancers (12, 13). This sampling design provides unbiased estimates of familial cancer risks that have greater precision than those obtained from a simple random sample of the same size. In stage 1, we screened patients by telephone to classify them into one of two categories: category A (patients whose cancers are likely to be hereditary) and category B (all other patients), hereafter called "hereditary" and "nonhereditary" categories. In stage 2, we invited all patients in category A and a random sample of those in category B to enroll in the Breast Cancer Family Registry. Category A patients were those who met at least one of the following criteria: (a) breast cancer diagnosis before age 35 years; (b) bilateral breast cancer with first diagnosis before age 50 years; (c) prior ovarian or childhood cancer; or (d) at least one first-degree relative with breast or ovarian cancer.

Ovarian cancer patients were enrolled in the Familial Registry of Ovarian Cancer, a research project funded by National Cancer Institute (14). Eligible patients were women diagnosed with invasive epithelial ovarian cancer or epithelial tumors of low malignant potential at ages 20 to 64 years during the period March 1, 1997, through July 31, 2001. Patients residing in the nine-county Greater San Francisco Bay Area at the time of diagnosis were identified by the cancer registry within 1 month of diagnosis using rapid case ascertainment and were invited to participate in the study, regardless of their personal or family cancer histories. The present analysis is restricted to non-Hispanic White women with invasive cancer.

Participants from both studies completed similar family history questionnaires and provided blood or mouthwash samples for DNA analysis. The family history questionnaires elicited information on date of birth, vital status, date of death or last observation, and dates and types of all site-specific cancers for each of the patient's first-degree relatives. When possible, reports of breast or ovarian cancer in relatives were verified either by interviewing the relatives themselves, if they were living, or by obtaining medical records, or both.

The present analysis is restricted to the families of non-Hispanic White patients with invasive cancers of the breast or ovary. Risk estimates for the relatives were obtained by weighting the observed and expected cancer counts among a patient's relatives according to the patient's category (hereditary or nonhereditary), with the weight for a category inversely proportional to the fraction of all screened patients in that category who completed a family history questionnaire. In the ovarian cancer study, patients whose cancers are likely to be hereditary (i.e., ovarian cancer diagnosis before age 40 years, prior breast cancer, or at least one first-degree relative with ovarian cancer or with breast cancer diagnosed before age 50 years) completed the family history questionnaires at a higher frequency (88%) than did other patients (84%). To reduce potential selection bias in mutation prevalence estimates that might arise from this differential testing rate, we also classified ovarian cancer patients as either "hereditary" (category A) or "nonhereditary" (category B), and weighted patient counts in each category in proportion to the inverse of the testing rate for the category.

Figure 1 gives the distribution of screened patients in the two categories (A and B) by cancer site. The figure also shows the numbers of patients who were invited to complete the family history questionnaires, the numbers who completed it, and of these, the numbers who were tested for BRCA1 and BRCA2 mutations. Further details concerning study protocol and response rates can be found in John et al. (11) for the breast cancer study and McGuire et al. (14) for the ovarian cancer



- a) All patients whose cancers are likely to be hereditary (see text).
- b) All other screened patients.
- c) Family History Questionnaire.
- d) Breast cancer patients were tested for BRCA1 and BRCA2 mutations. Ovarian cancer patients were tested only for BRCA1 mutations.
- e) Category A sampling weights:  $764/600 = 1.3$  for breast cancer patients and  $95/84 = 1.1$  for ovarian cancer patients.
- f) Category B sampling weights:  $1713/69 = 24.8$  for breast cancer patients and  $318/255 = 1.2$  for ovarian cancer patients.

**Figure 1.** Distribution of screened non-Hispanic White patients with invasive cancers of the breast or ovary. Breast cancer patients were tested for BRCA1 and BRCA2 mutations; ovarian cancer patients were tested only for BRCA2 mutations.

study. Both study protocols were approved by all the institutions involved with the research. These included the Northern California Cancer Center, Stanford University, and the Dana-Farber Cancer Institute for the breast cancer study; and Stanford University, the Northern California Cancer Center, and the Roswell Park Cancer Institute for the ovarian cancer study.

**Laboratory Analysis.** For ovarian cancer patients, heparinized peripheral blood or buccal cells from mouthwash rinse were obtained in study participants' homes or at other locations of their convenience. Genomic DNA was isolated from blood leukocytes using the Puregene kit (Gentra Systems, Minneapolis, MN) and from exfoliated cells in mouthwash samples (15). For breast cancer patients, genomic DNA was isolated from whole blood or lymphoblastoid cell lines using a modification of the Miller salting out procedure (16).

