Mutation Analysis of the BRCA1 Gene in Ovarian Cancers

Hiroyuki Takahashi, Kian Behbakh, Patricia E. McGovern, Hsiu-Chiang Chiu, Fergus J. Couch, Barbara L. Weber, Lori S. Friedman, Mary-Claire King, Masakuni Furusato, Virginia A. LiVolsi, Andrew W. Menzin, Paul C. Liu, Ivor Benjamin, Mark A. Morgan, Stephanie A. King, Beth Ann Rebane, Annmarie Cardonick, John J. Mikuta, Stephen C. Rubin, and Jeff Boyd

Division of Gynecologic Oncology, Department of Obstetrics and Gynecology [H. T., K. B., P. E. M., H-C. C., A. W. M., P. C. L., I. B., M. A. M., S. A. K., B. A. R., A. C., J. J. M., S. C. R., J. B.], Division of Hematology-Oncology, Department of Medicine [F. J. C., B. L. W.], Surgical Pathology Section, Department of Pathology and Laboratory Medicine [V. A. L.], and University of Pennsylvania Medical Center and University of Pennsylvania Comprehensive Cancer Center [B. L. W., V. A. L., I. B., M. A. M., J. J. M., S. C. R., J. B.], Philadelphia, Pennsylvania 19104; Department of Molecular and Cell Biology and School of Public Health, University of California, Berkeley, California 94720 [L. S. F., M-C. K.]; and Department of Pathology, Jikei University School of Medicine, Tokyo 105, Japan [M. F.]

Abstract

Germline mutations of the BRCA1 tumor suppressor gene on chromosome 17q are involved in a significant fraction of hereditary breast and ovarian cancers. Allelic deletions that include the BRCA1 locus are common in breast and ovarian cancers, implying that somatic mutations of this gene may play an important role in the more common sporadic forms of these tumors as well. The recent cloning of BRCA1 allows direct testing of this hypothesis. A combination of single strand conformation and sequencing analyses was used to examine the 22 coding exons and intronic flanking the gene, occur in 50–75% of breast carcinomas (11–13) and imply that somatic mutation of BRCA1 plays a role in the development of many sporadic breast cancers as well. Similarly, chromosome 17 is frequently affected by allelic deletion in ovarian carcinoma, with up to 75% of tumors exhibiting LOH on 17q (14–18).

The recent cloning and characterization of BRCA1 (19) now allows direct determination of the extent of BRCA1 involvement in hereditary and sporadic cancers. Inactivating mutations of this gene, consistent with its role as a putative tumor suppressor, were detected in the germlines of family members that segregate a BRCA1 allele with breast and ovarian cancer susceptibility (19, 20), but somatic mutations were not observed in 32 breast or 12 ovarian cancers with LOH in this region (20). Subsequent studies have confirmed the role of germline BRCA1 mutations in hereditary breast and ovarian cancers (21–24). These observations raise the possibility that BRCA1 plays an important role in hereditary but not in sporadic tumors. The purpose of this study was to examine a larger number of unselected ovarian carcinomas to further clarify the extent of BRCA1 involvement in both sporadic and familial forms of this tumor type.

Materials and Methods

Tissue Acquisition and DNA Preparation. epithelial ovarian carcinomas were obtained at the Hospital of the University of Pennsylvania and from the Gynecologic Oncology Group/Cohortive Human Tissue Network ovarian tissue bank (Columbus, OH). An Institutional Review Board-approved informed consent was obtained from each patient before tissue acquisition. The tumors used in this study were primary site cancers only that had not been treated previously with either chemotherapy or radiation and were representative for grade, stage, and histological subtype. Corresponding normal tissues consisted of either lymphocytes or uninvolved tissue from the reproductive tract removed at hysterectomy. Tissues were flash frozen in liquid nitrogen after pathological examination, and genomic DNA was prepared with the use of standard procedures (25).

