Genetic characterization of early onset ovarian carcinoma ☆

Sarah S. Bernards [a], Barbara M. Norquist [a], Maria I. Harrell [a], Kathy J. Agnew [a], Ming K. Lee [b], Tom Walsh [b], Elizabeth M. Swisher [a,b,*]

[a] Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, University of Washington, 1959 Northeast Pacific Street, Seattle, WA 98195, USA
[b] Division of Medical Genetics, Department of Medicine, University of Washington, 1959 Northeast Pacific Street, Seattle, WA 98195, USA

HIGHLIGHTS

• Women with ovarian carcinoma age ≤40 have a high rate of *BRCA1* mutations.
• Deleterious germline mutations were also identified in *BRCA2*, *MSH2*, and *RAD51D*.
• The family history did not predict mutation status in these young patients.

ABSTRACT

Objective. Ovarian carcinoma (OC) is rare in young women and the fraction of early onset OC attributable to inherited mutations in known OC genes is uncertain. We sought to characterize the fraction of OC that is heritable in women diagnosed with ovarian, fallopian tube, or peritoneal carcinoma at forty years of age or younger.

Methods. We sequenced germline DNA from forty-seven women diagnosed with OC at age 40 or younger ascertained through a gynecologic oncology tissue bank or referred from outside providers using BROCA, a targeted capture and massively parallel sequencing platform that can detect all mutation classes. We evaluated 11 genes associated with ovarian carcinoma (*BARD1*, *BRCA1*, *BRCA2*, *BRIP1*, *MLH1*, *MSH2*, *MSH6*, *PALB2*, *PMS2*, *RAD51D*, and *RAD51C*) and additional candidate genes in DNA repair (*ATM*, *BAP1*, *CHEK2*, *MRE11A*, *NBN*, *PTEN*, *TP53*). We counted only clearly damaging mutations.

Results. Damaging mutations in OC genes were identified in 13 of 47 (28%) subjects, of which 10 (77%) occurred in *BRCA1* and one each occurred in *BRCA2*, *MSH2*, and *RAD51D*. Women with a strong family history were no more likely to have an OC gene mutation (8/17, 47%) than those without a strong family history (9/30, 30%, P = 0.35). Additionally, damaging mutations in non-OC genes were identified, one in *NBN* and one in *CHEK2*.

Conclusions. A high proportion of young women with invasive OC have mutations in *BRCA1*, and a smaller fraction have mutations in other known OC genes. Family history was not associated with mutation status in these early onset cases.

© 2015 Published by Elsevier Inc.

1. Introduction

Of the approximately 22,000 cases of ovarian cancer that are diagnosed each year in the United States, about 2700 occur in women age forty-four or younger [1,2]. However, many of the early onset cases are germ cell or stromal malignancies and the proportion of epithelial ovarian cancer or ovarian carcinoma (OC) occurring in women under 40 years is <10%, with a median age of diagnosis in the early 60s [2]. In many solid malignancies, very early onset is a hallmark of hereditary predisposition [3,4]. However, most unselected OC series that evaluated for genetic predisposition have included few young women [2,5,6,7,8,9], leading to an incomplete understanding of the hereditary factors underlying early onset OC.

Studies of unselected women with OC suggest that inherited damaging mutations in *BRCA1* and *BRCA2* account for 13%–15% of all OC cases [5,6,7,9]. Women with *BRCA1* mutations are on average about 10 years younger than non-mutation carriers or those with *BRCA2* mutations [9,10]. A smaller subset of the inherited mutations that predispose to

OC fall under the definition of Lynch syndrome, also called hereditary non-polyposis colorectal cancer. Lynch syndrome is caused by mutations in the DNA mismatch repair genes *MLH1*, *MSH2*, *MSH6*, and *PMS2* [11]. OC patients with Lynch syndrome are often younger than average at diagnosis, with a median age at diagnosis of 43 years [11,12].

In addition to *BRCA1*, *BRCA2*, and the Lynch syndrome genes, additional genes associated with hereditary OC have been recently identified including *RAD51C*, *RAD51D*, and *BRIP1* [5,13,14,15,16,17,18,19]. The contribution of these genes to early onset OC has not been previously evaluated. One study of OC in women under 30 identified no *BRCA1* or *BRCA2* mutations and two possibly pathogenic *MLH1* mutations [20].

Another study of 52 women with OC age forty or younger found a *BRCA1* or *BRCA2* mutation in 19% (10/52; 8 *BRCA1*, 2 *BRCA2*, 95% confidence interval 11–32%) [21], similar to the contribution of *BRCA1* and *BRCA2* mutations in all-ages cohorts [5,6,7,8,21]. We sought to define the contribution of *BRCA1*, *BRCA2*, Lynch syndrome, and recently identified or suspected OC genes including *RAD51C*, *RAD51D*, *BRIP1*, *PALB2*, and *BARD1*, as well as other known breast cancer genes in women with early onset OC.