BRCA1 and BRCA2 testing was done using one of three methods: (a) full sequencing by Myriad Genetics; (b) two-dimensional gene scanning (17); and (c) and high-throughput heteroduplex detection. For ovarian cancer patients, BRCA1 testing was done using single-strand conformation polymorphism (18, 19) in conjunction with the protein truncation test (20). Specifically, SSCP was used to test the entire coding region of the gene, and exon 11 also was tested using protein truncation test. BRCA2 testing was not done. Variants were designated deleterious according to the criteria described on the Breast Cancer Information Core website.

**Statistical Analysis.** We used Poisson regression models to estimate standardized incidence ratios (SIRs) for breast and ovarian cancer in patients' relatives, and to evaluate how these SIRs vary with patient characteristics (cancer site, BRCA1 and BRCA2 mutation status, and age at diagnosis). We describe the methods for inferring breast cancer SIRs; the methods for ovarian cancer SIRs are similar.

**Table 1. Distribution of breast and ovarian cancer patients according to age at diagnosis and BRCA mutation status, by cancer site**

	Breast cancer patients, n (%)	Ovarian cancer patients, n (%)
Age (y)		
<40	150 (23)	29 (9)
40-49	176 (26)	91 (27)
50-65	343 (51)	219 (64)
BRCA status		
BRCA1-positive	18 (3)	29 (9)
BRCA2-positive	15 (2)	—*
Negative	491 (74)	259* (76)
Untested	145 (21)	51 (15)
All patients	669 (100)	339 (100)

\*BRCA2 testing was not done on ovarian cancer patients.

For a given model, we assigned to each patient a column vector  $x$  of coded covariates determined by the model. We then modeled the breast cancer incidence rate for a patient's relative as  $\lambda(x) = \lambda_0 \exp(\beta x)$ . Here,  $\lambda_0$  is the baseline rate in the female non-Hispanic White U.S. population, specific for the relative's age and calendar year,  $x$  is the patient's covariate vector, and  $\beta$  is a row vector of fitted regression coefficients. Under this model,  $\exp(\beta x)$  represents the SIR for relatives of patients with covariates  $x$ . We used as baseline rates Surveillance, Epidemiology, and End Results rates for breast and ovarian cancer for the period 1975 to 2000. For years before 1975, we used the Connecticut Tumor Registry rates. Because Connecticut Tumor Registry data before 1940 are considered less reliable than those in later years, we used 1940 to 1944 rates for all periods before 1940.

We assumed that, given the patient's covariates, her relatives' times to breast cancer are mutually independent, and independent of times to censoring. Under these assumptions, maximum likelihood estimates of the log relative risk  $\beta$  can be obtained by analyzing the total breast cancer count in a patient's relatives as a Poisson variable with mean  $\exp(\beta x)e$ , where  $e$  is the total expected breast cancer incidence in her relatives based on the U.S. incidence rates (21, 22). The expected breast cancer incidence for each relative was obtained by integrating the appropriate U.S. age- and calendar year-specific rates from birth until the relative's reference age. The latter was defined as her age at breast cancer diagnosis, at death, at age 85 years, or at the patient's cancer diagnosis, whichever was earliest. Because patients' mothers had to live long enough to bear them, we excluded cancers and time at risk contributed by mothers before the patients' births.

Each patient's contribution to the Poisson loglikelihood has the form  $o\beta x - \exp(\beta x)e$ , where  $o$  and  $e$  are the observed and expected cancer counts among her relatives. To accommodate the two-stage sampling of patients, we weighted this contribution by the inverse of the sampling fraction assigned to the patient, specific for her cancer site (breast versus ovary) and

sampling category (hereditary versus nonhereditary). We then estimated  $\beta$  by maximizing the resulting Horvitz-Thompson pseudo-loglikelihood. The maximum pseudo-loglikelihood estimate  $\hat{\beta}$  is asymptotically unbiased, provided that, within each sampling category, the breast and ovarian cancer histories in families of patients who completed a family history questionnaire represent those in families of all screened patients in the category (12, 13). A consistent estimate  $V$  for the asymptotic covariance matrix of  $\hat{\beta}$  is described in Appendix 1. Confidence intervals (CIs) for SIRs were obtained by exponentiating the CIs for their logs using standard errors determined by the matrix  $V$ . All  $P$  values are two-sided.

