Family History and BRCA1/BRCA2 Status Among Japanese Ovarian Cancer Patients and Occult Cancer in a BRCA1 Mutant Case

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Background: This study aimed to examine family history among Japanese ovarian cancer patients and to investigate the TP53 status of fallopian tube epithelial and ovarian cancer cells in a Japanese BRCA1 mutant case that may be associated with the transformed state in hereditary ovarian cancer.

Methods: One hundred and two primary ovarian cancer patients were retrospectively evaluated in this cross-sectional study. The family history of cancer was determined in probands. In a BRCA1 mutant case, p53 immunostaining and direct sequencing, followed by laser-capture microdissection, were performed for the fallopian tube, considered the origin of ovarian cancer.

Results: Nine of 102 (8.8%) families were regarded as having hereditary breast–ovarian cancer syndrome, two families (2.0%) were diagnosed with Lynch syndrome and six patients harbored BRCA1 or BRCA2 mutations. One case underwent risk-reductive salpingo-oophorectomy as a BRCA1 mutant carrier was retrospectively diagnosed as occult cancer. Common TP53 mutations were detected in cancer and fallopian tube epithelial cells in the case.

Conclusions: Here, we integrate family cancer history and histology in ovarian cancer cases as well as TP53 status in a BRCA1 mutant case into a discussion regarding carcinogenesis in a Japanese population. The TP53 status for the BRCA1 mutant case examined here supports the recently proposed theory that ovarian cancer develops because of BRCA1 or BRCA2 inactivation and/or TP53 mutations.


INTRODUCTION

Ovarian cancer (OC) is the sixth most common cancer in women and the seventh most common cause of cancer death worldwide, with ~204 000 new cases and 125 000 deaths reported annually (1). In general, OC has become more common in industrialized countries where parity is lower (1). However, the age-adjusted OC incidence rate in Japan has been increasing since the 1970s, and approximately half of all cancer deaths are attributed to cancer of the breast, uterus and ovary of female patients aged 40–49 years (http://ganjoho.jp/public/statistics/backnumber/2011_en.html). An increase in the incidence rate of OC was seen among female patients aged ≥15 years, and a clear increase is observed in Japanese female patients aged 50–54 years (http://ganjoho.jp/public/statistics/backnumber/2011_en.html). Although many factors influence a woman’s risk of developing OC, family history is believed to be the most important predictor of risk for the disease (2).
Inherited OC largely falls into two clinically defined syndromes: hereditary breast–OC syndrome (HBOC) and Lynch syndrome (LS). The majority of inherited OC cases are classified as HBOC, which is defined by inherited mutation of the *BRCA1* or *BRCA2* genes (*BRCA1/2*) (3,4). Conversely, LS is due to mutations in mismatch repair genes, mainly *MLH1*, *MSH2* and *MSH6*, and is responsible for a smaller proportion of hereditary OC. Individuals carrying germline mutations in the *BRCA1* or *BRCA2* genes have up to a 46% or 12% risk of developing OC by age 70, respectively (5,6), whereas the cumulative risk of OC in LS is estimated to be 8–10%, with an average onset age of 42 years (7).

Primary OC is a morphologically and biologically heterogeneous disease consisting of four major types: serous, mucinous, endometrioid and clear cell adenocarcinomas. Epithelial OC had long been thought to arise from the ovarian surface epithelium; however, recent studies have suggested a dualistic model of epithelial OC carcinogenesis. This model divides epithelial OCs into two broad categories, Types I and II, based on their clinicopathological features and characteristic molecular genetic changes (8). Type I includes mucinous, clear cell and low-grade endometrioid OCs and arises from a precursor lesion like endometriosis. Conversely, Type II tumors arise rapidly without a well-defined premalignant lesion and develop into high-grade serous or high-grade endometrioid OC. Interestingly, most germline *BRCA1/2* mutations are associated with Type II high-grade serous OC, and gene expression profiling of sporadic (non-germline mutant *BRCA1/2*) high-grade OC resembles germline mutant *BRCA1/2* OC (9,10). The identification of early serous carcinomas in the fallopian tube from risk-reductive salpingo-oophorectomies (RRSO) in women who were *BRCA1/2* mutation carriers (2,11) has shifted the paradigm of OC carcinogenesis to the idea that most epithelial OCs are extracellular in origin (8,11–13).

