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CLINICAL AND PATHOLOGICAL FEATURES OF OVARIAN CANCER IN WOMEN WITH GERM-LINE MUTATIONS OF *BRCA1*

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ABSTRACT

Background We tested the hypothesis that ovarian cancers associated with germ-line mutations of *BRCA1* have distinct clinical and pathological features as compared with sporadic ovarian cancers.

Methods We reviewed clinical and pathological data on patients with primary epithelial ovarian cancer found to have germ-line mutations of *BRCA1*. Survival among patients with advanced-stage cancer and such mutations was compared with that in control patients matched for age and stage, grade, and histologic subtype of the tumors. A combination of single-strand conformation and sequencing analyses was used to examine the 22 coding exons and intronic splice-donor and splice-acceptor regions of *BRCA1* for mutations in pathological specimens. Alternatively, some patients were known to be obligate carriers of the mutant *BRCA1* gene because of their parental relationships with documented mutant-gene carriers.

Results We identified 53 patients with germ-line mutations of *BRCA1*. The average age at diagnosis was 48 years (range, 28 to 78). Histologic examination in 43 of the 53 patients showed serous adenocarcinoma. Thirty-seven tumors were of grade 3, 11 were of grade 2, 2 were of grade 1, and 3 were of low malignant potential. In 38 patients, the tumors were of stage III; 9 patients (including those with tumors of low malignant potential) had stage I disease, 5 had stage IV, and 1 had stage II. As of June 1996, with a median follow-up among survivors of 71 months from diagnosis, 20 patients had died of ovarian cancer, 27 had no evidence of the disease, 4 were alive with the disease, and 2 had died of other diseases. Actuarial median survival for the 43 patients with advanced-stage disease was 77 months, as compared with 29 months for the matched controls ($P < 0.001$).

Conclusions As compared with sporadic ovarian cancers, cancers associated with *BRCA1* mutations appear to have a significantly more favorable clinical course. (N Engl J Med 1996;335:1413-6.)

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ADENOCARCINOMA of the ovary causes the death of more American women each year than all other gynecologic cancers combined. About 5 to 10 percent of ovarian cancers are familial, and several familial-cancer syndromes that include ovarian cancer have been identified.¹ In most families affected with the breast-and-ovarian-cancer syndrome or site-specific ovarian cancer, genetic linkage has been found to the *BRCA1* locus on chromosome 17q21.^{2,3} Allelic deletion at the *BRCA1* locus in tumors from these linked family members invariably involves the wild-type chromosome, suggesting that *BRCA1* functions as a tumor-suppressor gene.⁴ The cloning of *BRCA1*⁵ has allowed direct identification of *BRCA1* mutations in both sporadic and hereditary ovarian cancer. Although allelic deletions in the region of the *BRCA1* locus are common in sporadic ovarian cancers, the recent finding that *BRCA1* mutations in sporadic tumors are rare suggests the involvement of an additional tumor-suppressor gene in this region of chromosome 17q.⁶

The likelihood that distinct molecular abnormalities contribute to the pathogenesis of hereditary and

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sporadic ovarian cancers raises the question of whether these tumors also have distinct clinical and histopathological characteristics. In this report, we examine the clinical and pathological characteristics of ovarian cancers in 53 women with documented germ-line mutations of *BRCA1*.

METHODS

Mutational Analysis

The molecular-analysis technique used in our laboratory has been described in detail elsewhere.⁶ This technique, or minor variations of it, was also used at the centers participating in this study. In brief, specimens of epithelial ovarian cancer were obtained from the tissue-bank archives of the participating institutions. The study was approved by the respective institutional review boards, and informed consent was obtained before tissue collection and analysis. Corresponding normal tissues used for the analysis of germ-line DNA were either peripheral-blood lymphocytes or normal gynecologic tissues removed at the time of surgery. All tumors used in this study were obtained from primary-site ovarian cancers in previously untreated patients.

The polymerase chain reaction was used to amplify genomic DNA for single-strand conformation polymorphism analysis. Intron-based primers surrounding each of the 22 exons of *BRCA1* were used, with the exceptions that 16 overlapping primer sets were used to examine exon 11 and that 2 additional primer sets were designed to amplify exons 6 and 7 as two separate products. Exons 1 and 4 are noncoding and were not examined. All variants of single-strand conformation polymorphisms were examined by direct sequence analysis to determine mutations. Alternatively, some patients were obligate carriers of the mutant *BRCA1* gene, on the basis of their parental relationships with documented mutant-gene carriers, as determined by pedigree analysis.

Pathological and Clinical Analyses

At each participating center, investigators identified all the patients with ovarian cancer who had germ-line mutations of *BRCA1* and for whom the necessary pathological and clinical data were available, including the date of diagnosis; age; stage, histologic subtype, and grade of the tumor; and clinical follow-

up. It was not necessary for the patients to have been seen at the participating institution. In many instances, clinical care had been rendered in the community, and patients were identified as carriers of *BRCA1* mutations only after death, through molecular analysis of archival specimens or pedigree analysis initiated by a relative. The data were abstracted from the medical records and recorded in a spreadsheet for statistical analysis. Patients for whom histologic specimens were available and reviewed included 11 patients at the University of Pennsylvania and 3 at Duke University.