## Results

Table 1 shows the distribution of patients according to BRCA1 and BRCA2 mutation status and age at diagnosis, by cancer site (breast or ovary). Eighteen (2.7%) breast cancer patients and 29 (8.5%) ovarian cancer patients tested positive for deleterious germ line mutations of BRCA1. Fifteen (2.0%) breast cancer patients tested positive for mutations of BRCA2. None of the patients were found to carry mutations of both genes.

Table 2 shows SIRs and 95% CIs for breast cancer risk in families, according to the BRCA mutation status and cancer site of the index patient. Among families of patients with BRCA1 mutations, the SIR was 10.6 (95% CI, 5.2-21.6) for relatives of breast cancer patients, compared with 3.3 (95% CI, 1.4-7.3) for relatives of ovarian cancer patients ( $P = 0.02$  for difference by cancer site). No statistically significant differences in relatives' risk were seen with the patient's age at diagnosis, regardless of her cancer site. In families of breast cancer patients with BRCA2 mutations, the breast cancer SIR was 4.4 (95% CI, 1.1-16.9), independent of patients' ages at diagnosis. In families of patients who were negative or untested for BRCA mutations, breast cancer risks were elevated only if the patient was diagnosed with breast cancer. For these women, the breast cancer SIR was 1.5 (95% CI, 1.2-1.8) overall, and it was higher for relatives of patients diagnosed before age 40 years (SIR, 2.8; 95% CI, 1.5-5.3) than for relatives of patients diagnosed later (SIR, 1.4; 95% CI, 0.6-3.4,  $P < 0.01$  for difference by age at diagnosis).

SIRs for ovarian cancer are shown in Table 3. Among families segregating BRCA1 mutations, risk was higher for relatives of ovarian cancer patients (SIR, 11.3; 95% CI, 3.6-35.9) than for relatives of breast cancer patients (SIR, 7.9; 95% CI, 1.2-53.0), although the difference was not statistically significant ( $P = 0.37$ ). Among families of breast cancer patients with BRCA2 mutations, the ovarian cancer SIR was 4.6 (95% CI, 0.6-38.0). In families of patients who were negative or untested for BRCA mutations, ovarian cancer risks were elevated only if the patient was diagnosed with ovarian cancer. For these women, the ovarian cancer SIR was 1.9 (95% CI, 1.0-4.0). None of the ovarian cancer SIRs differed by the patient's age at diagnosis.

**Table 2. SIRs for breast cancer in first-degree relatives of cancer patients, by patients' cancer site and BRCA mutation status**

Patient's BRCA status	Patient's cancer site								$P^*$
	Breast cancer (n = 670)				Ovarian cancer (n = 342)				
	No.	O	E	SIR (95% CI)	No.	O	E	SIR (95% CI)	
BRCA1-positive	48	17	1.6	10.6 (5.2-21.6)	76	10	3.0	3.3 (1.4-7.3)	0.02
BRCA2-positive	47	12	2.7	4.4 (1.1-16.9)	—†	—	—	—	—
Negative/untested	1,883	387	258.0	1.5 (1.2-1.8)	881	52	47.3	1.1 (0.8-1.6)	0.12
All	1,978	416	260.0	1.6 (1.3-2.0)	957	62	51.7	1.2 (1.0-1.6)	

Abbreviations: O, number of cancers; E, expected number (based on U.S. incidence rates).

\*Two-tailed test of similar SIRs in relatives of breast and ovarian cancer patients.

†BRCA2 testing was not done among ovarian cancer patients.



**Table 3. SIRs for ovarian cancer in first-degree relatives of cancer patients, by patients' cancer site and BRCA mutation status**

Patient's BRCA status	Patient's cancer site								P*
	Breast cancer (n = 670)				Ovarian cancer (n = 342)				
	No.	O	E	SIR (95% CI)	No.	O	E	SIR (95% CI)	
BRCA1-positive	48	2	0.3	7.9 (1.2-53.0)	76	5	0.4	11.3 (3.6-35.9)	0.37
BRCA2-positive	47	2	0.4	4.6 (0.6-38.0)	—†	—	—	—	—
Negative/untested	1,883	34	37.8	0.9 (0.5-1.4)	881	13	6.8	1.9 (1.0-4.0)	0.04
All	1,978	38	42.2	1.0 (0.7-1.4)	957	18	7.2	2.5 (1.6-4.0)	

\*Two-tailed test of similar SIRs in relatives of breast and ovarian cancer patients.  
 †BRCA2 testing was not done among ovarian cancer patients.

