Germline BRCA1 and BRCA2 Mutations in Ovarian Cancer
Utility of a Histology-Based Referral Strategy

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OBJECTIVE: To estimate the frequency of BRCA1 and BRCA2 germline mutations in women with nonmucinous epithelial ovarian carcinoma unselected for a family history of breast or ovarian cancer.

METHODS: From 2004 to 2009, women undergoing surgical staging for nonmucinous epithelial ovarian carcinoma, including fallopian tube and primary peritoneal carcinoma, were invited to participate in tumor banking and genetic counseling for BRCA1 and BRCA2 mutations. Pathology and family history obtained by the gynecologic oncology surgeon and genetic counselors were reviewed.

RESULTS: Of 131 women fulfilling entry criteria, germline BRCA1 and BRCA2 mutations were found in 20% (26/131) and were exclusively associated with high-grade serous histology (26/103 [25%]). Restricting BRCA1 and BRCA2 testing to women with family histories of hereditary breast and ovarian cancer, as ascertained by the surgeon, missed 14 mutation carriers, lowering detection rates to 9% (12/131) or 11.6% (12/103) if only considering the patients with high-grade serous histology. This improved to 16% (21/131) or 20.4% (21/103) when ascertained by the genetic counselor; however, 5 of 26 (19%) mutation carriers did not have a family history of hereditary breast or ovarian cancer.

CONCLUSION: Germline BRCA1 and BRCA2 mutations in ovarian (pelvic) cancer are associated with high-grade serous histology. The high incidence (25%) of germline BRCA1 and BRCA2 mutations specific to the high-grade serous subtype suggests that genetic assessment of all women diagnosed with high-grade serous ovarian (pelvic) carcinoma will improve detection rates and capture mutation carriers otherwise missed by referral based on family history alone.

Obstet Gynecol 2012;120:235–40
DOI: 10.1097/AOG.0b013e31825f3576

LEVEL OF EVIDENCE: II

Germline mutations in BRCA1 and BRCA2 predispose to autosomal-dominant hereditary breast and ovarian cancer, in which determination of carrier status in women presenting with ovarian cancer may affect prognosis and influence treatment. Female carriers can be offered risk-reducing surgeries or can elect for heightened surveillance for breast and ovarian cancer. These implications extend to their families. Morphologic classification of epithelial ovarian carcinoma delineates five major histologic subtypes: high-grade serous, clear cell, endometrioid, mucinous, and low-grade serous. These constitute distinct clinical entities with differing presentation, prognoses, molecular, and immunohistochemistry profiles, reflecting aberration of specific molecular pathways such as DNA damage repair, cell cycle control, or promotion or control of growth and proliferation. Seventy percent of epithelial ovarian carcinoma is of the high-grade serous subtype. Germline BRCA1 and BRCA2 mutations account for 11.7–15% of invasive epithelial ovarian carcinoma.
Ovarian cancers in BRCA1 and BRCA2 mutation carriers show almost exclusive association with epithelial ovarian carcinoma in contrast to the low likelihood of germ cell or stromal tumors seen in this population.1–10 High-grade serous histology is reported in 76.7–93% of BRCA1 and BRCA2 mutation carriers in population-based series of invasive ovarian carcinoma.5,6,11,12 Our objective was to estimate the incidence of germline BRCA1 and BRCA2 mutation carriers in a consecutive series of women with nonmucinous ovarian cancer unselected for a personal or family history of breast cancer or ovarian cancer in British Columbia.

MATERIALS AND METHODS

From 2004 to 2009, patients were recruited from the Vancouver General Hospital and British Columbia Cancer Agency in Vancouver, British Columbia, Canada. Ethical approval was obtained from the University of British Columbia Ethics Board. All women who had been fully staged and were undergoing debulking surgery (primary or delayed) for cancers of ovarian, peritoneal, or fallopian tube origin were approached for informed consent for the banking of tumor tissue. Pathology review was performed in all cases. Women with borderline ovarian tumors or mucinous histology were excluded and those with endometrioid, clear cell, or serous carcinomas were referred to the British Columbia Cancer Agency Hereditary Cancer Program genetic counselors to discuss and perform germline BRCA1 and BRCA2 testing. Cascade genetic counseling of family members of BRCA1 and BRCA2-positive patients revealed from this study was offered through the same program. Carcinomas of mixed epithelial subtypes were included in the study, and those with a serous component were classified as high-grade serous. Press et al13 previously characterized the BRCA1 and BRCA2 abnormalities in the tumors of 49 of these cases and more recently the detailed immunohistochemical and molecular characterization of the primary tumors and clinical and outcome data related to the full cohort (n=131) has been published.14

The sequence of BRCA1 and BRCA2 was determined from peripheral blood-derived genomic DNA through standard bidirectional dideoxy sequencing of the entire coding and proximal intronic regions. The presence of large genomic rearrangements, conferred by deletions or duplications, was determined by multiplex ligation dependent probe amplification according to the manufacturer’s protocol.

