Am. J. Hum. Genet. 68:700–710, 2001

Prevalence and Penetrance of Germline *BRCA1* and *BRCA2* Mutations in a Population Series of 649 Women with Ovarian Cancer

Harvey A. Risch,[1] John R. McLaughlin,[2,3] David E. C. Cole,[4] Barry Rosen,[5] Linda Bradley,[5] Elaine Kwan,[6] Elaine Jack,[6] Danny J. Vesprini,[6] Graciela Kuperstein,[6] John L. A. Abrahamson,[6] Isabel Fan,[6] Betty Wong,[4] and Steven A. Narod[3,6]

[1]Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven; and [2]Samuel Lunenfeld Research Institute, Toronto, and University of Toronto Departments of [3]Public Health Sciences and [4]Clinical Biochemistry, [5]Toronto Hospital Department of Gynecology, and [6]Women's College Hospital Department of Medicine, Toronto

A population-based series of 649 unselected incident cases of ovarian cancer diagnosed in Ontario, Canada, during 1995–96 was screened for germline mutations in *BRCA1* and *BRCA2*. We specifically tested for 11 of the most commonly reported mutations in the two genes. Then, cases were assessed with the protein-truncation test (PTT) for exon 11 of *BRCA1*, with denaturing gradient gel electrophoresis for the remainder of *BRCA1*, and with PTT for exons 10 and 11 of *BRCA2*. No mutations were found in all 134 women with tumors of borderline histology. Among the 515 women with invasive cancers, we identified 60 mutations, 39 in *BRCA1* and 21 in *BRCA2*. The total mutation frequency among women with invasive cancers, 11.7% (95% confidence interval [95%CI] 9.2%–14.8%), is higher than previous estimates. Hereditary ovarian cancers diagnosed at age <50 years were mostly (83%) due to *BRCA1*, whereas the majority (60%) of those diagnosed at age >60 years were due to *BRCA2*. Mutations were found in 19% of women reporting first-degree relatives with breast or ovarian cancer and in 6.5% of women with no affected first-degree relatives. Risks of ovarian, breast, and stomach cancers and leukemias/lymphomas were increased nine-, five-, six- and threefold, respectively, among first-degree relatives of cases carrying *BRCA1* mutations, compared with relatives of noncarriers, and risk of colorectal cancer was increased threefold for relatives of cases carrying *BRCA2* mutations. For carriers of *BRCA1* mutations, the estimated penetrance by age 80 years was 36% for ovarian cancer and 68% for breast cancer. In breast-cancer risk for first-degree relatives, there was a strong trend according to mutation location along the coding sequence of *BRCA1*, with little evidence of increased risk for mutations in the 5′ fifth, but 8.8-fold increased risk for mutations in the 3′ fifth (95%CI 3.6–22.0), corresponding to a carrier penetrance of essentially 100%. Ovarian, colorectal, stomach, pancreatic, and prostate cancer occurred among first-degree relatives of carriers of *BRCA2* mutations only when mutations were in the ovarian cancer–cluster region (OCCR) of exon 11, whereas an excess of breast cancer was seen when mutations were outside the OCCR. For cancers of all sites combined, the estimated penetrance of *BRCA2* mutations was greater for males than for females, 53% versus 38%. Past studies may have underestimated the contribution of *BRCA2* to ovarian cancer, because mutations in this gene cause predominantly late-onset cancer, and previous work has focused more on early-onset disease. If confirmed in future studies, the trend in breast-cancer penetrance, according to mutation location along the *BRCA1* coding sequence, may have significant impact on treatment decisions for carriers of *BRCA1*-mutations. As well, *BRCA2* mutations may prove to be a greater cause of cancer in male carriers than previously has been thought.

Introduction

Germline mutations in *BRCA1* (MIM 113705) and *BRCA2* (MIM 600185) account for cancer predisposition in the majority of families with the breast ovarian-cancer syndrome (Narod et al. 1995; Frank et al. 1998). The probability of finding a mutation in a woman with ovarian cancer increases with the number of related cases of ovarian or early-onset breast cancer in her family. It is not yet clear what proportions of ovarian cancer in unselected general populations are due to mutations in these genes. Some estimates have been made for *BRCA1* (Matsushima et al. 1995; Takahashi et al. 1995; Stratton et al. 1997; Rubin et al. 1998; Janezic et al. 1999), but the fraction attributable to *BRCA2* is less well known (Foster et al. 1996; Takahashi et al. 1996; Khoo et al. 2000; Van der Looij et al. 2000). Furthermore, previous studies have been limited by small sample sizes and by

Received September 18, 2000; accepted for publication January 2, 2001; electronically published February 15, 2001.

Address for correspondence and reprints: Dr. Harvey A. Risch, Yale University School of Medicine, Department of Epidemiology and Public Health, 60 College Street, P.O. Box 208034, New Haven, CT 06520-8034. E-mail: harvey.risch@yale.edu

0002-9297/2001/6803-0015$02.00

potentially unrepresentative case sampling through the use of early-onset, hospital-based, prevalent or self-referred cases.

Accurate knowledge of the proportion of cases carrying mutations in these genes is important in order to offer genetic screening and counseling to women with either ovarian cancer or family histories of cancer. It is not yet known whether cases of ovarian cancer associated with *BRCA2* mutations differ from those associated with *BRCA1* mutations, in terms of age at diagnosis or of histological type, for example. To address these questions, we studied 649 unselected incident cases of ovarian cancer in Ontario and examined the presence of mutations with respect to age, histology, ethnicity, and family history.

Subjects and Methods

Subjects

All patients in the province of Ontario who had been diagnosed with epithelial ovarian tumors, from January 1995 through December 1996, were identified by monitoring of acquisitions at the Ontario Cancer Registry. For each case, the investigators reviewed pathology reports to determine eligibility and histological type. Patients were 20–79 years of age and were resident in Ontario at the time of diagnosis of a new primary borderline or invasive epithelial ovarian tumor. Of 1,024 eligible cases, we were able to obtain and test blood samples from 649 (63%). The reasons for nonparticipation of the other 375 cases included death (197 cases), subject refusal (76 cases), severity of illness (57 cases), physician refusal (5 cases), and inability to be found (8 cases). Family histories were taken by telephone interview. Styrofoam-packed venipuncture kits with consent forms were mailed to subjects, who had blood samples drawn locally and, along with signed consent forms, returned by prepaid courier. All participants were offered the option of receiving their genetic-testing results, in the context of a counseling clinic, via either the study team or counseling clinics elsewhere in the province. The study was approved by the institutional review boards of the University of Toronto and Yale University.

BRCA1 *and* BRCA2 *Analysis*

Lymphocyte DNA was prepared from whole blood by standard procedures. All samples were screened for 11 common mutations (seven in *BRCA1* and four in *BRCA2*). Included were the three mutations common to Ashkenazi Jews and others of eastern-European extraction, as well as the six mutations that previously had been identified in the French Canadian population (Tonin et al. 1998). These founder mutations were assayed by a rapid multiplex method (Kuperstein et al. 2000). We separately tested for the presence of the *BRCA1* exon 13 6-kb duplication (Puget et al. 1999) and for the *BRCA1* exon 7 mutation, G546T.

