Frequency of Germline and Somatic BRCA1 Mutations in Ovarian Cancer

Andrew Berchuck,[1] Kerrie-Ann Heron, Michael E. Carney, Johnathan M. Lancaster, Elisa G. Fraser, Vickie L. Vinson, Amie M. Deffenbaugh, Alexander Miron, Jeffrey R. Marks, P. Andrew Futreal, and Tom S. Frank

Departments of Obstetrics and Gynecology/Division of Gynecologic Oncology [A. B., K. H., M. C., J. M. L., P. A. F.], Surgery [A. M., J. R. M., P. A. F.], Duke University Medical Center, Durham, North Carolina 27710 and Myriad Genetic Laboratories, Salt Lake City, Utah 84108 [E. G. F., V. L. V., A. M. D., T. F.]

ABSTRACT

Germline mutations in the *BRCA1* tumor suppressor gene are thought to be the most common cause of hereditary ovarian cancer. The aim of this study was to explore further the role of BRCA1 alterations in the development of ovarian cancers. We sought to determine whether somatic BRCA1 mutations are ever present in ovarian cancers and whether mutation is always accompanied by loss of the wild-type allele. The entire coding region and intronic splice sites of BRCA1 were sequenced using genomic DNA samples from 103 unselected ovarian cancers. Thirteen clearly deleterious BRCA1 mutations and two variants of uncertain significance were found. Blood DNA was available in all but two cases and demonstrated that 4 of 13 mutations and both variants of uncertain significance were germline alterations, whereas in seven cases the mutation was a somatic change present only in the cancer. Using four microsatellite markers, loss of heterozygosity at the *BRCA1* locus was found in all 15 ovarian cancers with BRCA1 sequence alterations, compared with only 58% of ovarian cancers that did not have BRCA1 mutations. BRCA1-associated ovarian cancers were characterized by serous histology and moderate histological grade. These data confirm prior reports suggesting that germline mutations in BRCA1 are present in about 5% of women with ovarian cancer. In addition, somatic mutations in BRCA1 occur in the development of some sporadic cases. The finding that both germline and somatic BRCA1 mutations are accompanied by loss of heterozygosity, suggests that loss of this tumor suppressor gene is a critical event in the development of these cancers.

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[1] To whom requests for reprints should be addressed, at Division of Gynecologic Oncology, Duke University Medical Center, Box 3079, Durham, NC 21170.

INTRODUCTION

Germline mutations in the *BRCA1* gene are thought to be the most common cause of hereditary ovarian cancer. In one large study in the United Kingdom, it was estimated that about 5% of women with epithelial ovarian cancer carry mutations in BRCA1 (1). The fraction of ovarian cancers attributable to germline BRCA1 mutations may vary significantly between populations due to founder effects, however (2). In Ashkenazi Jews, it seems that about 20–30% of ovarian cancer cases arise in BRCA1 carriers (3, 4).

Although loss of heterozygosity at the *BRCA1* locus frequently occurs in sporadic ovarian cancers (5–7), it remains uncertain whether somatic mutations in BRCA1 play a role in their development. At the time of the identification of BRCA1 in 1994, Futreal *et al.* (8) completely sequenced BRCA1 in 12 ovarian and 32 breast cancers that had allele loss at this locus. Four BRCA1 mutations were found, but all of these mutations also were present in the germline. Subsequently, Merajver *et al.* (9) found somatic mutations in BRCA1 in 4 of 47 unselected ovarian cancers, and Hosking *et al.* (10) found one case with a somatic mutation among 17 ovarian cancers with allele loss at BRCA1; but more recent studies in which larger numbers of ovarian cancer were screened failed to identify somatic mutations (7, 8).

To determine the fraction of ovarian cancers in eastern North Carolina that are attributable to germline BRCA1 mutations and whether somatic mutations occur in the development of sporadic ovarian cancers, we performed DNA sequencing of the entire coding region and intronic splice sites using genomic DNA samples from 103 unselected snap-frozen epithelial ovarian cancers. In cases in which mutations were found, the corresponding blood DNA was examined to see if the mutation also was present in the germline. Before the identification of BRCA1, it had been reported that cancers in families demonstrating linkage to chromosome 17q21, invariably had lost the wild-type allele (11). This was interpreted as consistent with the hypothesis that *BRCA1* acts as a tumor suppressor gene. In the present study, we performed loss of heterozygosity analysis on tumors known to harbor BRCA1 mutations to address more definitively the issue of whether mutations always are accompanied by loss of the wild-type allele.

