

CONTRIBUTION OF *BRCA1* MUTATIONS TO OVARIAN CANCER

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**ABSTRACT**

**Background** Inherited mutations in the *BRCA1* gene confer a high risk of breast and ovarian cancer in some families. To determine the contribution of *BRCA1* mutations to ovarian cancer in the general population, we analyzed DNA samples from a consecutive series of women with ovarian cancer seen at one center.

**Methods** We studied 374 women who received a diagnosis of epithelial ovarian cancer before the age of 70 years and were treated at the Royal Marsden Hospital between July 1993 and September 1995. Genomic DNA was analyzed by multiplex heteroduplex analysis. Variants were further identified by sequencing.

**Results** Probable germ-line *BRCA1* mutations were identified in 13 of the 374 women (3 percent; 95 percent confidence interval, 2 to 6 percent). Six of the variants have not been described previously. Of the 13 mutations, 12 are predicted to result in a truncated protein product. An additional variant results in an in-frame deletion just outside the putative zinc-finger domain. Nine of the 12 women with truncating mutations had family histories of breast or ovarian cancer or both.

**Conclusions** Assuming that our method has a sensitivity of 70 percent, mutations in *BRCA1* occur in approximately 5 percent (95 percent confidence interval, 3 to 8 percent) of women in whom ovarian cancer is diagnosed before the age of 70 years. (N Engl J Med 1997;336:1125-30.)

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**T**HE *BRCA1* gene, which is located on chromosome 17q12-21, encodes a protein containing 1863 amino acids.<sup>1</sup> Germ-line mutations of the gene confer a lifetime risk of 85 percent for breast cancer and about 45 percent for ovarian cancer in families with multiple cases of such cancers.<sup>2</sup> However, the contribution of *BRCA1* mutations to the incidence of breast and ovarian cancer in the general population has not been precisely estimated. Langston et al.<sup>3</sup> found *BRCA1* mutations in 5 of 80 women (6 percent) who had received a diagnosis of breast cancer before the age of 35 years, only 1 of whom had no family history of breast or ovarian cancer. FitzGerald et al.<sup>4</sup> found *BRCA1* mutations in 5 of 30 women (17 percent) in whom breast cancer had been diagnosed before the age of 30 years. Matsushima et al.<sup>5</sup> identified 3 women with germ-line mutations and 1 with an

intronic variant in an unselected group of 76 Japanese women with ovarian cancer. Two of these women had relatives with either breast or ovarian cancer. Takahashi et al.<sup>6</sup> examined 115 ovarian tumors obtained from a tissue bank and found four frame-shift mutations and one missense mutation that were also in the germ line. All five women with mutations in that series had family histories of breast or ovarian cancer, or both. In contrast, Merajver et al.<sup>7</sup> found polymorphisms but no clear germ-line mutations in 47 women with ovarian cancer.

Using data from population-based studies, Ford et al.<sup>8</sup> estimated that the frequency of *BRCA1* mutations in the population is 0.0006 and that on the basis of this estimate, 2.8 percent of all ovarian cancers diagnosed in women younger than 70 years are attributable to such mutations. These estimates were based on the assumption that the excess risk of ovarian cancer in the relatives of patients with breast cancer and, similarly, the excess risk of breast cancer in the relatives of patients with ovarian cancer are due entirely to *BRCA1* mutations.

To evaluate more directly the frequency of *BRCA1* mutations in a general population of women with ovarian cancer, we tested samples from a large series of women at one hospital for germ-line *BRCA1* mutations. The women were not selected on the basis of a family history of cancer.

**METHODS****Patients**

The study group consisted of women with epithelial ovarian cancer (including borderline tumors) who were less than 70 years old at the time of the diagnosis and were seen at the Royal Marsden Hospital in London between July 1993 and September 1995. The study was approved by the ethics committee of the Royal Marsden Hospital.

The women were given an information sheet, and a research nurse explained the purpose of the study to them. Before written consent was obtained, the possibility that the results might indicate an increased risk of ovarian and possibly other cancers in family members was explained to each woman who agreed to enter the study. The women were given the option of being contacted if

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**TABLE 1. HISTOLOGIC DIAGNOSES IN 374 WOMEN WITH OVARIAN CANCER WHO WERE ENROLLED IN A STUDY OF MUTATIONS AND 108 WOMEN WITH OVARIAN CANCER WHO WERE NOT ENROLLED.\***