Exon 11 of *BRCA1* and exons 10 and 11 of *BRCA2* were then screened by the protein-truncation test (PTT). Primer sequences used to amplify overlapping fragments were obtained from the Breast Cancer Information Core (BIC). PTT was performed by the TNT™ rabbit reticulocyte lysate system (Promega), with [^{35}S]-methionine/cysteine (New England Nuclear) being incorporated for protein detection.

Cases that, by the previous testing, had been found not to carry mutations were then screened for additional *BRCA1* mutations, by fluorescent multiplex denaturing gradient gel electrophoresis (DGGE) (G.K., E.J., H.A.R., J.R.M., and S.A.N., unpublished data). All of the remaining coding exons, the exon-intron boundaries, and the beginning and end of exon 11 were included; noncoding exon 1a and 1b and the noncoding part of exon 24 were excluded.

In all cases, variants identified by PTT and DGGE were confirmed by direct DNA sequencing (Promega). We believe that all of the observed mutations are deleterious. The various founder mutations are known to be deleterious, and PTT identifies mutations associated with shortened, nonfunctional proteins. The specific mutations found by DGGE have all been seen previously and, as documented in the Breast Cancer Information Core (BIC) database, are known to be deleterious.

Statistical Analysis

Confidence limits for mutation frequencies were calculated under the assumption of binomial distributions of the observed numbers of cases. Relative risks (RRs) of cancer in family members, according to *BRCA1* and *BRCA2* mutation status of the proband, were calculated through proportional-hazards regression, with the baseline taken to be relatives of cases not carrying mutations. Each family member was assumed to be at risk until either diagnosis of the cancer of interest, death, or age at the time when the family history was reported. Cumulative incidence of cancer among relatives was obtained by calculating the estimated survival function to age 80 years, from the regression models, and then subtracting it from unity. Penetrance estimates for carriers of mutations were calculated by the method of Wacholder et al. (1998).

Results

Among the 515 women in our study who had invasive cancers, 60 mutations (11.7% [95% confidence interval [95%CI] 9.2%–14.8%) were identified, including 39 mutations in *BRCA1* and 21 in *BRCA2* (table 1). No

Table 1

***BRCA1* and *BRCA2* Mutations Detected in 649 Women with Ovarian Cancer**

Gene and Ethnicity (Age of Patient)[a]	Region	Mutation
BRCA1:		
Ashkenazi Jewish (37 years)	Exon 2	185delAG
Ashkenazi Jewish (41 years)	Exon 2	185delAG
Indo-Pakistani (46 years)	Exon 2	185delAG
Ashkenazi Jewish (59 years)	Exon 2	185delAG
Ashkenazi Jewish (66 years)	Exon 2	185delAG
British Isles (49 years)	Intron 4	IVS4−1 G→T
Slavic (45 years)	Exon 5	T300G
Mixed European (50 years)	Exon 5	T300G
Slavic (42 years)	Exon 11	962del4
Mixed European (65 years)	Exon 11	962del4
French Canadian (50 years)	Exon 11	G1081A
Mixed European (52 years)	Exon 11	G1081A
British Isles (60 years)	Exon 11	1294del40
Italian (43 years)	Exon 11	1479delAG
Italian (62 years)	Exon 11	1479delAG
Indo-Pakistani (49 years)	Exon 11	1768delA
Mixed European (47 years)	Exon 11	2080delA
British Isles (48 years)	Exon 11	2190delA
Mixed European (55 years)	Exon 11	2524delTG
French Canadian (52 years)	Exon 11	2800delAA
British Isles (42 years)	Exon 11	2819delTT
French Canadian (39 years)	Exon 11	2953delGTA/insC
Mixed European (78 years)	Exon 11	T3053G
British Isles (54 years)	Exon 11	3375insGA
French Canadian (53 years)	Exon 11	3768insA
Slavic (42 years)	Exon 11	3819delGTAAA
Italian (39 years)	Exon 11	3875delGTCT
Italian (57 years)	Exon 11	3875delGTCT
British Isles (46 years)	Exon 11	3879insT
Italian (71 years)	Exon 12	G4236T
British Isles (46 years)	Exon 13	6-kb duplication
Mixed European (46 years)	Exon 13	6-kb duplication
British Isles (49 years)	Exon 13	6-kb duplication
French Canadian (47 years)	Exon 13	C4446T
Mixed European (48 years)	Intron 16	IVS16+6 T→G
Mixed European (53 years)	Exon 20	C5370T
Slavic (46 years)	Exon 20	5382insC
Greek (50 years)	Exon 20	5382insC
Mixed European (72 years)	Exon 20	5382insC
BRCA2:		
Mixed European (45 years)	Exon 10	1257delA
Mixed European (51 years)	Exon 11	2814del7
British Isles (67 years)	Exon 11	3908delTG
British Isles (72 years)	Exon 11	4075delGT
Italian (62 years)	Exon 11	4510insT
British Isles (62 years)	Exon 11	4706del4
Philippines (49 years)	Exon 11	4859delA
British Isles (71 years)	Exon 11	T5087G
Mixed European (51 years)	Exon 11	5102delAA
British Isles (73 years)	Exon 11	5102delAA
Italian (65 years)	Exon 11	5302insA
British Isles (56 years)	Exon 11	C5910G
Mixed European (44 years)	Exon 11	6174delT
Ashkenazi Jewish (53 years)	Exon 11	6174delT
Mixed European (66 years)	Exon 11	6181delTC
British Isles (57 years)	Exon 11	6503delTT
Mixed European (51 years)	Exon 11	6602insA
Japanese (65 years)	Exon 11	6633del5 (delCTTAA)
Mixed European (60 years)	Exon 11	6872del4 (delACTC)
French Canadian (48 years)	Exon 20	8765delAG
Mixed European (40 years)	Exon 27	9894delT

[a] All ethnicities are non-Jewish, except as indicated.

mutations were seen in the 134 women with borderline tumors. All 60 women carrying mutations were unrelated. Of the 39 cases having *BRCA1* mutations, 11 were identified in the screens for individual mutations, 20 were identified by PTT of exon 11, and 8 were identified by DGGE. We observed 26 distinct *BRCA1* mutations, including 8 that were identified more than once. Two intronic mutations (IVS4−1 G→T and IVS16+6 T→G) were found.

In our case series, five women carried the *BRCA1* 185delAG mutation and three carried the 5382insC mutation. One of the five women with the 185delAG mutation was non-Jewish, of Pakistani ancestry. None of the three women with the 5382insC mutation reported being Jewish or were known to be Jewish. We also identified three women with the exon 13 6-kb duplication mutation. All three women had either complete or partial British Isles ancestry. Interestingly, we identified two women of Italian ethnicity who had the apparently novel 1479delAG mutation.