MATERIALS AND METHODS

Ovarian Cancers. The 103 snap-frozen ovarian cancers used were from women who had undergone surgery for epithelial ovarian cancer at Duke University between 1991 and 1996. The study group included 90 Whites, 11 African-Americans, and 2 Native Americans. The median age at diagnosis of the patients in this study was 64 years. Cancers were selected without knowledge of the age at ovarian cancer onset, history of prior breast cancer, or family history of cancer. In most cases, peripheral WBCs also had been obtained. Patients signed con-

Table 1 BRCA1 sequence variants in 103 unselected Duke ovarian cancers

Type	Age	Race	Exon or intron	Mutation	Other cancer	Family history cancer	Histology/grade
Germline mutations							
	49	W	intr5	IVS5-11T>G		Sister, breast Sister, ovarian Father, stomach	Serous/2
	39	W	11	3875del4		Aunt, breast Father, stomach	Serous/2
	46	B	19	5296del4	Breast	Sister, breast	Serous/3
	48	W	11	2576delC		Sister, breast Sister, ovarian Two brothers, colon	Serous/2
Germline variants of uncertain significance							
	40	W	2	D173G			Serous/2
	44	W	11	G1788V	Bilateral breast	Sister, breast Mother, "pelvic cancer"	Serous/2
Somatic mutations							
	49	W	intr2	IVS2+1 G>T			Serous/2
	72	W	11	2569delG			Serous/2
	64	B	11	2388delG			Endometrioid/2
	63	W	5	C61G			Serous/2[a]
	61	W	11	Q1420X		Grandfather, prostate	Serous/2[a]
	59	W	11	R1835X		Brother, lymphoma	Serous/3
	61	W	11	2388delG		Father, leukemia/lung	Serous/1
	70	W	11	2080insA			Serous/3
	56	W	11	E730X	Endometrium	Aunt, breast	Serous/2
Polymorphisms							
	1468	56	White 11	A1641T			Endometrioid/3
	1803	83	White 11	R1347G			Serous/2
	2252	72	White 11	S1512I			Endometrioid/2
	2369	66	White 11	R1347G	Endometrium		Endometrioid/2

[a] Presumed somatic mutations (germline DNA not available for analysis).

sent forms permitting the use of tissue and blood. Medical records were reviewed to collect relevant demographic and clinical information. All of the histology had been reviewed by gynecological pathologists at our institution.

BRCA1 Sequence Analysis. The entire coding region and intronic splice sites of the *BRCA1* gene were sequenced using genomic DNA isolated from 103 snap-frozen ovarian cancers. Sequence analysis of BRCA1 was performed at Myriad Genetic Laboratories. Briefly, exons 2–24 of the *BRCA1* gene were amplified using 35 pairs of PCR primers designed to avoid common polymorphisms that might inhibit equal amplification of both alleles. Dye primer sequencing was performed using fluorescent energy transfer primers (Amersham Life Science, Inc., Cleveland, OH), the mutant Taq polymerase F667Y, and a thermal stable pyrophosphatase (both from Perkin-Elmer Corp., Norwalk, CT). Sequencing reaction products were electrophoresed and detected using a Perkin-Elmer Applied Biosystems 377 sequencing gel. Analyses of sequence data were performed using software developed by Myriad Genetic Laboratories, Inc. All analyses demonstrating mutations were repeated for verification, and the appropriate segment of the *BRCA1* gene was sequenced in the corresponding WBC DNA.