Table 4 shows the distribution of patients with BRCA1 mutations according to the position of the mutation within the gene, by patients' cancer site. Relatives' breast and ovarian cancer risks did not differ by the position of the patients' mutation (in exon 11 or elsewhere). In addition, the differences in relatives' breast cancer risk by patients' cancer site remained significant after adjusting for mutation position (data not shown). In relatives of the 15 breast cancer patients who carried BRCA2 mutations, breast cancer risks tended to be lower when the patients' BRCA2 mutations lay in the ovarian cancer cluster region (nucleotides 3,059-6,629) than when the mutation did not. Ovarian cancer risks in relatives did not differ by the position of the patients' BRCA2 mutation. These comparisons are limited by the small number of BRCA2 mutation-positive patients.

**Discussion**

We estimated SIRs for breast and ovarian cancer in first-degree relatives of non-Hispanic White patients with invasive cancers of the breast or ovary. We found that relatives of patients who were negative or untested for BRCA1 and BRCA2 mutations had elevated risks only for the cancer site of the patient. A similar specificity was observed among relatives of patients with BRCA1 mutations: Although all had elevated SIRs for both breast and ovarian cancer, those ascertained through a breast cancer patient had a higher breast cancer SIR and a lower ovarian cancer SIR than did those ascertained through an ovarian cancer patient. We also found that, in families of breast cancer patients without identified BRCA mutations, the breast cancer SIR was higher for relatives of patients with early-onset than late-onset breast cancers. This inverse relationship between the patients' breast cancer ages and their relatives' risks was also found by the Collaborative Group on Hormonal Factors in Breast Cancer (8).

The site-specific correlations and absence of cross-site correlations in families of patients without BRCA mutations suggest the presence of other, unidentified genes responsible for breast or ovarian cancer, but not both. Similar breast-breast correlations have been noted in families ascertained through breast cancer patients and classified as BRCA-negative in Australia (6), Sweden (7), and the United States (10). Relatives'

**Table 4. Distribution of mutation position in patients with BRCA1 mutations, by cancer site**

Exon	Breast cancer, n (%)	Ovarian cancer, n (%)
1-5	7 (39)	12 (41)
6-10	2 (11)	1 (3)
11	5 (28)	12 (41)
12-16	1 (6)	2 (7)
17-24	3 (17)	2 (7)
Total	18 (100)	29 (100)

ovarian cancer risks were evaluated only in the Swedish and U.S. families, and no excess risks were seen, in agreement with the present findings. The findings from all four studies suggest that not all familial aggregation of breast and ovarian cancer can be explained by detectable mutations in BRCA1 and BRCA2. Further work is needed to determine how much of the remainder is due to undetected mutations in these genes and to mutations in other genes, acting either singly or in combination. The breast cancer clustering seen in families of early-onset BRCA-negative breast cancer patients is a promising springboard for such investigation.

We also found cancer site-specific correlations in families that segregate BRCA1 mutations. Specifically, the breast cancer risk was higher and the ovarian cancer risk was (nonsignificantly) lower in families ascertained through a breast cancer patient than in those ascertained through an ovarian cancer patient. These differences remained after adjusting for mutation position within the gene. Similar site specificity was seen for the breast cancer risk, but not for the ovarian cancer risk, among BRCA1-positive families in pooled data from several countries (9). The latter pooled data also showed higher breast cancer risk and lower ovarian cancer risk in BRCA2-positive families ascertained through a breast cancer patient compared with those ascertained through an ovarian cancer patient. Similar to the pooled analysis, this study found that in relatives of breast cancer patients who carry BRCA2 mutations, ovarian cancer risks do not depend on whether the patients' BRCA2 mutations lay in the ovarian cancer cluster region or not, whereas breast cancer risks appear lower if the patients' BRCA2 mutations lay within this region. These relationships suggest the presence of genes that modify the effects of BRCA1 and BRCA2 mutations on breast and ovarian carcinogenesis.