Family history acquired at the time of initial consultation by the gynecologic oncology surgical team (non-genetic counselor) and by the genetic counselor at the time of counseling for BRCA1 and BRCA2 mutation testing was reviewed compared with the British Columbia Cancer Agency’s referral guidelines for hereditary breast, ovarian cancer, or both (Table 1) [www.bccancer.bc.ca/HPI/CancerManagementGuidelines/HereditaryCancerProgram/referralinformation/hboccriteria.htm]. Family histories were considered in first- and second-degree relatives. Whether they would have been referred based on the finding of invasive, nonmucinous epithelial ovarian cancer alone in the proband compared with whether they would have met other criteria for testing was reviewed and compared with germline mutation status. This enabled us to determine the number of mutation carriers that were identified based on their history of ovarian cancer alone.

Statistical analysis was performed using SPSS 20.0.0 and OpenEpi 2.3.1. Ninety-five percent confidence intervals (95% CIs) for mutation frequencies were calculated under the assumption of binomial distributions of the observed numbers of cases using Fisher’s exact test. Age comparisons were performed using a Mann-Whitney U test.

Table 1. British Columbia Cancer Agency Referral Criteria for Hereditary Breast Cancer, Ovarian Cancer, or Both

<table>
<thead>
<tr>
<th>Previously known BRCA1 or BRCA2 mutation in a close family member</th>
<th>Confirmed BRCA1 or BRCA2 gene mutation*</th>
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<tr>
<td>Proband</td>
<td>Breast cancer at age 35 y or younger,* or Two breast cancers with at least one occurring at age 50 y or younger,* or Invasive, nonmucinous epithelial ovarian cancer, including cancer of the fallopian tubes or primary peritoneal cancer at any age</td>
</tr>
<tr>
<td>History in first-degree or second-degree relatives on same side of family†</td>
<td>One breast cancer—one ovarian cancer,* or One male breast cancer+breast or ovarian cancer,* or Two breast cancers, both at age 50 y or younger,* or Two ovarian cancers,* or Three breast cancers, one diagnosed at age 50 y or younger*</td>
</tr>
<tr>
<td>Ashkenazi Jewish heritage</td>
<td>Personal or family history of breast or ovarian cancer*</td>
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* Criteria relating to family history.
† History of cancer in cousins and more distant relatives from the same side of the family may also be relevant, although for the purpose of this study it was not included.
Of 131 women who participated in genetic counseling, 26 women (20%) were found to harbor germline mutations in either \textit{BRCA1} \((n=19)\) or \textit{BRCA2} \((n=7)\) (Fig. 1). Pathology review revealed that all 26 of these women had high-grade serous ovarian cancer (Table 2). Therefore, if we consider only the 103 cases of high-grade serous, referral to the Hereditary Cancer Program for genetic counseling and the option of \textit{BRCA1} and \textit{BRCA2} testing based on the criteria of high-grade serous histology alone would have revealed a \textit{BRCA1} and \textit{BRCA2} germline mutation rate of 25% in this cohort.

Current guidelines for referral to the British Columbia Cancer Agency Hereditary Cancer Program include the criterion of offering genetic counseling and testing to individuals with nonmucinous ovarian cancer diagnosed at any age. The importance of this criterion is evident, as even with a comprehensive family history obtained by a genetic counselor, germline \textit{BRCA1} and \textit{BRCA2} mutation carriers with high-grade serous histology and without a personal or family history consistent with hereditary breast and ovarian cancer, and \textit{BRCA1} and \textit{BRCA2} mutation carriers with high-grade serous histology, were not identified.