BRCA1 Loss of Heterozygosity. DNA was isolated as prescribed by Puregene (Gentra, Minneapolis, MN), and four microsatellite markers at the *BRCA1* locus were analyzed in paired normal/cancer DNA. PCR reactions were performed using 100 ng of DNA, 1 mM dNTP, 0.5 μg of primer, and 0.5 unit of AmpliTaq Gold in standard buffer with a total volume of 20 μl. Primers for markers flanking the *BRCA1* locus are: THRA1-AC (CTGCGCTTTGCACTATTGGG)/THRA1-TG (CGGGCAGCATAGCATTGCCT) and 658 (AGTCCTGTAGACAAAACCTG)/705 (CAGTTTCATACCAAGTTCCT). Primers intragenic to *BRCA1* include: 17S1322R (CTAGCCTGGGCAACAAACGA)/17S1322F(GCAGGAAGCAGGAATGGAAC) and 855R (ACACAGACTTGTCCTACTGCC)/855F (GGATGGCCTTTTAGAAAGTGG). The PCR reaction involved an initial denaturation step, 95°C/12 min, followed by 32 cycles of 94°C/1 min, 57°C/1 min, and 72°C/1 min. Reactions were then incubated at 72°C/5 min and stored at 4°C. The presence of the correct-sized PCR products was confirmed by electrophoresis on 2.5% agarose gels. For PCR-2, 3 μl of PCR-1 product was added to 1 mM CTP, 1 mM TTP, 1 mM GTP, and 0.1 mM ATP, 0.5 μCi of [α-^{32}P]dATP, 0.5 μg of primer, and 0.5 unit of Taq polymerase (Life Technologies, Inc.). PCR was initiated with a denaturation step of 95°C/5 min, 35 cycles of 95°C/45 s, 60°C/45 s, 72°C/1 min, and then 72°C/5 min. Products were run on a 5% polyacrylamide gel in Tris-borate EDTA buffer and visualized by autoradiography.

RESULTS

Clearly deleterious mutations in BRCA1 were found in 13 of 103 ovarian cancers (Table 1). Two variants of uncertain significance and four probable polymorphisms also were detected. Among the clearly deleterious mutations, 10 involved changes that predict a truncated protein product (7 frameshift, 3 nonsense). In addition, one missense mutation was found (C61G). This mutation, which disrupts a critical cysteine residue

in the zinc finger domain, has been reported previously (12). Two significant intronic splice site missense mutations were found. The mutation seen in intron 5 has been shown previously to represent a significant change that results in a splice variant encoding a truncated protein product (12). Similarly, the intron 2 mutation would be predicted to result in a truncated product or unstable mRNA transcript.

WBC DNA was available in 11 of 13 cases in which a clearly deleterious mutation was found. In four of these cases, the identical mutation was identified in the germline. All of these women developed ovarian cancer before age 50 and had a family history of breast or breast and ovarian cancer (Table 1). In seven cases, the BRCA1 mutation present in the cancer was not found in the corresponding blood DNA and presumably arose as a somatic event. The median age at onset in these seven women was in the early 60s, and none had a first-degree relative with breast and/or ovarian cancer. In two cases in which blood DNA was not available, the patients also had a later age at onset and lacked a family history of cancer. These also were classified as probable somatic mutations.

In addition, missense variants of uncertain significance were seen in two women and both were found in the germline. The D173G change, which was seen in a woman who developed ovarian cancer at age 40, results in substitution of an uncharged (glycine) for a charged (aspartic acid) amino acid. Although this may represent a disease causing mutation, it has not previously been reported. The G1788V mutation, in which valine is substituted for glycine, was seen in a woman with a prior history of bilateral breast cancer who developed ovarian cancer at age 44. In addition, she had a sister who developed breast cancer. Although this is not a striking sequence variant, neither is it a reported polymorphism. Overall, there were four definite and two probable germline BRCA1 mutations (6%) and seven definite and two probable somatic mutations (9%).

Loss of heterozygosity at one or more BRCA1 microsatellite markers was found in all 15 ovarian cancers with BRCA1 sequence alterations. In contrast, only 11 of 19 (58%) ovarian cancers that were completely sequenced and found to have wild-type BRCA1 had loss of heterozygosity at or near the *BRCA1* locus. Examples of ovarian cancers in which there is loss or retention of the wild-type *BRCA1* allele are demonstrated in Fig. 1. In all cases, analysis of the chromosome 17q BRCA1 markers and microsatellite markers D16S767, D19S601, and D3S1763 revealed identical-sized alleles in the paired normal/cancer DNAs confirming the identity of these samples. There was no evidence of microsatellite instability.

We examined the relationship between the status of the *BRCA1* gene and clinical features of ovarian cancers (Table 2). Because the two germline BRCA1 variants of uncertain significance occurred in women with early onset ovarian cancer, we considered them significant mutations for the purpose of this analysis. The median age of those with germline BRCA1 mutations (45 years) was younger than that of cases with somatic mutations (61 years) and cases not associated with BRCA1 mutations (64 years). All but one of the BRCA1-associated ovarian cancers were serous and all were stage III/IV, but they were less likely to be poorly differentiated (20%) relative to cases without mutations (45%). There was no difference between cases with germline mutations and those with somatic mutations with respect to stage or grade. Finally, BRCA1-associated ovarian cancers were about 3-fold more likely to have undergone a negative second-look laparotomy (Table 2).