HISTOLOGIC DIAGNOSIS	ENROLLED				NOT ENROLLED
	TOTAL	GRADE 1	GRADE 2	GRADE 3	
	number of women (percent)				
Serous cystadenocarcinoma	216 (58)	30 (14)	78 (36)	108 (50)	58 (54)
Endometrioid cystadenocarcinoma	56 (15)	10 (18)	29 (52)	17 (30)	11 (10)
Mucinous cystadenocarcinoma	45 (12)	19 (42)	21 (47)	5 (11)	20 (19)
Clear-cell carcinoma	23 (6)	0	16 (70)	7 (30)	1 (1)
Undifferentiated or unclassified	15 (4)	—	—	—	13 (12)
Borderline serous cystadenocarcinoma	12 (3)	—	—	—	3 (3)
Borderline mucinous cystadenocarcinoma	7 (2)	—	—	—	2 (2)

\*Unenrolled women included those who declined participation in the study and those who were excluded because they did not meet the criteria for enrollment. Grade 1 denotes well-differentiated tumors, grade 2 moderately differentiated tumors, and grade 3 poorly differentiated tumors. Percentages may not sum to 100 because of rounding.

“anything important was found.” Arrangements were made to provide genetic counseling for these women when the information became available. The women were asked to donate 10 ml of blood for genetic analysis and to complete a questionnaire on cancers in first- and second-degree relatives. The questionnaires were reviewed by a research nurse, who attempted to clarify ambiguous information and to confirm diagnoses of ovarian cancer in relatives on the basis of pathology reports or death certificates.

Of the 482 women who were eligible for the study, 386 (80 percent) agreed to participate; 134 of the women had just received a diagnosis of ovarian cancer, and 252 were being seen for follow-up. DNA samples from 12 women could not be amplified by polymerase chain reaction (PCR). Samples from the other 374 women were analyzed for mutations throughout the entire coding region of the *BRCA1* gene.

Family-history questionnaires were completed and returned by 280 (75 percent) of the 374 women. Thirty-one women reported family histories of ovarian cancer. In 15 cases the histories were confirmed. In two cases, a maternal aunt and a maternal grandmother reported as having had ovarian cancer actually had stomach cancer and pneumonia, respectively, according to the death certificates. The diagnoses reported for the relatives of the other 14 women could not be verified.

At the time of the diagnosis, 37 of the women (10 percent) were less than 40 years old, 82 (22 percent) were 40 to 49, 153 (41 percent) were 50 to 59, and 102 (27 percent) were 60 to 69. The age distribution of the 96 women who declined to enter the study and the 12 whose DNA samples could not be amplified by PCR was as follows: less than 40 years, 13 women (12 percent); 40 to 49, 25 (23 percent); 50 to 59, 33 (31 percent); and 60 to 69, 37 (34 percent). Table 1 shows the histologic diagnoses for the 374 women who participated in the study and the 108 who declined to participate or were excluded.

#### Analysis of the *BRCA1* Gene

Genomic DNA was extracted with the use of standard methods on an automated DNA extractor (Applied Biosystems). Twenty-

eight primer pairs were used to amplify the entire *BRCA1* coding sequence and intron-exon boundaries for screening by multiplex heteroduplex analysis. The primers were selected to produce PCR products of various sizes, ranging from approximately 640 to 170 bp. After amplification, four separate PCR products from the same woman were mixed together so that the fragments differed sufficiently in size to ensure adequate separation by electrophoresis.\* The mixed PCR products were denatured at 95°C for 10 minutes and cooled to 37°C over a period of 30 minutes to induce heteroduplex formation. The DNA fragments were subsequently electrophoresed through 20 cm×20 cm×0.1 cm, non-denaturing, 1× MDE gels (J.T. Baker), with the use of the Protean II vertical slab-gel apparatus (Bio-Rad). Electrophoresis was performed for 12 hours at a constant level of 250 V, with the gels cooled to 10°C.

For DNA detection, the gels were incubated in a 1:10,000 dilution of SYBR Green I Nucleic Acid Gel Staining (FMC Bio-products). The gels were then examined with an ultraviolet transilluminator and photographed with the SYBR Green gel stain photographic filter (Fig. 1).

#### Sequence Analysis

For each variant pattern identified by heteroduplex analysis, the putative mutated region was reamplified from the original genomic DNA sample. PCR products were prepared in volumes

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of 100  $\mu$ l. One oligonucleotide of each primer pair used for the amplification was biotinylated and purified by means of high-performance liquid chromatography. DNA fragments were immobilized on streptavidin-coated magnetic beads (Dynal) and denatured to produce single-stranded templates before fluorescent sequencing was performed with the use of a semiautomated sequencing system (model 373A, Applied Biosystems). The dideoxy chain-termination method was used with the Prism Sequenase Terminator Single-Stranded Sequencing Kit (Applied Biosystems).