In the testing for *BRCA2*, 19 of the 21 mutations were identified by PTT. One founder mutation (8765delAG) was observed, in a French Canadian woman. Nineteen of the 21 *BRCA2* mutations were unique. Two women with either British Isles or European ancestry carried 5102delAA mutations. The 6174delT mutation was also seen in two cases, one of whom was Jewish. Only 13 of the 21 *BRCA2* mutations occurred within the ovarian cancer–cluster region (OCCR; most recently defined as nucleotides 4075–6503 inclusive) of exon 11 (Thompson and Easton 2001).

Frequency of mutations, by ethnic group, is shown in table 2. High mutation frequency among these ovarian-cancer cases was seen for women of Jewish (26%), Italian (24%), and Indo-Pakistani (14%) ancestry. Total mutation frequency for women of British Isles ancestry was 4.7%:2.5% for *BRCA1* mutations and 2.2% for *BRCA2* mutations.

The presence of a mutation could be predicted, to some degree, on the basis of age at diagnosis, histological subtype, and family history. Age at diagnosis related both to the presence of a mutation and to the particular gene involved (table 3). Women diagnosed at age 40–50 years had the highest frequency of mutations (18.4%), which was more than double that of women whose age at diagnosis was outside this range (6.8%); women with cancer at age <40 years had a lower prevalence of mutations (4.2%).

There were marked differences between the age distribution of the cases with *BRCA1* mutations and that of cases with *BRCA2* mutations. The average age at diagnosis of the *BRCA1*-positive cases was 51.2 years, which was less than the age at diagnosis of the cases in whom mutations were not detected (55.6 years; $P = .041$); the average age at diagnosis of the 21 cases with

Table 2

Frequency of Mutations in Cases of Ovarian Cancer, by Ethnicity

	No. (%) Positive for Mutations in		
Ethnicity	*BRCA1*	*BRCA2*	Either
French Canadian (n = 60)	5 (8.3)	1 (1.7)	6 (10.0)
Ashkenazi Jewish (n = 19)	4 (21.1)	1 (5.3)	5 (26.3)
Indo-Pakistani (n = 14)	2 (14.3)	0 (0)	2 (14.3)
Chinese, Japanese, etc. (n = 19)	0 (0)	1 (5.3)	1 (5.3)
Italian (n = 29)	5 (17.2)	2 (6.9)	7 (24.1)
Hispanic (n = 17)	0 (0)	0 (0)	0 (0)
British Isles (n = 316)	8 (2.5)	7 (2.2)	15 (4.7)
Mixed European (n = 142)	10 (7.0)	8 (5.6)	18 (12.7)

BRCA2 mutations was 57.5 years, which was similar to the average age at diagnosis of other invasive-cancer cases in whom mutations were not identified (57.8 years). The majority (9/15) of mutations in women diagnosed with ovarian cancer at age >60 years were in *BRCA2;* in contrast, 24 of 29 mutations in women of age <50 years were in *BRCA1*.

All women with borderline or invasive cancers diagnosed in Ontario were eligible for inclusion in our study. Of the total of 649 cases, 134 (20.6%) had borderline tumors. As we have said, none of these women carried a mutation (table 4). Fifty-six (93%) of the 60 subjects with mutations had invasive serous cancers (this subgroup represents 53% of the total cases), and women with these cancers were almost twice as likely to carry *BRCA1* mutations as to carry *BRCA2* mutations. In addition, four women with endometrioid tumors were found to be carriers of mutations, and no woman with a mucinous tumor was found to be a carrier.

Family history also predicted the presence of a mutation (table 5). Women with first-degree relatives affected by breast or ovarian cancer had a mutation frequency of 19% (27/144). Mutations were also observed in 33 (6.5%) of 505 women who reported no first-degree relatives with breast or ovarian cancer. Using a definition of "potential familiality" to denote the presence of either (*a*) a first-degree relative either with ovarian cancer or with breast cancer at age <60 years or (*b*) a combination of two or more first- or second-degree relatives with breast or ovarian cancer, we found that 26% of such cases carry mutations. Women with both invasive serous cancers and potential familiality had the highest frequency of mutation, 36% (36/99). Twenty-eight (72%) of 39 women with *BRCA1* mutations had both invasive serous tumors and potential familiality, compared with 8 (38%) of 21 with *BRCA2* mutations (P = .011).

We estimated the cumulative incidence, by age 80 years, of various types of cancer among first-degree relatives of the women with ovarian cancer, according to the carriage of either *BRCA1* mutation or *BRCA2* mutation by the proband case (table 6). Compared with relatives of noncarriers, the RR for ovarian cancer among female relatives of carriers of *BRCA1* mutations was 8.6 (95%CI 4.1–18). This elevated risk did not differ significantly between mothers (RR 8.6 [95%CI 3.3–23]) and sisters (RR 7.1 [95%CI 2.3–22]). In total, 19% of female first-degree relatives of carriers of *BRCA1* mutations were estimated to be diagnosed with ovarian cancer by age 80 years, which gives a 36% lifetime penetrance for carriers.

A significant increase in risk of breast cancer in relatives of carriers of *BRCA1* mutations was also found (RR 4.8 [95%CI 3.0–7.6]), and this too was not significantly different between mothers (RR 3.5 [95%CI 1.7–7.2]) and sisters (RR 5.3 [95%CI 2.6–11]). In total, 39% of female first-degree relatives of carriers of *BRCA1* mutations got breast cancer by age 80 years, for an estimated carrier penetrance of 68%. As well, we observed significantly increased risks of stomach cancer and leukemias/lymphomas among relatives of carriers of *BRCA1* mutations (RR 6.2 [95%CI 2.0–19.0] and RR 2.6 [95%CI 1.02–6.6], respectively) and, among female first-degree relatives, increased risks for all cancer sites combined (RR 3.6 [95%CI 2.5–5.0]). The estimated penetrance by age 80 years, of cancer of any type among female carriers of *BRCA1* mutations, is thus nearly 100%.

A lesser increase in risk of ovarian cancer was observed among relatives of carriers of *BRCA2* mutations (RR 2.5 [95%CI 0.59–11]), and this also did not seem to differ between mothers and sisters. We did not find an excess of breast cancer among relatives of carriers of *BRCA2* mutations, with only 4 cases reported among 79 female first-degree relatives. However, significantly increased risk was seen for colorectal cancer (RR 2.5 [95%CI 1.02–6.3]), corresponding to a carrier lifetime-penetrance estimate of 16%. In addition, for all cancer sites combined, increased risk was observed among male first-degree relatives (RR 1.7 [95%CI 0.97–3.1]), for a

Table 3

Frequency of Mutations in Cases of Ovarian Cancer, by Age at Diagnosis

	No. (%) Positive for Mutations in		
Age Group	*BRCA1*	*BRCA2*	Either
≤40 years (n = 96)	3 (3.1)	1 (1.0)	4 (4.2)
41≤50 years (n = 136)	21 (15.4)	4 (2.9)	25 (18.4)
51≤60 years (n = 165)	9 (5.5)	7 (4.2)	16 (9.7)
>60 years (n = 252)	6 (2.4)	9 (3.6)	15 (6.0)
All (n = 649)	39 (6.0)	21 (3.2)	60 (9.2)

Table 4

Frequency of Mutations in Cases of Ovarian Cancer, by Histology

Histology	No. (%) Positive for Mutations in		
	BRCA1	*BRCA2*	Either
Invasive (n = 515)	39 (7.6)	21 (4.1)	60 (11.7)
Serous (n = 341)	37 (10.9)	19 (5.6)	56 (16.4)
Endometrioid (n = 94)	2 (2.1)	2 (2.1)	4 (4.3)
Mucinous (n = 44)	0 (0)	0 (0)	0 (0)
Other (n = 36)	0 (0)	0 (0)	0 (0)
Borderline (n = 134)	0 (0)	0 (0)	0 (0)

carrier lifetime penetrance of 53%, which is appreciably greater than the 38% calculated for females.