The long-term survival of the patients with mutant *BRCA1* genes was compared with that of a control group of patients with ovarian cancer selected from the clinical data base of the Society of Gynecologic Oncologists in use at the University of Pennsylvania since 1989, which currently contains data on 594 patients with ovarian cancer.⁷ Each patient was matched to a control subject for age (within five years) and tumor stage, histologic subtype, and grade. All the controls had had their disease diagnosed a minimum of three years before the time of analysis, and none had family histories suggestive of hereditary cancer syndromes. Survival data were calculated by the method of Kaplan and Meier⁸ and compared by the log-rank test.⁹

RESULTS

A total of 53 patients with ovarian cancer and germ-line mutations of *BRCA1* were identified: 29 from the University of Pennsylvania, 9 from Creighton University, 8 from Dana-Farber Cancer Institute, 4 from Duke University, and 3 from Brigham and Women's Hospital. *BRCA1* mutations were identified in 45 patients by molecular analysis, and 8 patients were determined to be obligate mutation carriers by pedigree analysis. The mean age at diagnosis among the 53 patients was 48 years (range, 28 to 78); the mean in the control group was 49 years. Table 1 shows the stage, histologic grade, and histologic subtype of the tumors from the 53 women. Two of the tumors of low malignant potential were of the serous type; one was mucinous.

The median follow-up for all surviving patients was 71 months from the time of diagnosis. As of June 1996, 20 patients had died of ovarian cancer a median of 35 months from diagnosis. Twenty-seven remained alive with no evidence of disease, four remained alive with ovarian cancer, and two had died of other diseases with no evidence of ovarian cancer present. Figure 1 shows the actuarial survival of the 43 patients with advanced-stage disease (38 with stage III and 5 with stage IV) as compared with the survival of the matched control group. The difference in survival is significant at the $P < 0.001$ level. There was no correlation between any of the clinicopathological features we examined and a particular *BRCA1* mutation.

DISCUSSION

In our study of the clinical features of ovarian cancers in women with documented germ-line mutations of the *BRCA1* gene, the average age at diagnosis, 48 years, was more than 10 years less than the mean age of 61 reported in a large sample of patients with ovarian cancer.¹⁰ Tumors of the serous

TABLE 1. STAGE, GRADE, AND HISTOLOGIC SUBTYPE OF OVARIAN CANCERS OCCURRING IN 53 WOMEN WITH GERM-LINE MUTATIONS OF *BRCA1*.

| CHARACTERISTIC | NO. OF PATIENTS |
|-------------------------|-----------------|
| Stage | |
| I | 9 |
| II | 1 |
| III | 38 |
| IV | 5 |
| Grade | |
| Low malignant potential | 3 |
| 1 | 2 |
| 2 | 11 |
| 3 | 37 |
| Histologic subtype | |
| Serous | 43 |
| Endometrioid | 3 |
| Mucinous | 3 |
| Undifferentiated | 4 |

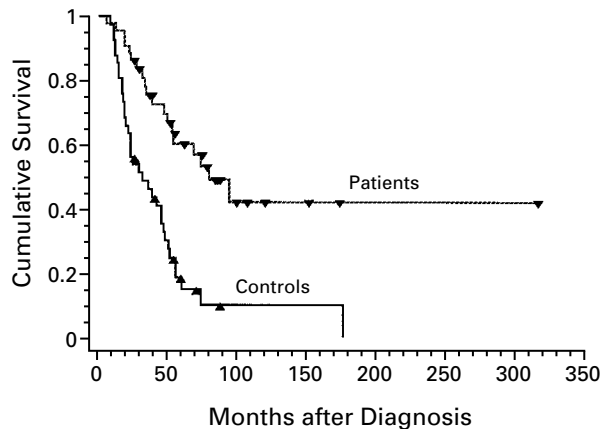


Figure 1. Actuarial Survival among 43 Patients with Advanced-Stage Ovarian Cancer and Germ-Line *BRCA1* Mutations, as Compared with Matched Controls without Such Mutations. $P < 0.001$ by the log-rank test. The triangles and inverted triangles indicate the durations of follow-up among surviving patients.

type, which constitute about 46 percent of all epithelial ovarian carcinomas,¹¹ predominate among women with *BRCA1* mutations. The distribution of patients according to stage and grade appears fairly typical, and it is apparent that tumors of low malignant potential can arise in association with *BRCA1* mutations. Because our patients were seen at several institutions and in some cases received primary treatment elsewhere, some relevant clinical information, including the extent of surgical tumor resection and the type of chemotherapy used, was not available. From the information we obtained, however, it appears that hereditary ovarian cancers arising in association with germ-line mutations of *BRCA1* may differ substantially from those that arise sporadically.