SSCP and Sequence Analyses. For SSCP analysis, PCR amplification of genomic DNA was performed with the use of intron-based primers surrounding each of the 22 exons (19, 20), with the exceptions that 16 overlapping primer sets were used to examine exon 11, and two additional primer sets were designed to amplify exons 6 and 7 as two separate products (21, 23). Exons 1 and 4 were not examined. PCR reactions were carried out in a volume of 10 μl containing 50 ng of genomic DNA, 1.5 mM MgCl₂, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 200 μM concentration of each dATP, dGTP, and dTTP, 20 μM dCTP, 1 μCi of [α-32P]dCTP (6,000 Ci/mmol; DuPont/NEN), each primer at 0.8 μM, and 0.75 units of Taq polymerase (Perkin Elmer Cetus). Amplification was for 35 cycles in a Perkin Elmer Cetus 9600 thermal cycler consisting of 30 s at 94°C, 1 min at 60°C, and 1 min at 72°C, with a 7 min extension at 72°C after the last cycle. A 2-μl aliquot of the product was diluted into 48 μl of denaturing loading buffer (95% formamide, 10 mm NaOH, 0.05% xylene cyanol FF, and 0.05% bromophenol blue), heated at 95°C for 10 min, and cooled to room temperature for 10 min. 7 μl of this solution was loaded for electrophoresis. SSCP gels consisted of 0.5× MDE solution (AT Biochem, Malvern, PA) in

Received 4/6/95; accepted 6/1/95.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 To whom requests for reprints should be addressed, at Department of Obstetrics and Gynecology, University of Pennsylvania Medical Center, 778 Clinical Research Building, 415 Curie Boulevard, Philadelphia, PA 19104.

2 The abbreviations used are: LOH, loss of heterozygosity; SSCP, single strand conformation polymorphism.
0.6 X Tris-borate EDTA buffer and were run in 0.6 X Tris-borate EDTA buffer (1 X concentration is 90 mm Tris-borate, 2 mm EDTA) at 8 W for 16-18 h at room temperature. After electrophoresis, gels were dried and exposed to reflection autoradiography film (DuPont NEN Research Products) for 3-16 h at room temperature.

After autoradiography for SSCP analysis, DNA bands of variant and wild-type mobility were excised from the gels and eluted into 50 µl of Tris-EDTA (IX concentration is 90 mM Tris-borate, 2 mM EDTA) at 8 W for 16-18 h at room temperature. After electrophoresis, gels were dried and exposed to reflection autoradiography film for 4-16 h at room temperature.

Allelotyping. LOH in the BRCA1 region was assessed with the use of the dinucleotide repeat markers THRA1, D17S1147, D17S855, D17S750, and D17S579. PCR reaction conditions were as described above for SSCP analysis, with the exceptions that all dNTPs were included at 200 µM and radiolabeled dCTP was omitted. One primer was end labeled with [γ-32P]ATP by polynucleotide kinase with the use of Wizard PCR Prep DNA Purification system (Promega), and subjected to cycle sequencing with the use of fmol DNA Sequencing system (Promega). Primer end-labeling and cycle-sequence reaction conditions were exactly as specified by the manufacturer. All templates were sequenced twice, once with each radiolabeled primer. After completion of the cycle sequencing reaction, 5 µl of denaturing loading buffer (as described earlier) were added to each sequencing reaction, and the samples were denatured at 95°C for 5 min, placed on ice, and 4 µl were loaded into a 6% polyacrylamide gel containing 7 M urea. Electrophoresis was at 70 W for 2-4 h at room temperature. Sequencing gels were fixed in 10% methanol-10% acetic acid, dried, and exposed to reflection autoradiography film for 1-4 days at room temperature.

Allelotyping. LOH in the BRCA1 region was assessed with the use of the dinucleotide repeat markers THRA1, D17S1147, D17S855, D17S750, and D17S579. PCR reaction conditions were as described above for SSCP analysis, with the exceptions that all dNTPs were included at 200 µM and radiolabeled dCTP was omitted. One primer was end labeled with [γ-32P]ATP by polynucleotide kinase with the use of Wizard PCR Prep DNA Purification system (Promega), and subjected to cycle sequencing with the use of fmol DNA Sequencing system (Promega). Primer end-labeling and cycle-sequence reaction conditions were exactly as specified by the manufacturer. All templates were sequenced twice, once with each radiolabeled primer. After completion of the cycle sequencing reaction, 5 µl of denaturing loading buffer (as described earlier) were added to each sequencing reaction, and the samples were denatured at 95°C for 5 min, placed on ice, and 4 µl were loaded into a 6% polyacrylamide gel containing 7 M urea. Electrophoresis was at 70 W for 2-4 h at room temperature. Sequencing gels were fixed in 10% methanol-10% acetic acid, dried, and exposed to reflection autoradiography film for 1-4 days at room temperature.