Racial and ethnic disparities exist in both Type I and II epithelial OCs. In general, approximately two-thirds of epithelial OC cases in Europe and the USA are of the high-grade serous type. Data from the British Columbia Cancer Agency (*n* = 2555) demonstrated that the overall frequency of tumor types was as follows: 68.1% high-grade serous, 3.4% low-grade serous, 12.2% clear cell, 11.3% endometrioid and 3.4% mucinous (14,15). This distribution was very similar to that observed in Europe and the USA (14–18). In contrast, Asian women were more commonly diagnosed with clear cell rather than serous type than Caucasian women (14,16,19–21). Clear cell subtypes are rare in Caucasian women, but represent over 25% of cases in Japan.

Importantly, although several studies have reported the prevalence of HBOC or LS family history (22–29), only a few reports have discussed the family history of Japanese OC cases (30–32).

Another factor that may influence the development of OC is the presence of p53-positive foci in the fimbria of the fallopian tubes from *BRCA1/2*-positive women and women without a history of pelvic cancer (2,33,34). Lee et al. (33) defined a ‘p53 signature’ as a focus of cells with strong nuclear p53 overexpression, but without increased proliferation or morphological abnormalities, and they proposed that the p53 signature is a precursor to invasive serous cancer. Although these factors have been implicated in the development of OC, their relationship to other risk factors for OC remains unclear.

Here, we evaluated the family history of Japanese OC patients and determined the TP53 status in the fallopian tubes of 1 Japanese *BRCA1* mutant case that may be associated with the transformed state in hereditary OC.

**PATIENTS AND METHODS**

**Family History Taking**

This study was conducted from 2009 until 2011. To be eligible for participation, patients were required to meet the following criteria: (i) diagnosis of epithelial OC, (ii) ≥20 years old, (iii) able to read and speak Japanese and (iv) not deemed ineligible for participation for any other reason.

Family history was taken from a total of 102 epithelial OC patients at the Department of Obstetrics and Gynecology, Keio University Hospital (Tokyo, Japan). OC patients were interviewed directly by the medical doctor, that is, face-to-face interaction between the interviewer and the interviewee, in order to obtain detailed family history in relatives. The interviewer inquired about the family history of HBOC-related cancers (breast, ovary, prostate and pancreas); LS-related cancers (colon, endometrium, stomach, ovary, small intestine, urinary tract/kidney, bile ducts, glioblastoma, sebaceous gland tumors and pancreas); other tumors; and the age of onset in probands, first- and second-degree relatives.

The diagnosis of HBOC is based on the ‘Hereditary Breast and/or Ovarian Cancer Syndrome Testing Criteria’ found in the NCCN Clinical Practice Guidelines in Oncology, Genetic/ Familial High-Risk Assessment: Breast and Ovarian (version 1, 2012) (http://www.nccn.org/index.asp), while LS diagnosis is based on the Amsterdam criteria II (35).

**BRCA1/2 Testing**

*BRCA1/2* testing has been performed for Japanese kindred suspicious of HBOC in association with the Center for Medical Genetics, Keio University School of Medicine (Tokyo, Japan) (36,37).

DNA was extracted from peripheral blood using a QIAamp DNA Blood Midi kit (QIAGEN). Direct sequencing of *BRCA1/2* was performed by FALCO Biosystems Ltd. (Kyoto, Japan). DNA was subjected to PCR amplification (35 reactions for *BRCA1*, 47 reactions for *BRCA2*). Each PCR product was sequenced by an ABI 3130xl genetic analyzer (Life Technologies). All variants detected by direct sequencing were interpreted according to the Myriad Genetics’ criteria (38).

Large rearrangement analysis of *BRCA1/2* genes were performed by Multiplex ligation-dependent probe amplification (MLPA) analysis (MRC–Holland, Amsterdam, The Netherlands) according to the manufacturer’s instructions.
PCR products were detected by an ABI 3130xl genetic analyzer (Life Technologies, Carlsbad, CA, USA). The relative copy number was determined by calculating the relative peak areas to the normal reference control.

**CLINICOPATHOLOGICAL FEATURE OF BRCA1/2 MUTATION CASES**

Clinicopathological features for 102 cases were determined. p53 protein expression and TP53 mutation status were examined in this case. Formalin-fixed paraffin-embedded tissues from one BRCA1 mutation carrier, who had undergone RRSO and in whom occult cancer was detected, were collected for immunostaining and TP53 genotyping. Immunostaining for p53 was performed as previously described (40) using a monoclonal antibody to p53 that targets an epitope in amino acids 37–45 of the protein (DO7: DAKO, Glostrup, Denmark). Categorization as p53 signature was made after review by two specialists using the published criteria, which requires 12 or more consecutive, strongly p53-positive secretory cell nuclei. In each case, one to four serial sections were taken from the tissue block (33,34,41) (http://www.nccn.org/index.asp). DNA from p53 signature-positive cells was analyzed for TP53 mutations, and normal DNA from blood served as a control. Laser-capture microdissection from p53 signature loci and fallopian tube epithelial cells were performed as previously described (40). TP53 sequences for both signature-positive foci, fallopian tube epithelial cells and DNA from blood were performed as previously described (40,42).