The survival of patients with *BRCA1* mutations and advanced-stage ovarian cancers appears to be notably longer than the survival of matched controls not known to have hereditary cancers. Our advanced-stage control group had an actuarial median survival of 29 months, similar to that typically seen in patients with advanced ovarian cancer before the advent of paclitaxel chemotherapy,¹² whereas the *BRCA1*-related cases have an actuarial median survival of 77 months ($P < 0.001$).

The selection of a historical control group must always be approached cautiously, but it is likely that our use of the control group we selected actually resulted in an underestimate of the magnitude of the survival difference. There is strong evidence from large-scale prospective, randomized trials that a patient's age is an important independent prognostic factor for survival in advanced-stage ovarian cancer.¹³ By selecting age-matched controls, we defined a population of relatively young patients with ovarian can-

cer, who on the basis of age alone would be expected to have more favorable outcomes than older patients.

The youth of the control group also increased the likelihood that patients with undetected *BRCA1* mutations were included in this group. In two recent studies of patients with breast cancer who were 35 years of age or younger, 7.5 percent of the women under 35¹⁴ and 13 percent of the women under 30¹⁵ were found to have *BRCA1* mutations that were unsuspected on the basis of their family histories. In view of the considerable survival advantage among patients known to have *BRCA1*-related ovarian cancers, it seems likely that a factor in the favorable prognosis of younger women with ovarian cancer is the relatively high frequency of *BRCA1* mutations in this population. Our control population was made up of women who were treated relatively recently by gynecologic oncologists at an academic medical center — a fact that might improve their survival relative to that of the patients with *BRCA1* mutations, many of whom were treated less recently in a community setting¹⁶: in 16 of the 43 patients (37 percent) with advanced-stage cancer in our series, as compared with 10 of 43 controls (23 percent), the disease was diagnosed before 1986.

The process we used to identify patients with *BRCA1*-related ovarian cancer was intended to yield a representative population, without favoring long-term survivors. All such patients identified at each participating institution for whom the basic clinical information was available were included. There was no requirement for the patients to have been treated at the institution. In many instances, cases of *BRCA1*-related ovarian cancer were identified by molecular analysis of tissue blocks at the request of relatives after the patients' deaths. In addition, the participation of several investigators at different institutions in the process of identifying patients should lessen the possibility of a selection bias.

In some previous reports of the clinical characteristics of cancers that arise in association with predisposing germ-line mutations, patients were identified by linkage analysis or examination of family pedigrees; this process left open the possibility that sporadic cases were also included. In a report on 35 patients with breast cancer who were probable carriers of a *BRCA1* mutation, on the basis of linkage analysis, Porter et al.¹⁷ suggested that these patients had a relatively indolent clinical course, but they did not include prognostic factors, such as tumor stage and grade. Eisinger et al.¹⁸ found a higher incidence of poorly differentiated tumors in 27 patients with breast cancer and documented germ-line *BRCA1* mutations but did not report on follow-up or survival in this group. Marcus et al.¹⁹ studied patients with *BRCA1*-related breast cancer and other forms of hereditary breast cancer, including *BRCA2*-related cases, and found that the *BRCA1*-related hereditary

breast cancers were more frequently aneuploid and showed higher tumor-proliferation rates than the other hereditary cases. Although there were adverse prognostic features in the *BRCA1*-related patients, they had paradoxically lower recurrence rates than the other patients with hereditary breast cancer.

Other evidence suggests a relatively favorable prognosis for hereditary colorectal cancer in patients carrying mutations of genes responsible for DNA mismatch repair. A recent study of data from the Finnish Cancer Registry showed significantly longer recurrence-free survival (at five years) in a group of patients with hereditary colorectal cancer than in a control group with sporadic colorectal cancer.²⁰

With regard to ovarian cancer, a previous pedigree analysis found that a relatively large proportion of possibly hereditary ovarian cancers are of the serous-cell subtype¹⁴ but did not provide information on survival. Interestingly, Buller et al.¹⁵ compared the survival of 11 patients with family histories of ovarian cancer with that among matched controls who had no such family history; they found longer survival among the women with the possibly familial cases.

Germ-line *BRCA1* mutations occur in only a small proportion of all ovarian cancers, but they appear to be the most important prognostic factor yet identified for patients with advanced-stage disease. The distinctive clinical behavior of *BRCA1*-related ovarian cancers could have implications for disease management and the design of clinical trials. The reasons for the relatively indolent course of *BRCA1*-related ovarian cancers are not immediately obvious from our data. We have been unable to obtain information about some well-known clinical prognostic factors, including the extent of tumor before and after initial surgery and the chemotherapy regimens used. It seems likely that explanations will come from a better understanding of the function of the *BRCA1* tumor-suppressor gene and that the difference in clinical behavior between *BRCA1*-related and sporadic ovarian cancers reflects distinct profiles of somatic mutations in other cancer-related genes.

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