Allelotyping. LOH in the BRCA1 region was assessed with the use of the dinucleotide repeat markers THRA1, D17S1147, D17S855, D17S750, and D17S579. PCR reaction conditions were as described above for SSCP analysis, with the exceptions that all dNTPs were included at 200 µM and radiolabeled dCTP was omitted. One primer was end labeled with [γ-32P]ATP by polynucleotide kinase with the use of Wizard PCR Prep DNA Purification system (Promega), and subjected to cycle sequencing with the use of fmol DNA Sequencing system (Promega). Primer end-labeling and cycle-sequence reaction conditions were exactly as specified by the manufacturer. All templates were sequenced twice, once with each radiolabeled primer. After completion of the cycle sequencing reaction, 5 µl of denaturing loading buffer (as described earlier) were added to each sequencing reaction, and the samples were denatured at 95°C for 5 min, placed on ice, and 4 µl were loaded into a 6% polyacrylamide gel containing 7 M urea. Electrophoresis was at 70 W for 2-4 h at room temperature. Sequencing gels were fixed in 10% methanol-10% acetic acid, dried, and exposed to reflection autoradiography film for 1-4 days at room temperature.

Allelotyping. LOH in the BRCA1 region was assessed with the use of the dinucleotide repeat markers THRA1, D17S1147, D17S855, D17S750, and D17S579. PCR reaction conditions were as described above for SSCP analysis, with the exceptions that all dNTPs were included at 200 µM and radiolabeled dCTP was omitted. One primer was end labeled with [γ-32P]ATP by polynucleotide kinase with the use of Wizard PCR Prep DNA Purification system (Promega), and subjected to cycle sequencing with the use of fmol DNA Sequencing system (Promega). Primer end-labeling and cycle-sequence reaction conditions were exactly as specified by the manufacturer. All templates were sequenced twice, once with each radiolabeled primer. After completion of the cycle sequencing reaction, 5 µl of denaturing loading buffer (as described earlier) were added to each sequencing reaction, and the samples were denatured at 95°C for 5 min, placed on ice, and 4 µl were loaded into a 6% polyacrylamide gel containing 7 M urea. Electrophoresis was at 70 W for 2-4 h at room temperature. Sequencing gels were fixed in 10% methanol-10% acetic acid, dried, and exposed to reflection autoradiography film for 1-4 days at room temperature.

Allelotyping. LOH in the BRCA1 region was assessed with the use of the dinucleotide repeat markers THRA1, D17S1147, D17S855, D17S750, and D17S579. PCR reaction conditions were as described above for SSCP analysis, with the exceptions that all dNTPs were included at 200 µM and radiolabeled dCTP was omitted. One primer was end labeled with [γ-32P]ATP by polynucleotide kinase with the use of Wizard PCR Prep DNA Purification system (Promega), and subjected to cycle sequencing with the use of fmol DNA Sequencing system (Promega). Primer end-labeling and cycle-sequence reaction conditions were exactly as specified by the manufacturer. All templates were sequenced twice, once with each radiolabeled primer. After completion of the cycle sequencing reaction, 5 µl of denaturing loading buffer (as described earlier) were added to each sequencing reaction, and the samples were denatured at 95°C for 5 min, placed on ice, and 4 µl were loaded into a 6% polyacrylamide gel containing 7 M urea. Electrophoresis was at 70 W for 2-4 h at room temperature. Sequencing gels were fixed in 10% methanol-10% acetic acid, dried, and exposed to reflection autoradiography film for 1-4 days at room temperature.

Allelotyping. LOH in the BRCA1 region was assessed with the use of the dinucleotide repeat markers THRA1, D17S1147, D17S855, D17S750, and D17S579. PCR reaction conditions were as described above for SSCP analysis, with the exceptions that all dNTPs were included at 200 µM and radiolabeled dCTP was omitted. One primer was end labeled with [γ-32P]ATP by polynucleotide kinase with the use of Wizard PCR Prep DNA Purification system (Promega), and subjected to cycle sequencing with the use of fmol DNA Sequencing system (Promega). Primer end-labeling and cycle-sequence reaction conditions were exactly as specified by the manufacturer. All templates were sequenced twice, once with each radiolabeled primer. After completion of the cycle sequencing reaction, 5 µl of denaturing loading buffer (as described earlier) were added to each sequencing reaction, and the samples were denatured at 95°C for 5 min, placed on ice, and 4 µl were loaded into a 6% polyacrylamide gel containing 7 M urea. Electrophoresis was at 70 W for 2-4 h at room temperature. Sequencing gels were fixed in 10% methanol-10% acetic acid, dried, and exposed to reflection autoradiography film for 1-4 days at room temperature.