Finally, we were able to examine cancer risk in first-degree relatives according to location of the *BRCA1* or *BRCA2* mutation within the coding sequence of the gene. For this analysis, we assumed the "location" of the *BRCA1* exon 13 6-kb duplication to be at nucleotide 4497, since it results in an abnormal ter1460 in the mRNA (Puget et al. 1999). For *BRCA1,* we found a strong trend for risk of breast cancer in family members to increase with more-downstream location of the mutation (continuous trend P = .0014; a 26% increase in risk with each additional 10% [=559 nucleotides] of downstream distance). There was little evidence to suggest that mutations in the 5′ fifth of the *BRCA1* coding sequence (nucleotides 120–1237) were associated with increased breast-cancer risk (RR 1.3 [95%CI 0.33–5.5]). Mutations in subsequent fifths, however, were increasingly so associated: RR 1.6 (95%CI 0.22–11) for mutations in nucleotides 1238–2355, RR 7.4 (95%CI 3.0–18) within nucleotides 2356–3474, RR 7.2 (95%CI 3.6–14) in nucleotides 3475–4592, and RR 8.8 (95%CI 3.6–22) in nucleotides 4593–5711. These RRs correspond to carrier penetrance estimates, by age 80 years, of 16%, 21%, 97%, 96%, and 100% for mutations in the successive fifths of *BRCA1.* Although, as noted above, we observed little elevation in risk of breast cancer in family members of cases with *BRCA2* mutations in general, we did see an increased risk associated with mutations outside the OCCR (RR 4.2 [95%CI 1.5–11]). This increase was essentially due to mutations occurring distal to the OCCR (RR 4.7 [95%CI 1.5–15]).

For ovarian cancer in first-degree relatives, we observed no differences in risk according to location of the *BRCA1* mutation. In *BRCA2,* ovarian cancers occurred among family members of cases carrying mutations only when the mutations were within the OCCR. For OCCR mutations, the RR was 3.6 (95%CI 0.85–15), which is of borderline statistical significance (P = .08).

We also examined mutation location with respect to colorectal, stomach, pancreatic, and prostate cancer in family members. There were no associations with location of *BRCA1* mutations. However, for probands carrying *BRCA2* mutations, colorectal cancer in family members occurred only when mutations were within the OCCR (RR 3.4 [95%CI 1.4–8.5]); stomach, pancreatic, and prostate cancers in family members also occurred for *BRCA2* mutations only within the OCCR. The RR of ovarian, colorectal, stomach, pancreatic, or prostate cancer in family members, for OCCR *BRCA2* mutations, was 3.1 (95%CI 1.7–5.7; P = .0003). This specificity for OCCR mutations also accounted for the

Table 5

Frequency of Mutations in Cases of Ovarian Cancer, by Family History of Breast or Ovarian Cancer

Family History	No. (%) Positive for Mutations in		
	BRCA1	*BRCA2*	Either
Mother with:			
Breast cancer (n = 58)	9 (15.5)	1 (1.7)	10 (17.2)
Ovarian cancer (n = 20)	6 (30.0)	1 (5.0)	7 (35.0)
Sister with:			
Breast cancer (n = 52)	5 (9.6)	3 (5.8)	8 (15.4)
Ovarian cancer (n = 17)	4 (23.5)	1 (5.9)	5 (29.4)
Any first-degree relative with breast or ovarian cancer (n = 144)	21 (14.6)	6 (4.2)	27 (18.7)
Proband with:			
Previous breast cancer (n = 30)	9 (30.0)	1 (3.3)	10 (33.3)
Potential familiality:[a]			
All case histologies (n = 145)	29 (20.0)	9 (6.2)	38 (26.2)
Invasive serous cases only (n = 99)	28 (28.3)	8 (8.1)	36 (36.4)
No potential familiality (n = 504)	10 (2.0)	12 (2.4)	22 (4.4)

[a] Defined as having either (*a*) a first-degree relative either with ovarian cancer or with breast cancer at age <60 years or (*b*) a combination of two or more first- or second-degree relatives with breast cancer or ovarian cancer.

Table 6

Cumulative Incidence and Relative Risk of Cancer among First-Degree Relatives, by Proband Mutation Status and Cancer Site

	Cumulative Incidence of Cancer, by Age 80 Years, in Relatives of Cases with[a]					
	No Mutations		*BRCA1* Mutations		*BRCA2* Mutations[b]	
Site	Incidence (%)	RR	Incidence (%)	RR (95%CI)	Incidence (%)	RR (95%CI)
Ovary	2.5	1.0	19.4	8.6 (4.1–18)	6.1	2.5 (.59–11)
Breast	9.9	1.0	39.1	4.8 (3.0–7.6)	11.9	1.2 (.45–3.3)
Colon/rectum	4.2	1.0	2.9	.70 (.17–2.8)	10.3	2.5 (1.0–6.3)
Stomach	.80	1.0	4.9	6.2 (2.0–19)	1.8	2.3 (.30–18)
Lung	3.7	1.0	4.5	1.2 (.38–3.9)	4.2	1.1 (.27–4.6)
Kidney, bladder	1.3	1.0	2.6	2.0 (.46–8.4)	1.9	1.4 (.19–11)
Leukemias, etc.	1.9	1.0	4.8	2.6 (1.0–6.6)	…	…
Prostate	6.3	1.0	3.1	.48 (.066–3.5)	9.8	1.6 (.38–6.5)
Pancreas	1.1	1.0	1.6	1.5 (.20–11)	2.2	2.1 (.27–16)
Uterus	1.5	1.0	2.3	1.5 (.20–12)	…	…
All cancers, in:						
Female relatives	26.3	1.0	66.3	3.6 (2.5–5.0)	32.1	1.3 (.69–2.3)
Male relatives	24.7	1.0	30.9	1.3 (.76–2.2)	38.9	1.7 (.97–3.1)

Note.—Cancers included are those reported in 291 relatives of cases with *BRCA1* mutations, 160 relatives of cases with *BRCA2* mutations, and 4,378 relatives of cases with no mutations. Analysis of ovarian, breast, and uterine cancers was in female relatives only; analysis of prostate cancer was in male relatives only; analysis of other cancers, as well as of all cancers combined, was in relatives of both sexes.