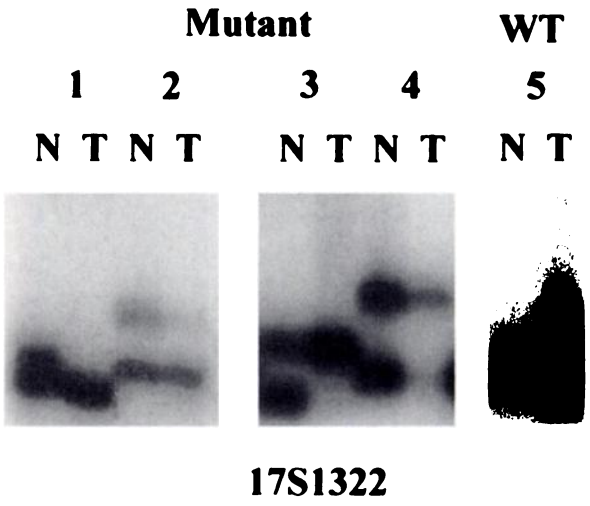

Fig. 1 Loss of heterozygosity at the *BRCA1* locus is seen in ovarian cancers with BRCA1 mutations (*Lanes 1-4*), whereas the ovarian cancer lacking a BRCA1 mutation (*Lane 5*) does not have loss of heterozygosity.

DISCUSSION

In a study from the United Kingdom in which blood cell DNA was screened using single-stranded conformation and heteroduplex analysis, 3% of 375 women with ovarian cancer were found to carry germline BRCA1 mutations (1). Because some mutations may be missed using these techniques, it was estimated that the true frequency of mutations probably was about 5%, but could be as high as 8%. The present study is the first in which the entire coding region and intronic splice sites of the *BRCA1* gene were sequenced in a large group of unselected ovarian cancers. Using this more sensitive detection strategy, we found clearly deleterious germline mutations in 4% of cases and 2% of cases with germline variants of uncertain significance that may represent deleterious mutations. Thus, the fraction of ovarian cancer cases attributable to germline BRCA1 mutations in eastern North Carolina is similar to that estimated for the population studied in the United Kingdom.

Our estimate that approximately 5% of ovarian cancer is attributable to inherited BRCA1 mutations likely represents a reasonable estimate for other areas of the United States as well, because the distribution of racial and clinical features in the study population was relatively representative. One possibly significant difference between our study and the general population is the absence of Ashkenazi Jewish women, who have a much higher incidence of germline BRCA1 mutations (20–30%; Refs. 3 and 4). This group is underrepresented in eastern North Carolina and none of the women in this study are known to be of Ashkenazi descent. Because only 2–3% of American women are of Ashkenazi heritage, even if they had been represented in our study in proportion to the United States population, the overall incidence of germline BRCA1 mutations would only be predicted to rise by 1–2%.

Table 2 Relationship between BRCA1 and clinical features of ovarian cancers

	BRCA1 mutation no./total (%)	P^a
Race		
White	11/90 (12%)	NS
African-American	2/11 (18%)	
Native American	0/2	
Age of onset		
≤50	7/19 (37%)	0.006
>50	8/84 (10%)	
Family history of breast/ovarian cancer		
No	9/75 (12%)	0.35
Yes	6/28 (21%)	
First-degree relative with breast/ovarian cancer		
No	11/90 (12%)	0.09
Yes	4/13 (31%)	
Histology		
Serous	14/80 (18%)	0.18
Endometrioid	1/7 (14%)	
Mucinous	0/4	
Clear cell	0/4	
Undifferentiated	0/8	
Grade		
Borderline	0/5	0.09
Well	1/4 (25%)	
Moderate	11/52 (21%)	
Poor	3/42 (7%)	
Stage		
I/II	0/14	0.12
III/IV	15/89 (17%)	
Second-look		
Negative	6/18 (33%)	0.07
Microscopic	1/9 (11%)	
Positive	3/26 (12%)	
Not done	5/48 (10%)	

[a] Fisher's exact test.