Allelotyping. LOH in the BRCA1 region was assessed with the use of the dinucleotide repeat markers THRA1, D17S1147, D17S855, D17S750, and D17S579. PCR reaction conditions were as described above for SSCP analysis, with the exceptions that all dNTPs were included at 200 µM and radiolabeled dCTP was omitted. One primer was end labeled with [γ-32P]ATP by polynucleotide kinase with the use of Wizard PCR Prep DNA Purification system (Promega), and subjected to cycle sequencing with the use of fmol DNA Sequencing system (Promega). Primer end-labeling and cycle-sequence reaction conditions were exactly as specified by the manufacturer. All templates were sequenced twice, once with each radiolabeled primer. After completion of the cycle sequencing reaction, 5 µl of denaturing loading buffer (as described earlier) were added to each sequencing reaction, and the samples were denatured at 95°C for 5 min, placed on ice, and 4 µl were loaded into a 6% polyacrylamide gel containing 7 M urea. Electrophoresis was at 70 W for 2-4 h at room temperature. Sequencing gels were fixed in 10% methanol-10% acetic acid, dried, and exposed to reflection autoradiography film for 1-4 days at room temperature.

Allelotyping. LOH in the BRCA1 region was assessed with the use of the dinucleotide repeat markers THRA1, D17S1147, D17S855, D17S750, and D17S579. PCR reaction conditions were as described above for SSCP analysis, with the exceptions that all dNTPs were included at 200 µM and radiolabeled dCTP was omitted. One primer was end labeled with [γ-32P]ATP by polynucleotide kinase with the use of Wizard PCR Prep DNA Purification system (Promega), and subjected to cycle sequencing with the use of fmol DNA Sequencing system (Promega). Primer end-labeling and cycle-sequence reaction conditions were exactly as specified by the manufacturer. All templates were sequenced twice, once with each radiolabeled primer. After completion of the cycle sequencing reaction, 5 µl of denaturing loading buffer (as described earlier) were added to each sequencing reaction, and the samples were denatured at 95°C for 5 min, placed on ice, and 4 µl were loaded into a 6% polyacrylamide gel containing 7 M urea. Electrophoresis was at 70 W for 2-4 h at room temperature. Sequencing gels were fixed in 10% methanol-10% acetic acid, dried, and exposed to reflection autoradiography film for 1-4 days at room temperature.
Three additional patients with germline inactivating mutations of BRCAl presented with unremarkable or modest family and medical histories. Patient 282 had no medical history of cancer and was from a Chinese family. Patient 291 had no remarkable family history of cancer but developed breast cancer at age 35 years and ovarian cancer at the relatively young age of 43 years. Less than 16% of ovarian cancers are diagnosed under the age of 44 years (1). It is noteworthiy that all six cases of ovarian cancer associated with germline BRCAl mutations were of serous histology, a subtype of epithelial ovarian cancer that constitutes 46% of all such cases generally (28). These results are consistent with those of previous studies, in which BRCAl have not commented on the histopathological features of ovarian cancers in the affected families (19-24), but our data are consistent with several epidemiological and linkage studies describing an extreme underrepresentation of mucinous tumors in all types of familial ovarian cancer (4, 5, 29–32). There was no obvious correlation between the type or distribution of mutations that we observed and the specificity for ovarian cancer. Two have been described thus far in eight families (24), including four unrelated Canadian families that appeared to share a common haplotype for four surrounding polymorphic markers (22). Our patient 363 is of Ashkenazi Jewish descent, and it would be interesting to determine if other families with the 23delAG mutation of similar ethnic origin.

In regard to clinical and histopathological correlates, it is noteworthy that all six cases of ovarian cancer associated with germline BRCAl mutations were of serous histology, a subtype of epithelial ovarian cancer that constitutes 46% of all such cases generally (28).