Most sequencing reactions were performed with the use of a nested oligonucleotide primer. If a suitable nested primer was not available, PCR products were gel-purified with the use of the Wizard PCR Prep system (Promega) before sequencing with the relevant primer used in the PCR reaction.

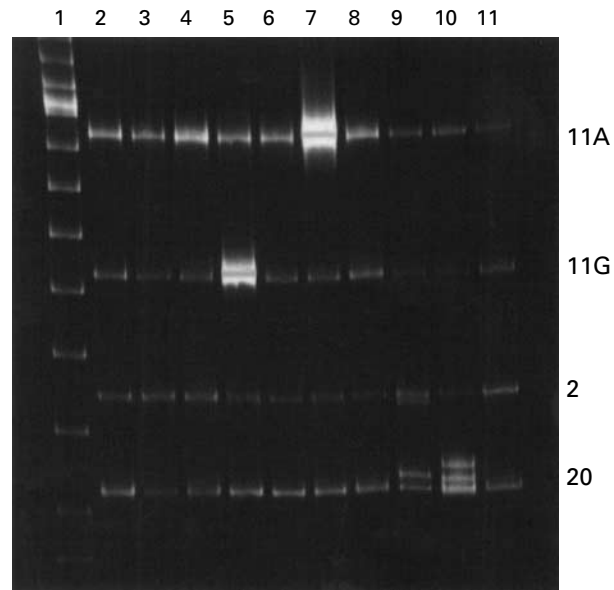
## RESULTS

We identified DNA sequence variations predicted to result in a truncated *BRCA1* protein and therefore probably of pathologic importance in 12 of the 374 women who were screened for mutations (3 percent; 95 percent confidence interval, 2 to 6 percent) (Table 2). Five of these variant sequences have not, to our knowledge, been previously described.

A further novel variant, a deletion of GAT at nucleotide 314 (314delGAT), resulted in an in-frame deletion adjacent to the ring-finger domain. The woman with this variant (Patient 154) received a diagnosis of ovarian cancer at the age of 51 years, and her father had had leukemia at the age of 54. She reported no family history of breast or ovarian cancer, but she had only one sister. This variant is probably of pathologic importance, because it was not found among 380 normal chromosomes from a similar population of 190 women. Moreover, the mouse *BRCA1* sequence also contains a charged residue (glutamic acid rather than aspartic acid) at this position. Most of the sequence variants in *BRCA1* for which there is evidence of pathologic importance occur at residues conserved in both mice and humans, whereas neutral polymorphisms tend to occur in nonconserved residues.<sup>9,10</sup> We have therefore included the woman with the 314delGAT mutation in our analysis.

Two women carried a 12-bp insertion in intron 20, which has been reported previously.<sup>9</sup> Neither of these women reported a family history of breast or ovarian cancer. This variant does not involve a splice site and is not predicted to alter the protein product. The functional importance of this variant is unknown, because no RNA was available to test for loss of transcript.<sup>11</sup>

Of the 12 women with truncating mutations, 9 reported a family history of breast or ovarian cancer, or both, and among these 9 women, 6 had affected first-degree relatives. Among the other three women, one had a paternal aunt with breast cancer diagnosed at the age of 38 years, one had a maternal aunt with ovarian cancer diagnosed at the age of 57, and one had a paternal aunt in whom breast cancer developed at 50 years. The diagnosis of either breast



**Figure 1.** Multiplex Heteroduplex Analysis of PCR Products Stained with SYBR Green.

Lane 1 contains a 100-bp ladder for orientation. DNA fragments are labeled at the right. Four variants are shown: a deletion of G at nucleotide 3869 (fragment 11G, lane 5); a substitution of T for C at position 1257, resulting in a premature stop codon (fragment 11A, lane 7); a 12-bp insertion in intron 20 (fragment 20, lane 9); and an insertion of C at position 5382 in exon 20 (fragment 20, lane 10).

or ovarian cancer was made before the age of 55 in 12 of the 14 affected relatives.

The mean age at the time of the diagnosis of ovarian cancer was 48 years among the women with *BRCA1* mutations and their first-degree relatives and 52 years among the noncarriers and their first-degree relatives — a statistically insignificant difference. The mean age at the time of the diagnosis of breast cancer also did not differ significantly between the two groups (45 years for the first-degree relatives of the carriers and 54 years for the first-degree relatives of the noncarriers). The prevalence of *BRCA1* mutations in cases of ovarian cancer diagnosed before the age of 50 years was 6 of 119 cases (5 percent; 95 percent confidence interval, 2 to 11 percent), as compared with 7 of 255 (3 percent; 95 percent confidence interval, 1 to 6 percent) for cases diagnosed at the age of 50 or older.