### Table 2. Histologic Subtypes and \textit{BRCA1} and \textit{BRCA2} Germline Mutation Results in a Study Cohort of Nonmucinous Epithelial Ovarian Cancer

<table>
<thead>
<tr>
<th>Histologic Subtype</th>
<th>No. of Cases</th>
<th>Age (y)</th>
<th>\textit{BRCA1} Mutation-Positive Cases</th>
<th>\textit{BRCA2} Mutation-Positive Cases</th>
<th>\textit{BRCA1} and \textit{BRCA2} Mutation-Positive Cases</th>
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<tbody>
<tr>
<td>High-grade serous</td>
<td>103</td>
<td>58 ± 11</td>
<td>19, 18% (12–27)</td>
<td>7, 7% (3–14)</td>
<td>26, 25% (17–34)</td>
</tr>
<tr>
<td>Combined non–high-grade serous</td>
<td>28</td>
<td>52 ± 11</td>
<td>0</td>
<td>0</td>
<td>0, 0 (0–12)</td>
</tr>
<tr>
<td>Low-grade serous</td>
<td>5</td>
<td>58 ± 8</td>
<td>0</td>
<td>0</td>
<td>0, 0 (0–52)</td>
</tr>
<tr>
<td>Clear cell</td>
<td>12</td>
<td>49 ± 12</td>
<td>0</td>
<td>0</td>
<td>0, 0 (0–26)</td>
</tr>
<tr>
<td>Endometrioid</td>
<td>11</td>
<td>52 ± 10</td>
<td>0</td>
<td>0</td>
<td>0, 0 (0–28)</td>
</tr>
<tr>
<td>Total</td>
<td>131</td>
<td>57 ± 11</td>
<td>19</td>
<td>7</td>
<td>26, 20% (13–28)</td>
</tr>
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</table>

Total number (n) of cases for each histology and mean age±standard deviation are shown. In columns 3, 4 and 5 the number of cases, mutation frequency (percentage of total no. of cases), and 95% confidence intervals are given.
family history suggestive of hereditary breast and ovarian cancer would not be identified. By showing the number of germline \( BRCA1 \) and \( BRCA2 \) mutation carriers with high-grade serous epithelial ovarian carcinoma meeting only the criterion of ovarian cancer in the proband, Figure 2 outlines which cases would be captured by family histories (by a genetic counselor or nongenetic counselor) as compared with histology-driven referral. Figure 2A–B shows the difference between family history ascertained by the gynecologic oncology surgeon (denoted as nongenetic counselor) and the genetic counselor. If the first family history was taken by another health care provider other than the gynecologic surgeon (eg, general practitioner), this was also recorded as a nongenetic counselor. Restricting \( BRCA1 \) and \( BRCA2 \) testing to women with family histories of hereditary breast and ovarian cancer, as ascertained by the surgeon, missed 14 mutation carriers, lowering detection rates to 9% (12/131) or 11.6% (12/103) if only considering the patients with high-grade serous histology. This improved to 16% (21/131) or 20.4% (21/103) when ascertained by the genetic counselor. Therefore, on reviewing the histories as obtained by the nongenetic counselor compared with those ascertained by the genetic counselor, it was apparent that 35% (9/26, 95% CI 17–56%) of \( BRCA1 \) and \( BRCA2 \) mutation carriers with high-grade serous epithelial ovarian carcinoma would have been missed if referrals for testing had only been based on having a family history suggestive of hereditary breast and ovarian cancer (Fig. 2). Even with the more extensive history obtained by a genetic counselor, 19% (5/26, 95% CI 6–39%) of mutation carriers did not meet the familial component of hereditary breast and ovarian cancer criteria and before the inclusion of the criterion addressing singleton cases of nonmucinous epithelial ovarian carcinoma in the proband would not have been offered testing. Capture of these probands was secondary to our research study protocol in which counseling and testing was offered to all patients and what was an emerging practice change to consider referral for all nonmucinous ovarian carcinomas. If considering only cases with high-grade serous epithelial ovarian carcinoma and without a family history of hereditary breast and ovarian cancer, 12% of women (5/42, 95% CI 4–26%) were mutation carriers. Furthermore, the number of mutation-positive women without an apparent family history increased to 14 individuals when the family history was taken by a nongenetic counselor. The average age of ovarian cancer diagnoses in women with high-grade serous epithelial ovarian carcinoma who did not have a family history of hereditary breast and ovarian cancer was 47.4 years in mutation-positive cases (ranging from 42 to 54 years) and 62 years in mutation-negative cases (ranging from 37 to 84 years). This difference was significant when compared using a Mann-Whitney \( U \) test \( (P=.003) \). Of the two mutation carriers that harbored the recurrent c.185 delAG mutation, only one was reported to be of Ashkenazi Jewish descent. Two unrelated individuals carried exon 13 duplications.
DISCUSSION

