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Advances in Brief

Frequency of the *BRCA1 185delAG* Mutation among Jewish Women with Ovarian Cancer and Matched Population Controls[1]

Michael G. Muto,[2] Daniel W. Cramer, Jacob Tangir, Ross Berkowitz, and Samuel Mok

Gynecologic Oncology Laboratory and Obstetrics-Gynecologic Epidemiology Center, Department of Obstetrics and Gynecology, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115

Abstract

Among women of Ashkenazi Jewish origin, a frameshift mutation of the *BRCA1* gene, designated *185delAG*, occurs with a carrier frequency of approximately 1% and is estimated to account for about 39% of ovarian cancer cases occurring prior to age 50 years. To determine the actual frequency of this mutation among Jewish women with ovarian cancer, we tested DNA collected as part of an ongoing population-based case-control study of genetic and environmental factors for epithelial ovarian cancer in eastern Massachusetts. Using single-stranded conformational polymorphism analysis followed by direct sequencing, we found that 6 (19.4%) of 31 Jewish patients were carriers for a *185delAG* mutation compared to 0 of 23 Jewish controls ($P = 0.03$). Using empiric logits, the estimated relative risk for ovarian cancer associated with a *185delAG* mutation is 12.0. The average age of the 6 patients with mutations was 48.3 years, significantly younger than the average of 57.4 years observed for the 25 patients without the mutation ($P = 0.05$). For ovarian cancer diagnosed prior to age 50 years, three (37.5%) of eight patients carried the mutation. None of the six patients with the mutation had a history consistent with hereditary breast ovarian cancer syndrome, although two had a personal history of prior cancer. Our results provide empiric confirmation of the estimated prevalence of *185delAG* mutations among Jewish women with ovarian cancer.

Introduction

Since the first breast ovarian cancer susceptibility gene *(BRCA1)* was cloned, over 38 distinct germ line mutations have been found among individuals with the hereditary breast/ovarian cancer syndrome (1). A frameshift mutation of *BRCA1*, due to deletion of 2 bp from exon 2 (designated *185delAG*), has been described in at least 10 families of Ashkenazi Jewish origin who have multiple cases of breast and/or ovarian cancer (2–4) and, recently, been found to occur with a prevalence of 0.9% in Jewish individuals seeking testing for Tay-Sachs or cystic fibrosis (5). Based on age-specific penetrance rates from the Breast Cancer Linakge Consortium, Struewing *et al.* (5) estimated that the *185delAG* mutation might account for 16% of breast cancers and 39% of ovarian cancers occurring in Ashkenazi women before age 50 years (5). However, these estimates have not been empirically confirmed by testing for this mutation among Jewish women with either breast or ovarian cancer. Here, we report on the frequency of *185delAG* mutations among Jewish women enrolled in a population-based study of ovarian cancer in Eastern Massachusetts and unselected on the basis of family history.

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[2] To whom requests for reprints should be addressed, at GYN Oncology Laboratory, Brigham and Women's Hospital, Harvard Medical School, 221 Longwood Avenue, Boston, MA 02115.

Materials and Methods

A population-based case-control study of ovarian cancer in eastern Massachusetts and New Hampshire was begun in April 1992. From the Massachusetts's component of the study, 622 women with ovarian cancer in Massachusetts had been identified as of September 30, 1995. Fifty-four (8.7%) of these women could not be located or had died before they could be studied. In 79 patients (12.7%), the primary physicians denied permission for study and in 41 patients (6.6%), the patient refused study. Excluding women pending study and those with nonepithelial ovarian cancers, there were 327 women with epithelial ovarian cancer including tumors of borderline malignancy who had been studied as of September 30, 1995. Age-matched control women are identified using a random digit dialing technique based on a patient telephone prefix. As of September 31, 1995, 388 eligible and potential controls had been identified of whom 71% agreed to be studied. Data were complete on 260 of these women.

Our study protocol included informed consent for collection of epidemiological information and blood for study of "epidemiological and biological correlates of ovarian cancer risk." Specimens suitable for DNA analysis included both a frozen buffy coat specimen and a portion of the buffy coat smeared on filter paper and refrigerated. Epidemiological information included age at diagnosis or study, medical history including previous cancers, and family history of cancer. We also inquired about "childhood religious upbringing" with "Jewish" as one category. Although our questionnaire did not further distinguish Ashkenazi from Sephardic, the majority of Jewish residing in the United States are European and, hence likely, Ashkenazi in origin (6). DNA specimens from the Jewish subjects, identified by number only, were examined for the presence of a *185delAG* mutation as described below.