Of the 226 women who reported having a first- or second-degree relative with any cancer, 90 had relatives with breast or ovarian cancer, or both (Table 3). Of the 54 women who reported no family histories of cancer, 2 had *BRCA1* mutations.

Second cancers developed in nine women. Five had endometrial cancer (diagnosed before the ovarian cancer in three women and as a second primary cancer concomitantly with the ovarian cancer in two). Two

**TABLE 2. GERM-LINE *BRCA1* MUTATIONS IN 13 WOMEN WITH OVARIAN CANCER.**

PATIENT No.	AGE AT DIAGNOSIS (YR)	EXON	NUCLEOTIDE CHANGE AND POSITION	FAMILY HISTORY*		HISTOLOGIC DIAGNOSIS AND GRADE
				BREAST OR OVARIAN CANCER		
				OTHER CANCERS		
274	43	3	Deletion of AA at 230	Paternal aunt (breast, 50 yr)	None	Serous cystadenocarcinoma, 2
154	51	5	Deletion of GAT at 314	None	Father (leukemia, 54 yr)	Serous cystadenocarcinoma, 3
14	64	11	4-bp deletion at 1942	2 sisters (ovarian, 38 and 45 yr)	None	Serous cystadenocarcinoma, 3
371	48	11	Deletion of AA at 2069	None	Daughter (Hodgkin's, 25 yr) Father (colon, 56 yr)	Serous cystadenocarcinoma, 3
7	40	11	4-bp deletion at 3452	Mother (ovarian, 51 yr) Maternal aunt (ovarian, 34 yr)	None	Serous cystadenocarcinoma, 3
34	61	11	Deletion of G at 3869	None	None	Serous cystadenocarcinoma, 2
335	44	11	Deletion of GT at 4287	Paternal aunt (breast, 38 yr)	None	Borderline mucinous cystadenocarcinoma
120	36	11	C→T at 1257 (premature stop codon)	None	None	Serous cystadenocarcinoma, 3
126	56	13	C→T at 4446 (premature stop codon)	Maternal aunt (ovarian, 57 yr) Paternal grandmother (breast, 60 yr)	None	Serous cystadenocarcinoma, 2
41	52	13	T→C at 4476+6	Sister (breast, 47 yr) Maternal aunt (breast, 43 yr) Maternal aunt (ovarian, 43 yr)	None	Serous cystadenocarcinoma, 2
219	53	17	4-bp deletion at 5149	Mother (breast, 44 yr)	Son (testicular, 22 yr)	Serous cystadenocarcinoma, 2
86	44	20	Insertion of C at 5382	Mother (ovarian, 44 yr)	Brother (stomach, 48 yr)	Serous cystadenocarcinoma, 3
279	51	24	Deletion of G at 5629	Sister (ovarian, 40 yr)	None	Serous cystadenocarcinoma, 2

\*The type of cancer is shown in parentheses, followed by the age at the time of diagnosis.

**TABLE 3. FAMILY HISTORIES OF OVARIAN, BREAST, COLORECTAL, AND PROSTATE CANCER, ACCORDING TO THE PRESENCE OR ABSENCE OF *BRCA1* MUTATIONS.\***

TYPE OF CANCER IN RELATIVE	FIRST-DEGREE RELATIVE		SECOND-DEGREE RELATIVE		FIRST- OR SECOND-DEGREE RELATIVE	
	ALL WOMEN	WOMEN WITH MUTATIONS	ALL WOMEN	WOMEN WITH MUTATIONS	ALL WOMEN	WOMEN WITH MUTATIONS
		number of women (percent)				
Ovarian	19	4 (21)	14	3 (21)	29	6 (21)
Breast	31	2 (6)	40	4 (10)	65	5 (8)
Diagnosed at age ≤60 yr	15	2 (13)	14	3 (21)	28	4 (14)
Diagnosed at age >60 yr	16	0	26	1 (4)	37	1 (3)
Colorectal	22	1 (5)	32	0	50	1 (2)
Prostate	7	0	8	0	14	0
Cancer other than breast or ovarian					190	3 (2)

\*Some women had more than one affected relative.

women had breast cancer, diagnosed at 55 and 43 years of age, respectively (their ovarian cancers were diagnosed at 56 and 60 years, respectively). One woman had a leiomyosarcoma of the thoracic cavity, and one had a basal-cell cancer, both of which were diagnosed simultaneously with the ovarian cancer. No *BRCA1* mutations were detected in these nine women.