We also cannot exclude the possibility that BRCA1 is inactivated in some women with ovarian cancer by other genetic mechanisms. Although it is unlikely that we missed mutations in the BRCA1 coding sequence or splice sites, it has been documented that regulatory mutations affecting the promoter can occur and these would not be detected using DNA sequencing (13). In addition, large genomic deletions in BRCA1 have been reported that are not detectable with the conventional PCR-based DNA sequencing mutation detection techniques (14). Additional studies in the United States population are needed to determine whether these other types of mutations contribute significantly to the development of any ovarian cancers.

Initially, two small studies reported somatic mutations in BRCA1 in about 10% of 54 ovarian cancers (9, 10). Larger studies published subsequently did not confirm this finding, however. Although BRCA1 mutations were seen in 7 of 115 and 4 of 76 ovarian cancers, all of the mutations were also present in the germline, and it was concluded that somatic mutations rarely occur (7, 8). Similarly, somatic mutations in BRCA1 have not been described in breast cancers (8, 13). In the present study, sequencing of the *BRCA1* gene in 103 ovarian cancers revealed mutations in at least 7, and possibly 9, cases that were present in the cancer but not in paired normal WBC DNA. The frequency of somatic BRCA1 mutations in the present study (7–9%) is similar to that reported in the two earlier small studies (10%; Refs. 9 and 10).

In contrast to women with germline BRCA1 mutations whose median age at ovarian cancer diagnosis was in the mid-40s, the median age of women with somatic mutations was about 60 years. This is consistent with the "multi-hit" theory of carcinogenesis in which several genetic alterations are requisite for tumorigenesis. Women with an inherited mutation in BRCA1 have a head start along this pathway and develop ovarian cancer at an earlier age, but additional genetic alterations must occur for malignant transformation to take place. In this regard, other genetic alterations have been demonstrated in BRCA1-associated breast cancers (15). In addition, it has been suggested that BRCA1 may be involved in DNA recombination and repair pathways (16). Thus, mutations in this gene may serve not only as the first hit in the transformation process, but also to accelerate the rate of accumulation of alterations in other cancer causing genes.

Before the identification of BRCA1, it had been anticipated that somatic mutations in BRCA1 would be common in ovarian cancers, because more than half of these cancers exhibit loss of heterozygosity on chromosome 17q (5–7). In a previous study from our group, loss of heterozygosity on chromosome 17q was seen in 64 of 120 (53%) cases (5). In 56 cases with allele loss, all informative markers on 17q were lost whereas focal losses were seen in only 8 cases. In the present study, we found that loss of the wild-type *BRCA1* allele invariably accompanied both germline and somatic BRCA1 mutations in 15 cases, whereas in a control group of 19 ovarian cancers lacking mutations 58% were found to have 17q allele loss similar to our prior study (5). Loss of the wild-type allele also was found in all eight breast and ovarian cancers that developed in women with germline BRCA1 mutations in a Japanese study (17) and in six ovarian cancers in a study from the United States (7). These data are supportive of the hypothesis that loss of BRCA1 function occurs by way of the classic tumor suppressor paradigm with mutation of one copy and deletion of the other.

In summarizing four prior hospital-based studies (1, 7, 17, 18) and the present study, 94% (59 of 63 cases) of BRCA1-associated ovarian cancers have been serous. Although this is the most commonly observed histological type of epithelial ovarian cancer, it generally accounts for only about one-half to two-thirds of cases. In view of the consistency of these reports, it now seems reasonably certain that serous histology is a hallmark of ovarian cancers with BRCA1 mutations.

Rubin *et al.* (19) found that survival of 43 BRCA1 carriers with advanced ovarian cancer in a collaborative study in the United States was markedly better than that of carefully matched sporadic cases. Actuarial median 5-year survival was 77 months in carriers and 29 months in noncarriers. Similarly, a Japanese group reported 79% survival in 13 stage III BRCA1-associated ovarian cancers treated with cisplatin-containing regimens, compared with 30% in matched controls (20). The present study suggests that BRCA1-associated ovarian cancers have a more favorable phenotype regardless of whether the mutation is germline or sporadic. Although all of the BRCA1-associated cases in this report were advanced stage, they were half as likely to be poorly differentiated and twice as likely to have a patho-

logically negative second-look operation after completion of primary chemotherapy, compared with cases without BRCA1 mutations. In contrast, a recent population-based study from Sweden noted an initial survival advantage in BRCA1-associated cases, but this did not persist over time (21). None of the studies performed to date have been optimally designed to assess survival and have included only small numbers of cases, however. Previous studies have demonstrated associations between alterations in the *p53* (22) and HER-2/*neu* (23) genes and high-risk clinical features and poor outcome. Thus, it would not be surprising if other genetic alterations, such as BRCA1 mutation, are associated with a more favorable clinical phenotype in ovarian cancer.