SSCP[3] was performed as described previously (7). Primers flanking exon 2 of the *BRCA1* gene (8) were first radiolabeled with 5 units T4 polynucleotide kinase (Boehringer Mannheim, Indianapolis, IN) and 0.5 μl [γ-^{32}P]ATP (7000 Ci/mmol; ICN Biomedicals, Costa Mesa, CA) in 5 μl at 73°C for 30 min. The reaction mixture was then diluted into a final volume of 400 μl of primer-PCR mixture containing 40 μl 10 × PCR buffer (0.1 M Tris-HCl-0.5 M KCl, pH 8.3), 20 mM $MgCl_2$, 20 ml 1.25 mM deoxynucleotide triphosphate mixture, and 2 μl (10 units) Taq polymerase (Perkin Elmer/Cetus, Norwalk, CT). The PCR was carried out with 50 ng genomic DNA in 5 μl using DNA from the filter paper blood specimens and 35 cycles of PCR (denaturation at 95°C for 30 s annealing at 52°C for 30 s and polymerization at 72°C for 45 s). The PCR products were then diluted with 45 μl loading buffer containing 95% formamide, 20 mM EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol FF (Sigma Chemical, St. Louis, MO). Three μl of the final mixture were loaded onto a 5% polyacrylamide (49:1), acrylamide:bisacrylamide gel with 6% glycerol, and electrophoresed at 30 W at 4°C for 2 to 3 h. The gel was dried on a filter paper with a vacuum gel dryer and exposed to X-ray film for 2 h with an intensifying screen.

Mobility shift band from the SSCP analysis was dissected out, and the DNA was extracted with 50 μl water at 65°C for 2 h as described previously (8). Eluted DNA was reamplified using the same PCR conditions as described above in a total volume of 100 μl. The PCR products were purified by running on a 5% nondenaturing polyacrylamide gel, extracted with phenol/chloroform, and precipitated with 2 volumes of absolute alcohol. Either the stream or downstream primer of exon 2 of the *BRCA1* gene was end labeled with

[3] The abbreviations used are: SSCP, single-strand conformation polymorphism; USB, United States Biochemical.

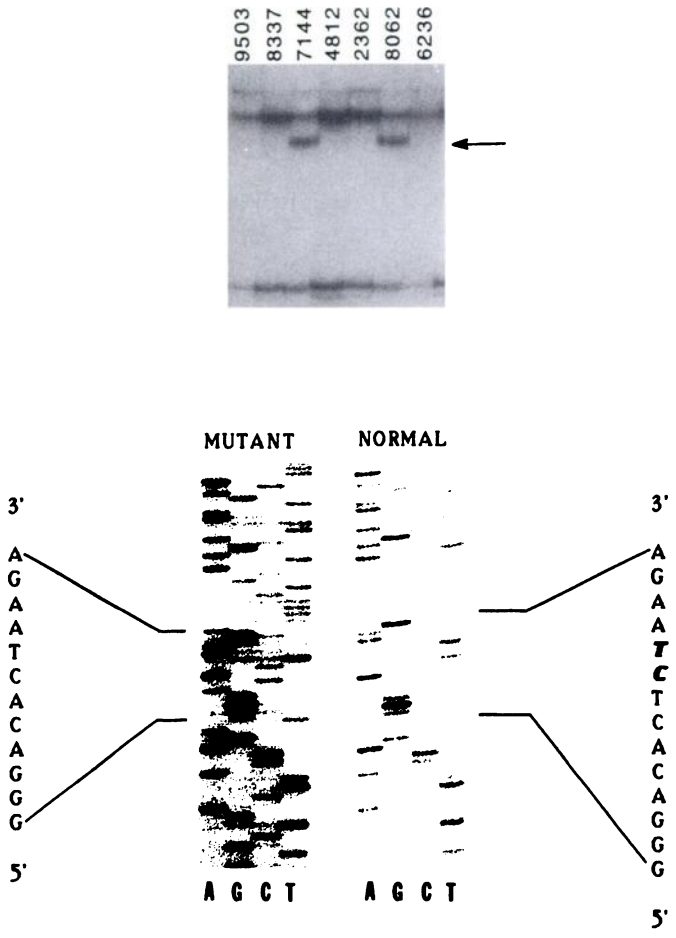

Fig. 1. SSCP and mutational analysis of BRCA1 exon 2 *185delAG* mutation. *Upper panel,* autoradiograph of SSCP gel showing two of seven subjects who displayed altered mobility *(arrow). Lower panel,* autoradiograph of sequencing gel. Results of BRCA1 exon 2 reverse-primed DNA from samples 7144 *(mutant)* and 4812 *(normal)* are shown. The mutant sequence displayed a 2-bp deletion *(TC)* when compared to the normal sequence *(bold letters).* Since the nucleotides sequence belongs to the complementary strand, the original strand displayed an AG deletion.