These results suggest that germline *BRCA1* and *BRCA2* mutations are confined to the high-grade serous histologic subtype, reflecting our own and other’s experiences of frequent aberration of the *BRCA1* and *BRCA2* pathways within this subtype, but not in non-high-grade serous subtypes. The same series reported here was also analyzed for somatic mutations in *BRCA1* and *BRCA2* with five additional women discovered to have mutations of clinical significance in their tumors (germline DNA normal). *BRCA1* and *BRCA2* germline or somatic mutation frequency of high-grade serous cancers was therefore 30% (31/103) and similar to the reported findings by Hennessy et al\(^{15}\) (somatic and germline mutations in *BRCA1* and *BRCA2* in 23% of high-grade serous epithelial ovarian carcinoma). Methylation of *BRCA1* was found in an additional 20% of high-grade serous tumors bringing the total percentage of high-grade serous tumors showing *BRCA1* and *BRCA2* abnormalities to 50%.\(^{14}\)

Population-based studies have shown the incidence of germline *BRCA1* and *BRCA2* mutations associated with high-grade serous histology to be 16.5–18%.\(^{7,11}\) Although studies have identified *BRCA1* and *BRCA2* mutations in subtypes other than high-grade serous, the classification of tumors was based on pathology reports without review and use of current diagnostic criteria.\(^{2,6,11}\) In the Cancer Genome Atlas, germline *BRCA1* and *BRCA2* mutations were found in only 15% of high-grade serous carcinomas (47/316 cases). The lower mutation rate may have related to case selection and the technical aspects of the sequencing and mutation analysis.\(^{10}\) Recently, Arnold et al\(^{17}\) reported a 22.2% frequency of germline *BRCA1* and *BRCA2* mutations in patients with high-grade epithelial ovarian carcinoma with a serous component and no Ashkenazi Jewish heritage. Walsh et al\(^{18}\) reported a one in four *BRCA1* and *BRCA2* mutation frequency in patients with high-grade serous epithelial ovarian carcinoma, although also identified mutations in undifferentiated carcinomas, endometrioid and clear cell subtypes, possibly relating to the method and extent of pathology review, which was not specified. With the evolution of histopathologic subtype diagnosis for ovarian carcinoma, and the appreciation of defining molecular abnormalities, specific subtypes can now be very reproducibly diagnosed with high interobserver agreement using a combination of morphologic appearance and immunophenotyping.\(^{2,19,20}\) In our cohort, we found germline *BRCA1* and *BRCA2* mutations to be exclusive to the high-grade serous subtype, suggesting that non-high-grade serous epithelial ovarian carcinomas are at very low risk of hereditary breast and ovarian cancer and thus may not require referral to the Hereditary Cancer Program for genetic counseling. These results will need to be validated in a larger population-based series that also includes careful pathologic review of the primary tumors; however, if the association holds true, histology-based referral to a Hereditary Counseling Program should be incorporated into the current referral schema. This would result in cost savings and improved use of resources, preventing unnecessary referrals in non-high-grade serous cases in which the incidence of *BRCA1* and *BRCA2* mutations of clinical significance is low or nil. It may also, as outlined, reduce the likelihood of missed probands when referral is based on family history alone. Thorough pathology assessment, using morphologic, molecular, and immunohistochemical methodology,\(^{2,5}\) is essential to the success of this strategy. Although clinical criteria for referral for hereditary breast and ovarian cancer syndrome exist and include referral of women with epithelial ovarian, fallopian tube, or primary peritoneal cancer (National Comprehensive Cancer Network Guidelines Version 1.2011),\(^{21}\) also with the serous subtype being specified in the 2009 American College of Obstetricians and Gynecologists guidelines,\(^{22}\) currently there is no mechanism to ensure that they are being referred for genetic counseling regarding their risk for germline *BRCA1* and *BRCA2* mutations. One way to address this would be the inclusion of reflex recommendations on the pathology report that recommend that genetic assessment be offered to all women with high-grade serous ovarian, fallopian tube, or peritoneal carcinoma. This uniform referral strategy may lead to an increased opportunity for determination of *BRCA1* and *BRCA2* carrier status in women belonging to patient populations that may be underreferred for genetic counseling. Further education in the community for referring family practitioners and general gynecologists regarding the importance of referral for genetic counseling for patients with high-grade serous ovarian carcinoma will also help ensure patients and families potentially at high risk for breast, ovarian, and other related cancers are not missed.

In view of the strong association and high incidence (25%) of underlying *BRCA1* and *BRCA2* mutations in women with high-grade serous ovarian (pelvic) carcinoma, genetic assessment for consideration of *BRCA1* and *BRCA2* germline testing should be offered to all women diagnosed with this histologic subtype of ovarian cancer regardless of age or family history.
REFERENCES