The study was conducted with approval of the ethics committee of the School of Medicine, Keio University (approval number: 20030097, 20070090, 20070081).

**RESULTS**

**FAMILY HISTORY**

Clinicopathological features for OC cases are shown in Table 1. The mean age of the patients was 55 years (range 32–79 years). All patients underwent staging laparotomy/debulking surgery including peritoneal cytology, and 85 patients (83.3%) received platinum-based chemotherapy after surgery. A pathologist classified all 102 tumors by histology according to the World Health Organization (WHO) criteria (43). Thirty (29.4%) cases were serous adenocarcinoma, 12 (11.8%) were mucinous adenocarcinoma, 25 (24.5%) were endometrioid adenocarcinoma and 31 (30.4%) were clear cell adenocarcinoma. The remaining four (3.9%) were classified as other types of epithelial OC. Thirty-nine cases (38.2%) had a history of endometriosis, and 12 probands (11.8%) had other cancer history, in addition to OC. Seven of these 12 had a history of breast cancer (BC), whereas five had a history of endometrial cancer (EMC). Surgical staging was based on the International Federation of Gynecology and Obstetrics staging system (44): Stage I, 66 (64.7%) patients; Stage II, 9 (8.8%) patients; Stage III, 24 (23.5%) patients and Stage IV, 3 (2.9%) patients. Nine of 102 (8.8%) families were diagnosed with HBOC, and two families (2.0%) were diagnosed with LS. Peutz–Jeghers syndrome, Li–Fraumeni syndrome or other hereditary cancer syndromes associated with ovarian tumors were not found.

Table 2 shows HBOC- or LS-related cancer history in the first- and second-degree relatives of OC patients. Twenty-two cases (21.6%) had a family history of BC. Ten of these cases (9.8%) had a family history of BC in first-degree relatives, and

<table>
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<th>Table 1. Clinicopathological characteristics of 102 OC patients</th>
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<td>Cancer in proband’s history</td>
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<td>HBOC (<a href="http://www.nccn.org/index.asp">http://www.nccn.org/index.asp</a>)</td>
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OC, ovarian cancer; BC, breast cancer; EMC, endometrial cancer; HBOC, hereditary breast–ovarian cancer; LS, Lynch syndrome.
12 cases (11.8%) had a family history of BC in second-degree relatives. Ten cases (9.8%) had a family history of OC, with five cases (4.9%) each in the first-degree and second-degree relatives. Four cases (3.9%) had a family history of both BC and OC, with two cases (2.0%) each in first-degree and second-degree relatives, while seven cases (6.9%) had a family history of EMC in the first- or second-degree relatives.

HBOC-related cancer history in the first- and second-degree relatives of OC patients is summarized in Table 3. Eight patients (7.8%) had one first-degree relative with HBOC-related cancer, and 11 (10.8%) had one second-degree relative with HBOC-related cancer. Two patients (2.0%) had two or more first-degree relatives with HBOC-related cancer and six (5.9%) had two or more second-degree relatives with HBOC-related cancer.

CLINICOPATHOLOGICAL FEATURE OF BRCA1/2 MUTATION CASES

Four cases were of serous adenocarcinoma, and two cases were of endometrioid carcinoma. Clinicopathological features for the six BRCA1/2-positive cases are listed in Table 5.

The Case 1 patient underwent RRSO as a BRCA1 mutation case (37) and was diagnosed as having occult serous adenocarcinoma on the surface of the right ovary based on the final retrospective pathology analysis.

DISCUSSION

For this study, the family history of Japanese OC patients was taken via direct interviews, and the p53 status in BRCA1 mutation cases was analyzed. A direct interview should provide the most accurate family history information to help distinguish HBOC and LC cases. Importantly, TP53 mutation was found
in ‘normal’ fallopian tube epithelial cells as well as in cancer cells in a BRCA1 mutation patient.

Only a few reports to date have described the HBOC-related family history of Japanese OC cases (30–32). For example, Komata et al. collected family history of HBOC using a self-administered questionnaire from 289 OC patients (30). In the previous study, 3% of OC patients had a family history of OC in second-degree relatives, and 16% of OC patients had a family history of BC in second-degree relatives. Only 1% of OC patients had a family history of both OC and BC in first-or second-degree relatives. In addition, Mori et al. collected data on reproductive, genetic and dietary risk factors from 110 epithelial OC patients and reported 2.7% BC and 0.9% OC cases among close relatives (45). van Altena et al. evaluated the adequacy of family history taking in OC patients and concluded that adequate documentation on family history was present in only 41% of all medical records of EOC patients (46). Based on this study, we used direct interviews to take family history in order to obtain more accurate information compared with self-administered questionnaires.