Table 1. Summary of BRCAl mutations

<table>
<thead>
<tr>
<th>Patient</th>
<th>Codon/nucleotide</th>
<th>Mutation</th>
<th>Medical history</th>
<th>Family history</th>
</tr>
</thead>
<tbody>
<tr>
<td>73</td>
<td>Intron 20+48</td>
<td>12-bp duplication/insertion</td>
<td>BrCa, a age 38 yr</td>
<td>M: BrCa, CA: (x4), MA: BrCa</td>
</tr>
<tr>
<td>281</td>
<td>61/300</td>
<td>Missense TGT(cys) → GGT(gly)</td>
<td>BrCa, a age 36 yr</td>
<td>M: BrCa, a age 48 yr</td>
</tr>
<tr>
<td>282</td>
<td>24–25/191–192</td>
<td>2-bp deletion</td>
<td>None</td>
<td>M: OvCa, a age 41 yr</td>
</tr>
<tr>
<td>291</td>
<td>1290/3989–3990</td>
<td>2-bp deletion</td>
<td>OvCa, a age 30 yr, OvCa, a age 40 yr, OvCa, a age 41 yr</td>
<td>None</td>
</tr>
<tr>
<td>300</td>
<td>197/710</td>
<td>Silent</td>
<td>None</td>
<td>M: OvCa, a age 41 yr</td>
</tr>
<tr>
<td>337</td>
<td>1234–1235/3820–3824</td>
<td>5-bp deletion GTA/AAC → C (1242 stop)</td>
<td>OvCa, a age 43 yr</td>
<td>M: BrCa, a age 43 yr</td>
</tr>
<tr>
<td>355</td>
<td>1257/3890–3891</td>
<td>2-bp deletion GAG → G (1266 stop)</td>
<td>BrCa, a age 30 yr, OvCa, a age 41 yr</td>
<td>M: BrCa, a age 69 yr</td>
</tr>
<tr>
<td>363</td>
<td>23/187–188</td>
<td>2-bp deletion GAG → G (39 stop)</td>
<td>OvCa, a age 47 yr</td>
<td>BrCa (x10), age 30–45 yr, OvCa (x7), age 45–58 yr, PrCa (x5), age 47–65 yr, CoCa (x1), age 55 yr</td>
</tr>
</tbody>
</table>

a Numbered according to nucleotide sequence under GenBank accession no. U14680 on October 18, 1994.
b OvCa, ovarian cancer; BrCa, breast cancer; EmCa, endometrial cancer; CxCa, cervical cancer; PrCa, prostate cancer; CoCa, colon cancer; M, mother; MA, maternal aunt; MC, maternal cousin.

20, it is possible that this alteration prevents the correct splicing of exons 20 and 21. We were unable to obtain additional fresh tissue from this patient for use in the preparation of RNA that would have allowed testing of this hypothesis.

All of the germline sequence variants shown in Table 1 occurred in individuals with medical and/or family histories of breast and/or ovarian cancer. Three of these patients had medical histories of dual primary cancers of the breast and ovary, with an average age of diagnosis of 35 years for breast cancer and an average age of 44 years for ovarian cancer. We have shown previously that LOH in the BRCAl region of chromosome 17q is common in tumors from patients with dual primary breast and ovarian cancers, and that the average age of diagnosis is significantly lower in patients with BRCAl-region LOH in both tumors compared to patients with BRCAl-region LOH in one or the other tumor only (26). Furthermore, the same allele is deleted in all cases of dual primary cancers with 17q LOH (26). Taken together, these data indicate that the inheritance of a mutant BRCAl allele plays a critical role in a significant fraction of dual primary breast and ovarian cancer cases. All three of these patients had mothers with breast cancer; patient 73 had four maternal aunts and a maternal cousin with breast cancer. Interestingly, there was no previous history of ovarian cancer evident in any of these three families.

Three additional patients with germline inactivating mutations of BRCAl presented with unremarkable or modest family and medical histories. Patient 282 had no medical history of cancer and was screened based on a maternal history of ovarian cancer at age 41 years. The identification of a severe germline BRCAl mutation in a disease-free individual such as this has significant implications for genetic counseling and clinical management (27). Patient 337 had a mother diagnosed with breast cancer at age 43 years and presented with ovarian cancer at the relatively young age of 43 years. Less than 16% of ovarian cancers are diagnosed under the age of 44 years (1). Patient 291 had no remarkable family history of cancer but developed breast cancer at age 35 years, and ovarian cancer at age 43 years, and a cervical carcinoma at age 41 years. Although tissues were not available from the cervical and endometrial tumors for an assessment of LOH, these observations are consistent with the possibility that BRCAl may also be involved in the etiology of endometrial and cervical cancer.