[a] RRs and 95%CIs were obtained from proportional-hazards regression models, with relatives of cases not carrying mutations as baseline; in the regression models, the cumulative incidence was obtained by subtracting, from unity, the estimated survival (to age 80 years).

[b] An ellipsis (…) indicates that no cancers were reported among family members of cases.

increased risk of cancers at all sites that was seen among males (RR 1.9 [95%CI 1.00–3.6]; penetrance 55%).

Discussion

Our study of 649 unselected cases of ovarian cancer, is the largest population-based series to date and is comprehensively representative of all incident ovarian cancers, both borderline and invasive, arising in a defined geographic area of North America. We did not restrict probands to early-onset cases. Our study tested both for mutations in *BRCA1* and for mutations in *BRCA2* and obtained detailed family histories of all cases. Because our study was population based, the family histories are representative of those of all cases of ovarian cancer—and not of families selected for high occurrence of cancer, as is observed in studies of subjects in genetic-testing clinics.

Nevertheless, our overall case-participation rate (63%) was a little lower than desired, and the 19% nonparticipation due to death prior to subject contact slightly increased the proportion of cases with borderline versus invasive histology. It is possible that our enrolled subjects could therefore underrepresent those cases of ovarian cancer that had worse prognoses, such as cases occurring at a younger age or having poor-prognosis histological types (e.g., clear cell and mucinous). The age distribution of our cases (table 3), however, is similar to that seen in ovarian cancer in general in the United States (Ries et al. 1994), although the percentage of cases of age <50 years was slightly greater, and the percentage of cases at age >60 years was slightly smaller, than that in the United States. Our distribution of histologies (clear cell 4.5%; mucinous 15%) is also very similar to distributions in the United States and Canada (Risch et al. 1996). Our sample of cases thus appears to be highly representative of cases of ovarian cancer in North America.

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. Such information may be less valid than confirmed cancer identifications, but errors are likely to be nondifferential between carriers of mutations and noncarriers and among carriers of the various types of mutations, tending to produce observed associations shifted toward the null. We restricted our penetrance analyses to use only information on first-degree relatives, for whom the reported cancer histories are likely to be the most accurate.

Finally, even though we tested 649 subjects, only 60 with mutations were identified, limiting the statistical power for studying certain associations. In particular, the numbers of families with cases carrying *BRCA2* mutations and ovarian, colorectal, stomach, pancreatic, or

prostate cancers in relatives were small; in most instances, no more than one of these cancers occurred in any given family, making our estimates of confidence intervals and statistical significance reasonably valid. In conclusion, because of the size and strengths of this study, we believe that our results provide the most accurate description of *BRCA1* and *BRCA2* associations in ovarian cancer that thus far has been published.

In genetic screening of the 649 cancer cases, we found that the hereditary proportion of invasive ovarian cancer in Ontario was >11%; for the large subgroup of serous cancers, the frequency reached 16%. Our strategy using PTT and DGGE, combined with specific assays for a number of founder mutations, was designed to be reasonably rapid and inexpensive and, at the same time, comprehensive. It is possible, however, that some mutations could have been missed in this screen—and that the hereditary fraction could thus be somewhat higher, perhaps 10%–15% greater for *BRCA1* and 20%–25% greater for *BRCA2*. The sensitivity of mutation detection has been found to be similar for direct sequencing compared with other standard methods (Ford et al. 1998). As does sequencing, the testing strategy employed here misses genomic rearrangements, which are thought to account for <10% of *BRCA1* mutations and for an even smaller fraction of *BRCA2* mutations (Unger et al. 2000).

Previous estimates of the hereditary fraction of ovarian cancer in general populations have typically been less than ours. Takahashi et al. (1995) found germline *BRCA1* mutations in 7 (6.1%) of 115 women diagnosed with invasive cancer in a multi-institutional U.S. hospital–based pathology series. Matsushima et al. (1995) found 4 cases (5.3%) with mutations among 76 women with resected ovarian cancers, in a hospital series in Japan. Stratton et al. (1997) identified *BRCA1* mutations in 13 (3.5%) of 374 women diagnosed with ovarian tumors, both borderline and invasive, in a single hospital in England. Rubin et al. (1998) found germline *BRCA1* mutations in 10 (8.8%) of 113 cases of ovarian cancer in a Philadelphia-hospital series; Van der Looij et al. (2000) identified *BRCA1* mutations in 10 (11%) of 90 cases of ovarian cancer, in a hospital-based series in Hungary; and Khoo et al. (2000) found *BRCA1* mutations in 6 (11%) of 53 hospital cases in Hong Kong; however, Janezic et al. (1999) found *BRCA1* mutations in only 2 (1.9%) of 107 cases in a population-based study in southern California. With regard to *BRCA2,* Foster et al. (1996) observed germline *BRCA2* mutations in 2 (4%) of 50 cases of ovarian cancer, in a combined sample from Australia, the United Kingdom, and the United States; Takahashi et al. (1996) identified mutations in 4 (3.1%) of 130 cases in their extended pathology series, Van der Looij et al. (2000) none in their hospital series, and Khoo et al. (2000) 1 (2.3%) in their 43 hospital cases tested. In the Rubin et al. (1998) study, only one *BRCA2* mutation was observed in the 113 cases, and this woman also carried a *BRCA1* mutation. Rubin et al. (1998) concluded that the contribution of *BRCA2* to ovarian cancer is minimal, but the sample sizes of studies to date have been small. In our much larger study, *BRCA2* mutations clearly account for an appreciable fraction of hereditary cases.

For both *BRCA1* and *BRCA2,* it is likely that mutation-frequency differences according to ethnic group produce some of the observed differences between the various studies. This is seen, in the Stratton et al. (1997) report, in the low fraction of cases with *BRCA1* mutations, which is consistent with the low percentage with such mutations that is present among our cases of British Isles ancestry. The mutation fraction given by Stratton et al. (1997) may be an underestimate, however, because those authors did not look for the *BRCA1* exon 13 6-kb duplication, which may be a founder mutation in the population that they studied (The *BRCA1* Exon 13 Duplication Study Group 2000). Our finding of greater frequency of mutations among cases of ovarian cancer that are of Italian and Indo-Pakistani extraction than among British or mixed northern- or western-European ethnicity is interesting and suggests that ethnic composition needs further examination in population-based studies of mutation frequency.

The importance of *BRCA2* as an appreciable contributor to hereditary ovarian cancer has not been well recognized. This is largely because women with cancers attributable to *BRCA2* are not young and because an appreciable number do not have strong family histories. Our data show that *BRCA2*-associated ovarian cancer occurs at the same ages as does sporadic invasive ovarian cancer. Although our numbers are somewhat small, the majority of hereditary cancers in women diagnosed at age >60 years were in *BRCA2* carriers. This finding, in our general-population series, is similar to the predominance, in Ashkenazi Jews, of *BRCA2* mutations among ovarian cancer–mutation carriers who are age >60 years (Boyd et al. 2000; Moslehi et al. 2000). As well, these observations are consistent with the Breast Cancer Linkage Consortium (Ford et al. 1995) report based on linkage using markers on chromosome 13, which estimated the cumulative incidence of ovarian cancer among *BRCA2* carriers to be 0.4% by age 50 years but 27% by age 70 years. The majority of *BRCA2*-associated cancers are thus expected to appear at age 50–70 years, and this is consistent with our observations (table 3).