Of the 102 patients, Stage I disease was noted in 64.7%, whereas Stage III or IV disease was seen in 26.4%. HBOC-related OC appears to be of a high grade with advanced-stage serous tumors in Caucasian populations (1,2). A confounding factor was that almost all of the cases for which the family history was documented were of cancer survivors without tumors. This may explain the stage distribution of the patients as well as the racial differences.

Our BRCA1/2 mutant cases consisted of four high-grade serous adenocarcinomas and two high-grade endometrioid carcinomas without coexistence of endometriosis and were recognized as Type II. The ratio of Type II in Japanese OC is smaller than that in Caucasian populations; nevertheless, the number of BRCA1/2 germline mutations associated with Type II OC in our study was not smaller than that observed in Caucasian populations. Our previous study revealed that the prevalence of deleterious BRCA1/2 mutations in a Japanese population was significantly higher than that found in non-Ashkenazi individuals (36).
Recent studies have revealed small, linear p53-positive foci, termed ‘p53 signature’, in the fimbria of the fallopian tubes from BRCA1/2-positive women and women without a history of pelvic cancer (2,33). P53 signature is presumed to be an early cancer precursor and often associated with TP53 mutations (2). The Case 1 patient, a BRCA1 mutant carrier, was the first patient to undergo RRSO in Japan (37), and this case may support the recent theory that Type II OC develops due to BRCA1/2 inactivation and/or TP53 mutations (8).

Common TP53 gene mutations, such as codon273 CGT to CAT, were detected in microdissected cells from occult cancer and fallopian tube epithelial cells in this case (Fig. 3). Levine et al. noted at least three mutation ‘hotspots’ affecting residues 175 248, and 273 of TP53 in human cancers. The highest percentage of mutations (13%) has been found at position 273 (47).

Occult cancer is identified in 2.3–17% (averaging 5–6%) of BRCA1/2 mutation carriers who undergo RRSO (2). RRSO reduces the risk of OC by 85 to 90%, making it the most efficacious method for OC risk reduction in women who are BRCA1/2 mutation carriers. The Case 1 patient was diagnosed with occult serous adenocarcinoma on the surface of the right ovary based on the final retrospective pathology analysis. Nevertheless, her ovaries and fallopian tubes appeared to be normal on a gross level at the time of RRSO. Our clinical experience should be worth reporting as early experience to detect occult cancer from RRSO-underwent case in this country. This case would have developed progressive OC without RRSO because occult cancer cells had been detected.
on RRSO. The evidence of RRSO for prevention effect has been established in HBOC. RRSO should be performed not only to detect occult cancer but also to prevent cancer in Japan.

OC is significantly associated with the development of synchronous EMC in premenopausal Caucasian women (48–51). Gitsch et al. reported that synchronous OC was found in 29.4% of EMC cases (49). In addition, Walsh et al. reported that 25% out of 102 premenopausal women who underwent hysterectomy for EMC had coexisting OC (50). The nationwide Swedish Family-Cancer Database of 10.2 million individuals, which includes 19,128 invasive EMC and 19,440 OC cases, established a strong relationship between EMC and OC (51). However, coexistence of EMC and OC was found to be lower in Japanese than in Caucasian populations (52,53). Here, we found only five OC cases (4.9%) with EMC in the proband’s history. The lower incidence of EMC and OC coexistence may reflect the low incidence rate of EMC in Japan.

Most inherited OC is clinically defined as HBOC; however, ~2% of colorectal cancers associated with EMC and OC can be attributed to Lynch syndrome (54,55). Our finding that two families (2.0%) were diagnosed with LS suggests that clinical and genetic aspects of LS-related OC in Japan may be similar to those of Caucasian populations, although clinical aspects of HBOC-related OC were different from those of Caucasians.

The phenotypes of HBOC-related or LS-related OC are undistinguishable from each other and sporadic OCs. Clinicians should take note that the prevalence of deleterious BRCA1/2 mutations is reported to be significantly higher in Japanese individuals than in non-Ashkenazi individuals (36). These observations underline the importance of obtaining an adequate family history using direct interviews for OC cases.

Our report is the first report to find an occult cancer case of a BRCA1 mutant carrier who underwent RRSO in Japan. And it is unique in that it integrates family cancer history, and histology in OC cases as well as TP53 status in a BRCA1 mutant case into a discussion regarding carcinogenesis in a Japanese population.

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Conflict of interest statement
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References