[γ-^{32}P]ATP by polynucleotide kinase in a total volume of 10 μl. The labeled primer was subsequently diluted 5-fold with sterile distilled water. One μl of the diluted labeled primer was mixed with 0.1 μg purified template DNA, 2 μl reaction buffer [160 mM Tris-HCl (pH 9.5) and 65 mM $MgCl_2$], and 8 units δ Taq Version 2.0 DNA polymerase (USB), Cleveland, OH) in a total volume of 17 μl. Then 4 μl of the labeling mixture were transferred to each of the four termination mixtures (A, G, C, and T) as described in the Taq Cycle Sequencing kit (USB). The termination reactions were incubated in a DNA thermal cycler using the same conditions as described above and stopped with 45 μl stop solution (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol FF; USB). All samples were boiled for 10 min, and 2 μl of each sample were loaded onto an 8% sequencing gel.

Results and Discussion

Among the 327 patients, there were 31 (9.5%) women who identified their childhood religious background as Jewish. Among the 260 controls, there were 23 (8.9%) who identified their background as Jewish. Fig. 1 illustrates SSCP analysis on DNA from subjects. The normal band in five subjects compared to the band shift associated with a presumptive *185delAG* mutation in two subjects are illustrated in the *upper panel* of Fig. 1. Sequencing to confirm the mutation was performed for all subjects displaying the band shift and is illustrated in the lower panel of Fig. 1 for patient 7144.

Altogether 6 (19.3%) of 31 Jewish women with epithelial ovarian cancer were found to have a confirmed *185delAG* mutation compared to 0 of the 23 controls (P = 0.03) using Fisher's exact test. For ovarian cancer diagnosed prior to age 50 years, three patients (37.5%) of eight carried the mutation. From this case-control data, the exposure odds ratio can be used to estimate the relative risk for ovarian cancer associated with possession of a *185delAG* mutation. The empiric logit method must be used to estimate risk when there are no controls who have the exposure of interest (9). Using this method, the relative risk is estimated to be 12.0, although the 90% confidence limits would be wide based on the small sample size (1.0, 138.7).

Table 1 lists some characteristics among Jewish patients with and without the mutation and all controls. The average age at diagnosis for patients with a mutation was 48.3 compared to 57.4 for patients without the mutation (P = 0.05). Histological types of epithelial ovarian cancer among Jewish patients having the mutation included four serous tumors (1 of which was a borderline malignancy) and 2 endometrioid cancers; serous type cancers also predominated (18/25) among the patients not possessing the mutation. Among patients with the mutation, one woman had a personal history of breast cancer and one had a neuroendocrine malignancy compared to two women with previous breast carcinomas among Jewish patients without the mutation. Four of the 6 patients with the mutation had no family history of breast, prostate, or ovarian cancer while one patient had a family history of ovarian cancer and one had a history of breast and prostate cancers among primary relatives. These family histories did not differ significantly compared either with that in Jewish patients lacking the mutation (4/25 of whom had breast, ovarian, or prostate among primary relatives) or that in Jewish control women 4/23 of whom had a family history of either breast or ovarian cancer among primary relatives). None of the Jewish patients with *185delAG* mutations could be described as coming from high-risk pedigrees (*i.e.,* two or more primary relatives with ovarian or premenopausal breast cancer). Among women who have ovarian cancer, family history does not

Table 1 *Characteristics of Jewish women with epithelial ovarian cancer and controls*

Characteristics	Patients (n = 31)		Controls (n = 23)
185delAG mutation	Present in 6	Absent in 25	Absent in 23
Average age, yr (±SE)	48.3 (2.0)	57.4 (2.1)	47.3 (3.0)
Histological type of ovarian cancer	Serous (3) Serous borderline (1) Endometrioid (2)	Serous (15) Serous Borderline (3) Endometrioid (2) Mucinous (2) Other (3)	Not applicable
Personal history cancer	Breast (1) Neuroendocrine (1)	Breast (2)	None
Family history cancer	Ovarian (1) Breast (1) Prostate (1)	Ovarian (1) Breast (1) Prostate (2)	Ovarian (2) Breast (2)

appear to help identify those who are likely to have a *185delAG* mutation.

We have found that approximately 20% of women who identify their childhood religious background as Jewish and who developed ovarian cancer have *185delAG* mutations of the *BRCA1* gene, and the proportion is almost double that for Jewish women who developed their ovarian cancer prior to age 50 years. The patients in this study were selected only on the basis of residence in greater Boston and were not referred because of strong family histories. Our findings add impetus to the need for programs capable of screening for specific *BRCA1* mutations, including *185delAG*. These programs should be established in the context of clinics that are capable of providing genetic counseling, screening for both breast and ovarian cancer, and appropriate gynecological and surgical consultation. Properly conducted trials will be necessary to assess the impact of such testing on mortality associated with breast and ovarian cancer.

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