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REFERENCES

1. Stratton, J. F., Gayther, S. A., Russell, P., Dearden, J., Gore, M., Blake, P., Easton, D., and Ponder, B. A. Contribution of BRCA1 mutations to ovarian cancer. N. Engl. J. Med., *336:* 1125–1130, 1997.

2. Szabo, C. I., and King, M. C. Population genetics of BRCA1 and BRCA2. Am. J. Hum. Genet., *60:* 1013–1020, 1997.

3. Abeliovich, D., Kaduri, L., Lerer, I., Weinberg, N., Amir, G., Sagi, M., Zlotogora, J., Heching, N., and Peretz, T. The founder mutations 185delAG and 5382insC in BRCA1 and 6174delT in BRCA2 appear in 60% of ovarian cancer and 30% of early-onset breast cancer patients among Ashkenazi women. Am. J. Hum. Genet., *60:* 505–514, 1997.

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

5. Jacobs, I. J., Smith, S., Wiseman, R., Futreal, A., Berchuck, A., Ponder, B. J., and Bast, R. C. A deletion unit on chromosome 17q defined by loss of heterozygosity in epithelial ovarian cancer and a benign serous cystadenoma. Cancer Res., *53:* 1218–1221, 1993.

6. Schildkraut, J. M., Collins, N. K., Dent, G. A., Tucker, J. A., Barrett, J. C., Berchuck, A., and Boyd, J. Loss of heterozygosity on chromosome 17q11–21 in cancers of women who have both breast and ovarian cancer. Am. J. Obstet. Gynecol., *172:* 908–913, 1995.

7. Takahashi, H., Behbakht, K., McGovern, P. E., Chiu, H. C., Couch, F. J., Weber, B. L., Friedman, L. S., King, M. C., Furusato, M., and Livolsi, V. A. Mutation analysis of the *BRCA1* gene in ovarian cancers. Cancer Res., *55:* 2998–3002, 1995.

8. Futreal, P. A., Liu, Q., Shattuck-Eidens, D., Cochran, C., Harshman, K., Tavtigian, S., Bennett, L. M., Haugen-Strano, A., Swensen, J., and Miki, Y. BRCA1 mutations in primary breast and ovarian carcinomas. Science (Washington DC), *266:* 120–122, 1994.

9. Merajver, S. D., Pham, T. M., Caduff, R. F., Chen, M., Poy, E. L., Cooney, K. A., Weber, B. L., Collins, F. S., Johnston, C., and Frank, T. S. Somatic mutations in the BRCA1 gene in sporadic ovarian tumours. Nat. Genet., *9:* 439–443, 1995.

10. Hosking, L., Trowsdale, J., Nicolai, H., Solomon, E., Foulkes, W., Stamp, G., Signer, E., and Jeffreys, A. A somatic BRCA1 mutation in an ovarian tumour. Nat. Genet., *9:* 343–344, 1995.

11. Smith, S. A., Easton, D. F., Evans, D. G., and Ponder, B. A. Allele losses in the region 17q12–21 in familial breast and ovarian cancer involve the wild-type chromosome. Nat. Genet., *2:* 128–131, 1992.

12. Shattuck-Eidens, D., Oliphant, A., McClure, M., McBride, C., Gupte, J., Rubano, T., Pruss, D., Tavtigian, S. V., Teng, D. H., Adey, N., Staebell, M., Gumpper, K., Lundstrom, R., Hulick, M., Kelly, M., Holmen, J., Lingenfelter, B., Manley, S., Fujimura, F., Luce, M., Ward, B., Cannon-Albright, L., Steele, L., Offit, K., Gilewski, T., Norton, L., Brown, K., Schulz, C., Hampel, H., Schluger, A., Giulotto, E., Zoli, W., Ravaioli, A., Nevanlinna, H., Pyrhonen, S., Rowley, P., Loader, S., Osborne, M. P., Daly, M., Tepler, I., Weinstein, P. L., Scalia, J. L., Michaelson, R., Scott, R. J., Radice, P., Pierotti, M. A., Garber, J. E., Isaacs, C., Peshkin, B., Lippman, M. E., Dosik, M. H., Caligo, M. A., Greenstein, R. M., Pilarski, R., Weber, B., Burgemeister, R., Frank, T. S., Skolnick, M. H., and Thomas, A. BRCA1 sequence analysis in women at high risk for susceptibility mutations. Risk factor analysis and implications for genetic testing. J. Am. Med. Assoc., *278:* 1242–1250, 1997.