On the other hand, ovarian cancers occurring among carriers of *BRCA1* mutations were diagnosed, on average, ~4–5 years earlier than those among women not found to have mutations and ~7 years earlier than sporadic invasive ovarian cancer. *BRCA1*-associated cases

typically occur during a patient's 40s and 50s, with <10% being diagnosed at age <40 years. Other studies have also shown only a small percentage of cases occurring at age <40 years (Stratton et al. 1999; Boyd et al. 2000).

Histology of cases is also useful in distinguishing whether a mutation is likely to be present. Elsewhere, we have suggested that mucinous ovarian cancer is etiologically distinct from nonmucinous cancer (Risch et al. 1996), and this observation appears to carry over to the presence of *BRCA1* or *BRCA2* mutations. In the present study, we observed no mutations among 44 women with invasive mucinous tumors. The study by Stratton et al. (1997) apparently found one *BRCA1* mutation among 52 cases with mucinous tumors, and a study in Scandinavia (Jóhannsson et al. 1997) found no women with mucinous tumors among 15 cases with *BRCA1* mutations. In the Gynecologic Oncology Group case series, no women with mucinous tumors were found among either six cases with *BRCA1* mutations or four cases with *BRCA2* mutations (Takahashi et al. 1995, 1996); nor were any found in two studies of nearly 100 Ashkenazi Jewish women with *BRCA1*-associated ovarian cancer (Muto et al. 1996; Boyd et al. 2000). Finally, a collected series of 68 Ashkenazi Jewish women with ovarian cancer and carrying germline *BRCA1* or *BRCA2* mutations apparently found one case with a mucinous tumor (Moslehi et al. 2000). In the present study, mutations also were not seen among cases of ovarian cancer of borderline invasiveness (low malignant potential), and this is consistent with other reports as well (Gotlieb et al. 1998).

Because of the population-based sampling of our case series and the complete reporting of cancer histories among first-degree relatives, we were able to estimate the lifetime penetrance of breast cancer and of ovarian cancer associated with carriage of germline mutations. The lifetime penetrance of breast cancer among carriers of *BRCA1* mutations, 68%, is within the 45%–74% range that has been estimated elsewhere (Whittemore et al. 1997; Antoniou et al. 2000); also, the lifetime penetrance of ovarian cancer among carriers of *BRCA1* mutations, 36%, is within the 28%–66% range established elsewhere (Whittemore et al. 1997; Antoniou et al. 2000). However, these ranges are wide. The estimates by Whittemore et al. (1997) were made on the basis of population-based case-control studies that had no mutation testing and that therefore required that assumptions about the carriage of mutations be based on family history. The estimates by Antoniou et al. (2000) were made on the basis of modeling of both an unselected case series and a set of highly affected families. Our estimated penetrance values are based solely on a large population series of unselected cases—and, therefore, of families unselected for cancer incidence. It is nevertheless possible that the probands that we studied could have underreported the existence of cancers among their first-degree relatives; however, the cumulative incidences of ovarian and breast cancer among noncarrier-case relatives—2.5% and 9.9%, respectively—are similar to general-population lifetime risks and suggest that the underreporting of affected relatives is not an appreciable issue here. Our penetrance estimates are therefore likely to be reasonably valid.

An interesting new finding in the present study is the trend of breast-cancer penetrance increasing according to more-downstream mutation location within the *BRCA1* coding sequence. Shattuck-Eidens et al. (1995) did not find any apparent clustering of breast cancers in families of carriers by mutation location in the *BRCA1* coding sequence. Studies of Ashkenazi Jews show that the 5382insC mutation may have a breast-cancer penetrance similar to that of the 185delAG mutation (Levy-Lahad et al. 1997; Moslehi et al. 2000); however, if, as has been suggested, the prevalence of the 5382insC mutation in the general Ashkenazi community proves to be only ~10% of that of the 185delAG mutation (Tonin et al. 1996), then its penetrance for breast cancer could be appreciably greater. Gayther et al. (1995) found a significant correlation between the location of the *BRCA1* mutation in the gene and the breast:ovary ratio of cancer incidence in the family, with 3′-third mutations associated with a lower proportion of ovarian cancer. In a series with ovarian cancer uniformly present (as in the present study), this would result in an excess of breast cancer cases in families with mutations in the 3′ third, as we have found. Whether there should also be a relative deficit of breast cancer in families with 5′ mutations is unclear; only two breast cancers were reported among 42 female first-degree relatives of the 12 cases with 5′ *BRCA1* mutations, including the 5 with the 185delAG mutation.

It is also interesting that overall we did not find an excess of breast cancer among family members of cases carrying *BRCA2* mutations. This appears to be a result of our study sampling, in which at least one case of ovarian cancer (the proband) exists in each family, giving a moderately high proportion (62%) of *BRCA2* mutations within the OCCR. By considering OCCR and non-OCCR *BRCA2* mutations separately, we did see a significant excess of breast cancer in families with non-OCCR mutations. In a large set of families with breast-ovarian cancer and germline *BRCA2* mutations, Thompson and Easton (2001) have recently also found only non-OCCR mutations to be associated with increased risk of breast cancer.

Our finding that an elevated risk of colorectal, stomach, pancreatic, and prostate cancer is associated specifically with OCCR *BRCA2* mutations is also new. Although, in some studies (e.g., Lehrer et al. 1998; Sin-

clair et al. 2000), prostate cancer has not been associated with *BRCA2* mutations, the Breast Cancer Linkage Consortium (1999) analysis of 173 families with *BRCA2* mutations shows that prostate, as well as colorectal, stomach, and pancreatic cancer, seems to be more frequent among carriers of *BRCA2* mutations than among noncarriers. Whether the increase in risk of cancers at these sites is due essentially to OCCR mutations remains to be seen. Thompson and Easton (2001) have observed greater increased risk of prostate cancer with non-OCCR mutations than with OCCR mutations. Phelan et al. (1996) did find a few families with non-OCCR *BRCA2* mutations and cancers of these various sites. Overall, the issue does not appear to have been systematically studied. Our finding of increased cancer risks specifically with OCCR mutations is probably not attributable to the *BRCA2*-screening methods that we used, since the OCCR as considered here—that is, nucleotides 4075–6503, inclusive (Thompson and Easton 2001)—comprises only 40% of the screened coding length of exons 10 and 11. Indeed, in 8 (38%) of our 21 cases with *BRCA2* mutations, the mutations were outside the OCCR, and this fraction is very similar to the 41% that Thompson and Easton (2001) have seen among 119 cases of ovarian cancer with *BRCA2* mutations.