Patient 363 presented with ovarian cancer at age 47 years and a classical breast-ovarian cancer family history, including 17 cases of primarily early onset breast or ovarian cancer and 5 cases of prostate cancer. The codon 23 mutation in this individual is one of the two most common BRCAl mutations reported previously, having been described thus far in eight families (24), including four unrelated Canadian families that appeared to share a common haplotype for four surrounding polymorphic markers (22). Our patient 363 is of Ashkenazi Jewish descent, and it would be interesting to determine if other families with the 23delAG mutation are of similar ethnic origin.

In regard to clinical and histopathological correlates, it is noteworthy that all six cases of ovarian cancer associated with germline BRCAl mutations were of serous histology, a subtype of epithelial ovarian cancer that constitutes 46% of all such cases generally (28). The six other published reports describing germline mutations in BRCAl have not commented on the histopathological features of ovarian cancers in the affected families (19–24), but our data are consistent with several epidemiological and linkage studies describing an extreme underrepresentation of mucinous tumors in all types of familial ovarian cancer (4, 5, 29–32). There was no obvious correlation between the type or distribution of mutations that we observed and the specificity for ovarian cancer. Two have been described previously (23delAG and Cys61Gly) and five are novel; the mutations were in exons 2, 5, and 11, all occurring in the 5'-half of the gene. There is suggestive evidence that mutations nearer the 3' end of the gene are associated with a lower incidence of ovarian cancer in affected families (24), and these data are consistent with that trend.

Corresponding normal tissue was available from 89 of the 115 ovarian cancer cases screened for BRCAl mutations in this study. These tumors were analyzed for LOH in the BRCAl region of chromosome 17q with the use of a panel of five dinucleotide repeat markers flanking and intragenic to BRCAl. Sixty of 89 tumors (67%) exhibited a pattern of LOH that included the BRCAl gene (Fig. 3). These results are consistent with those of previous studies, in which 17q LOH has been observed in 39–77% of epithelial ovarian carcinomas studied (14–18). When considered together with the results of Futreal et al. (20), who found no somatic mutations of BRCAl in 12
ovarian carcinomas with LOH that included the BRCA1 locus, our data suggest that somatic mutations of BRCA1 in sporadic ovarian carcinomas are rare, and that mutation of an additional tumor suppressor gene(s) underlies the frequent LOH seen on chromosome 17q in ovarian cancers. Fine deletion mapping on chromosome 17q is difficult in ovarian cancers because the majority of tumors with LOH have lost the entire chromosome arm. However, interstitial or partial deletions in a small number of ovarian cancer cases have implicated one or perhaps two distinct regions distal to BRCA1 harboring presumptive tumor suppressor genes that may account for much of the 17q LOH seen in ovarian cancers (13, 33, 34). Alternatively, at least one additional gene in the BRCA1 region, that encoding N-acetylglucosaminidase, has been shown to contain inactivating mutations in sporadic breast cancers (35).

These findings are in contrast with two recent reports describing somatic mutations of the BRCA1 gene in approximately 10% of sporadic ovarian carcinomas (36, 37). In one of these studies, however, only 1 mutation was seen in 17 tumors preselected for 17q LOH (36), again suggesting that an additional tumor suppressor gene for sporadic ovarian cancers exists on chromosome 17q. Thus, although it is likely that BRCA1 mutations will continue to be documented in a small fraction of sporadic ovarian cancers, our data support the hypothesis that germline mutations of this gene play a significant role in hereditary ovarian carcinomas but that somatic mutations in sporadic tumors are rare. The mutational analysis of a larger number of ovarian cancers, perhaps with the use of more sensitive screening techniques, will be necessary to determine with certainty the frequency of BRCA1 mutations in sporadic and hereditary ovarian carcinomas.

References
Mutation Analysis of the *BRCA1* Gene in Ovarian Cancers

Hiroyuki Takahashi, Kian Behbakht, Patricia E. McGovern, et al.


Updated version  Access the most recent version of this article at:  
http://cancerres.aacrjournals.org/content/55/14/2998

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.