Some limitations of this study warrant comment. First, although we attempted to control false-positive reports of relatives' breast and ovarian cancers by verifying such reports whenever possible, we could have missed some unreported cancers, leading to false negatives. Underreporting of ovarian cancer by the breast cancer patients and of breast cancer by the ovarian cancer patients might explain some of the observed cancer site specificity within families. However, the extent of such underreporting, if any, is likely to be small. Comparisons of self-reported histories of breast and ovarian cancer to reports by first-degree relatives have shown high reliability for both cancer sites (23; data not shown). Second, we could not adjust the SIR estimates for unmeasured nongenetic risk factors, such as oral contraceptive use, age at menarche, and age at first birth. Although unmeasured shared risk factors specific for cancer site might account for some of the site specificity within families, the magnitude of their contribution is likely to be small (24). Third, we could not test all patients for BRCA1 and BRCA2 mutations: Some did not provide DNA and the ovarian cancer patients who did provide DNA were tested only for BRCA1 mutations. Moreover, among tested patients, the laboratory methods used

to identify BRCA mutations are not perfectly sensitive. For example, reports of the sensitivity of single-strand conformation polymorphism have varied from 65% (25) to 94% (26), a variability that may reflect differences in the primers used, the electrophoresis conditions and the investigator's experience. BRCA1 testing in the ovarian cancer patients was done using single-strand conformation polymorphism augmented by protein truncation test in exon 11 (which comprises 60% of the coding region). The sensitivity of this combination has not been evaluated. This incomplete testing contaminates the subgroup of BRCA1-negative and BRCA2-negative and untested patients with unidentified mutation carriers and could have induced elevated SIR estimates among their relatives. However, the magnitude of the contamination and resulting bias are unlikely to be large because mutation frequencies are low, even among cancer patients. For example, using the BRCA2 prevalence and penetrance estimates of Antoniou et al. (9), we estimate that the present sample of ovarian cancer patients contains at most two to three unidentified BRCA2 mutation carriers. Thus, any contamination by unidentified BRCA2 carriers is unlikely to have had a major effect on the results.

In conclusion, the findings of this study concerning the magnitudes of risks in first-degree relatives of patients with breast and ovarian cancer, specific for BRCA1 and BRCA2 mutation status, may help guide risk counseling strategies. The findings support the accumulating body of data suggesting that, regardless of her BRCA1 and BRCA2 mutation status, relatives of a breast cancer patient have higher breast cancer risk and lower ovarian cancer risk than do relatives of an ovarian cancer patient. This cancer site specificity suggests the presence of risk-modifying genes whose roles are specific to breast or ovarian cancer, some of which interact with mutations of BRCA1 or BRCA2. Further research is needed to identify these genes and to understand how they act. Such information should elucidate breast and ovarian cancer carcinogenesis and refine cancer prevention strategies.

## Appendix 1

Here, we describe how we estimated the asymptotic covariance matrix of the maximum pseudolikelihood estimate for  $\hat{\beta}$ , the vector of regression coefficients in the log SIR. To do so, we let  $\bar{Q}_{ij}$  denote the set of patients with cancer site  $i$  ( $i = 1$  for breast cancer and  $i = 0$  for ovarian cancer) who belong to sampling category  $j$  ( $j = A$  for hereditary patients and  $j = B$  for nonhereditary patients). We also let  $p_{ij}$  denote the proportion of patients who completed a family history questionnaire among all screened patients with site  $i$  in category  $j$ . Let  $o_n$  and  $e_n$  denote the observed and expected cancer counts, respectively, among the relatives of the  $n$ th patient; let  $x_n$  denote her covariate vector; and let  $a_n = \sum_{i=0,1} \sum_{j=A,B} p_{ij}^{-1} 1(n \in \bar{Q}_{ij})$  denote the inverse of her sampling fraction. Here,  $1(E)$  is the indicator function assuming the value 1 if event  $E$  is true and 0 otherwise. With this notation, our estimate is

$$V = X^{-1} + X^{-1} Y X^{-1}$$

where

$$X = \sum_n a_n e_n \exp(\beta x_n) x_n x_n^T$$

and

$$Y = \sum_{i=0,1} \sum_{j=A,B} p_{ij}^{-1} (p_{ij}^{-1} - 1) S_{ij}$$

with

$$S_{ij} = \sum_{n \in \bar{Q}_{ij}} [o_n - \exp(\beta x_n) e_n]^2 x_n x_n^T$$

In these expressions,  $\beta$  is evaluated at  $\hat{\beta}$

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