There are a number of potential clinical recommendations that can be inferred from our results. Risk-factor analysis has been used to predict the presence of *BRCA1* mutation in high-risk subjects (Shattuck-Eidens et al. 1997). This method provides a suitable threshold on the basis of which genetic testing can be offered, but it cannot reassure individual women that, if tested, they would not be found to be carrying mutations. The same may be said for age at diagnosis, family history, or ethnicity, in counseling cases of ovarian cancer. However, only ovarian cancers of invasive nonmucinous histology are likely to be associated with *BRCA1* or *BRCA2* mutations. The frequency of mutations in general North American populations thus suggests that it is reasonable to offer genetic testing to all women with invasive nonmucinous ovarian cancer. It would seem not to be prudent to exclude patients from screening because they lack a family history. In our study, 33 of 60 mutations would have been missed if we had restricted screening to women with at least one affected first-degree relative. In contrast, none of the mutations would have been missed if our efforts had been limited to the subgroup of invasive nonmucinous tumors, and we would have reduced the extent of screening by 33%. It also appears worthwhile to test all women with invasive nonmucinous ovarian cancer for the three common Ashkenazi Jewish founder mutations, since they occur not infrequently in other ethnic groups. In addition, testing should include examination of the *BRCA1* exon 13 6-kb duplication mutation, which is missed by standard assay methods, including direct sequencing (Puget et al. 1999).

If confirmed in future studies, the finding that penetrance of breast cancer increases with more-distal mutation location along the *BRCA1* coding sequence may be important for genetic counseling. If, in the general population, the risk of breast cancer proves not to be appreciably elevated with mutations in the proximal end of the gene, then it is possible that women carrying such mutations may be able to avoid disfiguring surgery. Our study did not find a *BRCA1*-location effect on penetrance of ovarian cancer, but the number of cases among family members of carriers was small. The *BRCA1*-mutation penetrance for ovarian cancer appears to be sufficiently great that prophylactic oophorectomy or other prevention strategies are warranted. Finally, for cancers at all sites combined, if future studies show the penetrance of *BRCA2* mutations, particularly OCCR mutations, is high in male carriers, as it is in female carriers, then males will need to be included in genetic-testing programs whenever the presence of a *BRCA2* mutation is suspected.

Acknowledgment

This work was supported in part by National Cancer Institute grants R01 CA 63682 (to H.A.R.) and R01 CA 63678 (to S.A.N.).

Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Breast Cancer Information Core (BIC), http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for *BRCA1* [MIM 113705] and *BRCA2* [MIM 600185])

References

Antoniou AC, Gayther SA, Stratton JF, Ponder BAJ, Easton DF (2000) Risk models for familial ovarian and breast cancer. Genet Epidemiol 18:173–190

Boyd J, Sonoda Y, Federici MG, Bogomolniy F, Rhei E, Maresco DL, Saigo PE, Almadrones LA, Barakat RR, Brown CL, Chi DS, Curtin JP, Poynor EA, Hoskins WJ (2000) Clinicopathologic features of *BRCA*-linked and sporadic ovarian cancer. JAMA 283:2260–2265

BRCA1 Exon 13 Duplication Screening Group, The (2000) The exon 13 duplication in the *BRCA1* gene is a founder mutation present in geographically diverse populations. Am J Hum Genet 67:207–212

Breast Cancer Linkage Consortium (1999) Cancer risks in *BRCA2* mutation carriers. J Natl Cancer Inst 91:1310–1316

Ford D, Easton DF, Peto J (1995) Estimates of the gene frequency of *BRCA1* and its contribution to breast and ovarian cancer incidence. Am J Hum Genet 57:1457–1462

Ford D, Easton DF, Stratton M, Narod S, Goldgar D, Devilee P, Bishop DT, et al (1998) Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. Am J Hum Genet 62:676–689

Foster KA, Harrington P, Kerr J, Russell P, DiCioccio RA, Scott IV, Jacobs I, Chenevix-Trench G, Ponder BAJ, Gayther SA (1996) Somatic and germline mutations of the *BRCA2* gene in sporadic ovarian cancer. Cancer Res 56:3622–3625

Frank TS, Manley SA, Olopade OI, Cummings S, Garber JE, Bernhardt B, Antman K, et al (1998) Sequence analysis of *BRCA1* and *BRCA2:* correlation of mutations with family history and ovarian cancer risk. J Clin Oncol 16:1–10

Gayther SA, Warren W, Mazoyer S, Russell PA, Harrington PA, Chiano M, Seal S, Hamoudi R, van Rensburg EJ, Dunning AM, Love R, Evans G, Easton D, Clayton D, Stratton MR, Ponder BAJ (1995) Germline mutations of the *BRCA1* gene in breast and ovarian cancer families provide evidence for a genotype-phenotype correlation. Nat Genet 11:428–433

Gotlieb WH, Friedman E, Bar-Sade RB, Kruglikova A, Hirsh-Yechezkel G, Modan B, Inbar M, Davidson B, Kopolovic J, Novikov I, Ben-Baruch G (1998) Rates of Jewish ancestral mutations in *BRCA1* and *BRCA2* in borderline ovarian tumors. J Natl Cancer Inst 90:995–1000

Janezic SA, Ziogas A, Krumroy LM, Krasner M, Plummer SJ, Cohen P, Gildea M, Barker D, Haile R, Casey G, Anton-Culver H (1999) Germline *BRCA1* alterations in a population-based series of ovarian cancer cases. Hum Mol Genet 8:889–897

Jóhannsson ÓT, Idvall I, Anderson C, Borg Å, Barkardóttir RB, Egilsson V, Olsson H (1997) Tumour biological features of *BRCA1*-induced breast and ovarian cancer. Eur J Cancer 33:362–371

Khoo U-S, Ngan HYS, Cheung ANY, Chan KYK, Lu J, Chan VWY, Lau S, Andrulis IL, Ozcelik H (2000) Mutational analysis of *BRCA1* and *BRCA2* genes in Chinese ovarian cancer identifies 6 novel germline mutations. Hum Mutat 16:88–89

Kuperstein G, Foulkes WD, Ghadirian P, Hakimi J, Narod SA (2000) A rapid fluorescent multiplexed-PCR analysis (FMPA) for founder mutations in the *BRCA1* and *BRCA2* genes. Clin Genet 57:213–220

Lehrer S, Fodor F, Stock RG, Stone NN, Eng C, Song HK, McGovern M (1998) Absence of 185delAG mutation of the *BRCA1* gene and 6174delT mutation of the *BRCA2* gene in Ashkenazi Jewish men with prostate cancer. Br J Cancer 78:771–773

Levy-Lahad E, Catane R, Eisenberg S, Kaufman B, Hornreich G, Lishinsky E, Shohat M, Weber BL, Beller U, Lahad A, Halle D (1997) Founder *BRCA1* and *BRCA2* mutations in Ashkenazi Jews in Israel: Frequency and differential penetrance in ovarian cancer and in breast-ovarian cancer families. Am J Hum Genet 60:1059–1067