13. Couch, F. J., and Weber, B. L. Mutations and polymorphisms in the familial early-onset breast cancer (BRCA1) gene. Hum. Mutat., *8:* 8–18, 1996.

14. Petrij-Bosch, A., Peelen, T., van, V. M., van, E. R., Olmer, R., Drusedau, M., Hogervorst, F. B., Hageman, S., Arts, P. J., Ligtenberg, M. J., Meijers-Heijboer, H., Klijn, J. G., Vasen, H. F., Cornelisse, C. J., van't, V. L. J., Bakker, E., van, O. G. J., and Devilee, P. BRCA1 genomic deletions are major founder mutations in Dutch breast cancer patients. Nat. Genet., *17:* 341–345, 1997.

15. Tirkkonen, M., Johannsson, O., Agnarrsson, B. A., Olsson, H., Ingvarsson, S., Karhu, R., Tanner, M., Isola, J., Barkardottir, R. B., Borg, A., and Kallioniemi, O. P. Distinct somatic genetic changes associated with tumor progression in carriers of BRCA1 and BRCA2 germ-line mutations. Cancer Res., *57:* 1222–1227, 1997.

16. Scully, R., Chen, J., Plug, A., Xiao, Y., Weaver, D., Feunteun, J., Ashley, T., and Livingston, D. M. Association of BRCA1 with Rad51 in mitotic and meiotic cells. Cell, *88:* 265–275, 1997.

17. Takano, M., Aida, H., Tsuneki, I., Takakuwa, K., Hasegawa, I., Tanaka, H., Saito, M., Tsuji, S., Sonoda, T., Hatae, M., Chen, J. T., Takahashi, K., Hasegawa, K., Toyoda, N., Saito, N., Yakushiji, M., Araki, T., and Tanaka, K. Mutational analysis of BRCA1 gene in ovarian and breast-ovarian cancer families in Japan. Jpn. J. Cancer Res., *88:* 407–413, 1997.

18. Matsushima, M., Kobayashi, K., Emi, M., Saito, H., Saito, J., Suzumori, K., and Nakamura, Y. 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, 1995.

19. Rubin, S. C., Benjamin, I., Behbakht, K., Takahashi, H., Morgan, M. A., Livolsi, V. A., Berchuck, A., Muto, M. G., Garber, J. E., Weber, B. L., Lynch, H. T., and Boyd, J. Clinical and pathological features of ovarian cancer in women with germ-line mutations of BRCA1. N. Engl. J. Med., *335:* 1413–1416, 1996.

20. Aida, H., Takakuwa, K., Nagata, H., Tsuneki, I., Takano, M., Tsuji, S., Takahashi, T., Sonoda, T., Hatae, M., Takahashi, K., Hasegawa, K., Mizunuma, H., Toyoda, N., Kamata, H., Torii, Y., Saito, N., Tanaka, K., Yakushiji, M., and Araki, T. Clinical features of ovarian cancer in Japanese women with germ-line mutations of BRCA1. Clin. Cancer Res., *4:* 235–240, 1998.

21. Johannsson, O. T., Ranstram, J., Borg, A., and Olsson, H. Survival of BRCA1 breast and ovarian cancer patients: a population-based study from southern Sweden. J. Clin. Oncol., *16:* 397–404, 1998.

22. Hartmann, L., Podratz, K., Keeney, G., Kamel, N., Edmonson, J., Grill, J., Su, J., Katzmann, J., and Roche, P. Prognostic significance of p53 immunostaining in epithelial ovarian cancer. J. Clin. Oncol., *12:* 64–69, 1994.

23. Berchuck, A., Kamel, A., Whitaker, R., Kerns, B., Olt, G., Kinney, R., Soper, J. T., Dodge, R., Clarke-Pearson, P., Marks, S., McKenzie, S., Yin, S., and Bast, R. C., Jr. Overexpression of HER-2/*neu* is associated with poor survival in advanced epithelial ovarian cancer. Cancer Res., *50:* 4087–4091, 1990.

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