## DISCUSSION

In this large study of germ-line *BRCA1* mutations in 374 women with ovarian cancer who were unselected for family history, we found probable mutations in 13 of the women (3 percent). However, some mutations were undoubtedly missed. Of the 132 *BRCA1* mutations reported by Couch and Weber,<sup>9</sup>

62 percent were frame-shift mutations, 19 percent were nonsense mutations, 9 percent were splice-site mutations, and 8 percent were missense mutations. In our hands, heteroduplex analysis detects over 98 percent of frame-shift mutations,<sup>12</sup> but its sensitivity in detecting other types of mutations is unknown.<sup>13-15</sup> Among women with familial ovarian or breast cancer, which has a clear link to *BRCA1* mutations, approximately 20 percent have no detectable coding-sequence alterations.<sup>1,16-18</sup> Assuming a sensitivity of 70 percent for the detection of point mutations, we estimate the overall sensitivity of heteroduplex analysis for the detection of coding alterations as follows:

$$80\% \times [(98\% \times 62\%) + (70\% \times 38\%)] = 70\%.$$

With a sensitivity of 70 percent, the true proportion of ovarian cancers attributable to *BRCA1* mutations would be 5 percent (95 percent confidence interval, 3 to 8 percent). This value is consistent with the prevalence estimated by Ford et al.<sup>8</sup> Given the genetic heterogeneity of the British population, the prevalence of *BRCA1* mutations among women with ovarian cancer in most other populations is likely to be similar. The results reported by Matsushima et al.<sup>5</sup> and Takahashi et al.<sup>6</sup> in Japan support this conclusion. Nevertheless, our findings are not applicable to populations that are genetically isolated, such as Ashkenazi Jews.

We based our study on a series of ovarian cancers that included both incident and prevalent cases. The study is therefore biased toward ovarian cancers with better prognoses, which may have influenced our prevalence estimates if *BRCA1* mutations are related to the prognosis. Rubin et al.<sup>19</sup> have suggested a survival advantage for women with ovarian cancer who carry *BRCA1* mutations, but they studied small numbers of cases, and their findings have yet to be confirmed. Moreover, the similar prevalence of mutations in the women with incident cancers (4 of 134 women, or 3 percent) and those with prevalent cancers (9 of 250, or 4 percent) in our series suggests that any effect of survival is small.

Our finding of *BRCA1* mutations throughout the length of the gene in families with predominantly ovarian cancers seems inconsistent with the reported connection between the risk of ovarian cancer and mutations in the 5' region of the gene.<sup>12</sup> However, our numbers are too small to permit inferences about the importance of the location of *BRCA1* mutations.

Of the 13 women with *BRCA1* mutations, 12 had serous cystadenocarcinomas of the ovary, which is consistent with previous reports that most ovarian cancers in carriers of *BRCA1* mutations are serous cystadenocarcinomas.<sup>20</sup> The frequencies of the various histologic types of tumors shown in Table 1 suggest that a woman with a serous cystadenocarcinoma is eight times as likely to have a *BRCA1* mutation as

a woman with another type of ovarian cancer (95 percent confidence interval, 1 to 62;  $P < 0.05$ ).

One *BRCA1* carrier (Patient 335) had a borderline mucinous tumor, which is the fifth reported case of a borderline tumor in a woman with a *BRCA1* mutation, and the second reported case of a mucinous tumor.<sup>6,19</sup> The relation of borderline tumors to invasive tumors is unclear. These five cases suggest that some borderline and invasive tumors can be related to similar mutations.

*BRCA1* mutations account for a substantial proportion of familial ovarian cancers, but it is not possible to estimate precisely the role of other genes in familial cases. *BRCA2*, as well as the mismatch-repair genes *hMSH2* and *hMLH1*, which are responsible for hereditary nonpolyposis colorectal cancer, also confer a predisposition to ovarian cancer.

Our results suggest some advantages and disadvantages of various strategies of screening women for *BRCA1* mutations. A policy of restricting genetic testing to families with at least one case of breast cancer diagnosed before the age of 60 years or one case of ovarian cancer in a first- or second-degree relative, in addition to the index case, would have detected the majority of *BRCA1* mutations in our series (9 of 13). With such a policy, only 20 percent of women with ovarian cancer would be screened (55 of the 280 women in our study from whom family histories were available met these criteria). On the basis of our findings, a further selection for serous cystadenocarcinoma would not provide any advantage, because 47 of the 55 women in our study who met the family-history criteria also had serous tumors. Restricting screening even further, to families with three or more cases of ovarian or breast cancer (including the index case), would not be effective, because the majority of *BRCA1* mutations would be missed (10 of 13 in our study).

Primer sequences are available by e-mail from Dr. Stratton at <jfs20@cam.ac.uk>.

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