Matsushima M, Kobayashi K, Emi M, Saito H, Saito J, Suzumori K, Nakamura Y (1995) Mutation analysis of the *BRCA1* gene in 76 Japanese ovarian cancer patients: four germline mutations, but no evidence of somatic mutation. Hum Mol Genet 4:1953–1956

Moslehi R, Chu W, Karlan B, Fishman D, Risch H, Fields A, Smotkin D, Ben-David Y, Rosenblatt J, Russo D, Schwartz P, Tung N, Warner E, Rosen B, Friedman J, Brunet J-S, Narod SA (2000) *BRCA1* and *BRCA2* mutation analysis of 208 Ashkenazi Jewish women with ovarian cancer. Am J Hum Genet 66:1259–1272

Muto MG, Cramer DW, Tangir J, Berkowitz R, Mok S (1996) Frequency of the *BRCA1* 185delAG mutation among Jewish women with ovarian cancer and matched population controls. Cancer Res 56:1250–1252

Narod S, Ford D, Devilee P, Barkardottir RB, Eyfjord J, Lenoir G, Serova O, Easton D, Goldgar D (1995) Genetic heterogeneity of breast-ovarian cancer revisited. Breast Cancer Linkage Consortium. Am J Hum Genet 57:957–958

Phelan CM, Lancaster JM, Tonin P, Gumbs C, Cochran C, Carter R, Ghadirian P, Perret C, Moslehi R, Dion F, Faucher M-C, Dole K, Karimi S, Foulkes W, Lounis H, Warner E, Goss P, Anderson D, Larsson C, Narod SA, Futreal PA (1996) Mutation analysis of the *BRCA2* gene in 49 site-specific breast cancer families. Nat Genet 13:120–122

Puget N, Sinilnikova OM, Stoppa-Lyonnet D, Audoynaud C, Pagès S, Lynch HT, Goldgar D, Lenoir GM, Mazoyer S (1999) An Alu-mediated 6-kb duplication in the *BRCA1* gene: a new founder mutation? Am J Hum Genet 64: 300–302

Ries LAG, Miller BA, Hankey BF, Kosary CL, Harras A, Edwards BK (eds) (1994) SEER cancer statistics review, 1973–1991: tables and graphs. National Institutes of Health publ 94-2789. National Institutes of Health, Bethesda

Risch HA, Marrett LD, Jain M, Howe GR (1996) Differences in risk factors for epithelial ovarian cancer by histologic type: results of a case-control study. Am J Epidemiol 144: 363–372

Rubin SC, Blackwood MA, Bandera C, Behbakht K, Benjamin I, Rebbeck TR, Boyd J (1998) *BRCA1, BRCA2,* and hereditary nonpolyposis colorectal cancer gene mutations in an unselected ovarian cancer population: Relationship to family history and implications for genetic testing. Am J Obstet Gynecol 178:670–677

Shattuck-Eidens D, McClure M, Simard J, Labrie F, Narod S, Couch F, Hoskins K, et al (1995) A collaborative survey of 80 mutations in the *BRCA1* breast and ovarian cancer susceptibility gene: implications for presymptomatic testing and screening. JAMA 273:535–541

Shattuck-Eidens D, Oliphant A, McClure M, McBride C, Gupte J, Rubano T, Pruss D, et al (1997) *BRCA1* sequence analysis in women at high risk for susceptibility mutations: risk factor analysis and implications for genetic testing. JAMA 278:1242–1250

Sinclair CS, Berry R, Schaid D, Thibodeau SN, Couch FJ (2000) *BRCA1* and *BRCA2* have a limited role in familial prostate cancer. Cancer Res 60:1371–1375

Stratton JF, Gayther SA, Russel PL, Dearden J, Gore M, Blake P, Easton D, et al (1997) Contribution of *BRCA1* mutations to ovarian cancer. N Engl J Med 336:1125–1130

Stratton JF, Thompson D, Bobrow L, Dalal N, Gore M, Bishop DT, Scott I, Evans G, Daly P, Easton DF, Ponder BAJ (1999) The genetic epidemiology of early-onset epithelial ovarian

cancer: a population-based study. Am J Hum Genet 65: 1725–1732

Struewing JP, Abeliovich D, Peretz T, Avishai N, Kaback MM, Collins FS, Brody LC (1995) The carrier frequency of the *BRCA1* 185delAG mutation is approximately 1 percent in Ashkenazi Jewish individuals. Nat Genet 11:198–200

Takahashi H, Behbakht K, McGovern PE, Chiu H-C, Couch FJ, Weber BL, Friedman LS, King M-C, Furusato M, LiVolsi VA, Menzin AW, Liu PC, Benjamin I, Morgan MA, King SA, Rebane BA, Cardonick A, Mikuta JJ, Rubin SC, Boyd J (1995) Mutation analysis of the *BRCA1* gene in ovarian cancers. Cancer Res 55:2998–3002

Takahashi H, Chiu H-C, Bandera CA, Behbakht K, Liu PC, Couch FJ, Weber BL, LiVolsi VA, Furusato M, Rebane BA, Cardonick A, Benjamin I, Morgan MA, King SA, Mikuta JJ, Rubin SC, Boyd J (1996) Mutations of the *BRCA2* gene in ovarian carcinomas. Cancer Res 56:2738–2741

Thompson D, Easton D (on behalf of the Breast Cancer Linkage Consortium) (2001) Variation in cancer risks, by mutation position, in BRCA2 mutation carriers. Am J Hum Genet 68:410–419

Tonin PN, Mes-Masson A-M, Futreal PA, Morgan K, Mahon M, Foulkes WD, Cole DEC, Provencher D, Ghadirian P, Narod SA (1998) Founder *BRCA1* and *BRCA2* mutations in French Canadian breast and ovarian cancer families. Am J Hum Genet 63:1341–1351

Tonin P, Weber B, Offit K, Couch F, Rebbeck TR, Neuhausen S, Godwin AK, et al (1996) Frequency of recurrent *BRCA1* and *BRCA2* mutations in Ashkenazi Jewish breast cancer families. Nat Med 2:1179–1183

Unger MA, Nathanson KL, Calzone K, Antin-Ozerkis D, Shih HA, Martin A-M, Lenoir GM, Mazoyer S, Weber BL (2000) Screening for genomic rearrangements in families with breast and ovarian cancer identifies *BRCA1* mutations previously missed by conformation-sensitive gel electrophoresis or sequencing. Am J Hum Genet 67:841–850

Van der Looij M, Szabo C, Besznyak I, Liszka G, Csokay B, Pulay T, Toth J Devilee P, King M-C, Olah E (2000) Prevalence of founder *BRCA1* and *BRCA2* mutations among breast and ovarian cancer patients in Hungary. Int J Cancer 86:737–740

Wacholder S, Hartge P, Struewing JP, Pee D, McAdams M, Brody L, Tucker M (1998) The kin-cohort study for estimating penetrance. Am J Epidemiol 148:623–630

Whittemore AS, Gong G, Itnyre J (1997) Prevalence and contribution of *BRCA1* mutations in breast cancer and ovarian cancer: results from three U.S. population-based case-control studies of ovarian cancer. Am J Hum Genet 60:496–504