2. Methods

All patients in this study were diagnosed with ovarian, fallopian tube, or peritoneal carcinoma at age 40 years or younger and provided informed consent on a protocol approved by the institutional review board. Patients had either enrolled in genomics research at the time of diagnosis at the University of Washington or were referred by outside providers to the study based on an early age of diagnosis. Patients were excluded if they had OC diagnosed at the time of planned risk-reducing surgery. A "strong family history" was defined as a relative with OC, a relative with breast cancer <50 years of age, or two relatives with breast cancer at any age.

Germline DNA was extracted from blood and sequenced using BROCA, a targeted capture, massively parallel sequencing test developed at the University of Washington [22]. For this study, we sequenced *ATM*, *BAP1*, *BARD1*, *BRCA1*, *BRCA2*, *BRIP1*, *CHEK2*, *MLH1*, *MSH2*, *MSH6*, *MRE11A*, *NBN*, *PALB2*, *PMS2*, *PTEN*, *RAD51C*, *RAD51D*, and *TP53*. 1 μg of DNA was used to create paired-end libraries with ~200 base pair (bp) inserts, hybridized to a custom pool of oligonucleotides for the genomic regions of interest using the SureSelectXT enrichment system on a Bravo liquid handling instrument (Agilent). Samples were then barcoded and sequenced on a HiSeq (Illumina) with 2 × 101 bp paired end reads and a 7 bp index read. Sequencing reads were aligned to the human reference genome (hg19) using BWA (Burrows–Wheeler alignment). Variants were identified using GATK37 and Pindel after indel realignment and base quality recalibration. Variants from low quality (≤50) and low depth of coverage regions (<5 reads) were filtered out. Single nucleotide variants, insertions and deletions, and copy number variations were detected as previously described [22]. Missense mutations were only included if proven to be deleterious (i.e., *BRCA1* C61G). All mutations were validated with Sanger sequencing.

Patients were classified as having an OC gene mutation if they had a damaging mutation in one of 11 genes: *BRCA1*, *BRCA2*, *BRIP1*, *RAD51C*, *RAD51D*, *PALB2*, *BARD1*, *MSH2*, *MLH1*, *PMS2*, and *MSH6*. Mutations in other candidate genes were not assumed to be causative.

Secondary mutations that restore *BRCA2* were evaluated in one case as previously described [23].

3. Results

A total of 47 young women with OC were evaluated, 38 from consecutive enrollment at the University of Washington and 9 from outside referrals. Eleven cases from the UW series were previously reported (Supplemental Table S1) [5,13]. Table 1 compares the clinical characteristics of University of Washington patients to those of the outside

Table 1
Clinical characteristics of study patients by mode of referral.

	UW tissue bank	Outside referral
Number of subjects	38	9
Median age at dx (range)	37 (27–40)	37 (28–40)
Strong family history[a]	11 (29.7%)	6 (75%)
WT[b]	25 (65.8%)	7 (77.8%)
BRCA mutation[c]	10 (26.3%)	1 (11.1%)
Mutations in non-BRCA OC genes[c]	2 (5.3%)	0
Mutations in other genes[d]	1 (2.6%)	1 (11.1%)
Histology		
HG serous	15 (39.5%)	6 (66.7%)
undifferentiated carcinoma	2 (5.3%)	0
LG serous	6 (15.8%)	1 (11.1%)
Endometrioid	7 (18.4%)	2 (22.2%)
Clear cell	5 (13.1%)	0
Mucinous	3 (7.9%)	0
FIGO stage[e]		
Stage I	6 (17.1%)	1 (11.1%)
Stage II	5 (14.3%)	0
Stage III	21 (60%)	6 (66.7%)
Stage IV	3 (8.6%)	2 (22.2%)

Abbreviations:
UW = University of Washington, dx = diagnosis, WT = wildtype, OC = ovarian carcinoma, HG serous = high grade serous (grade 2 or 3), LG serous = low grade serous (grade 1).
[a] A relative with OC, a relative with br ca <50 years of age, or two relatives with br ca at any age.
[b] No damaging mutations detected in any genes tested.
[c] MSH2 and RAD51D.
[d] CHEK2 and NBN.
[e] Stage was not available for three cases.

referral patients. Eight of 9 outside cases had been previously tested and all but one were negative for *BRCA1* and *BRCA2* mutations at the time of referral. In this young population of women with OC, histology other than high-grade serous was relatively common (Table 2) and accounted for 26 (55%) of the carcinomas, including 2 undifferentiated (4%), 9 endometrioid (19%), 5 clear cell (11%), 3 mucinous (6%) and 7 low grade serous carcinomas (15%). No mutations were identified amongst low grade serous and mucinous carcinomas. Endometrioid OC had the highest mutation rate (4/9, 44%) and the most diverse gene distribution (2 *BRCA1*, 1 *MSH2*, 1 *RAD51D*, Table 2).

Damaging mutations in OC genes were identified in 12 of 38 (32%) of UW subjects, of which 10 (83%) occurred in *BRCA1* or *BRCA2*, and one each occurred in the non-BRCA OC genes *MSH2* and *RAD51D*. The *RAD51D* 5810delA frameshift mutation was previously reported by Wickramanayake et al. and occurred in an individual with synchronous endometrioid ovarian and endometrial carcinomas [13]. This individual had insufficient knowledge of her family history of cancer to generate a pedigree. The subject with the *MSH2* mutation meets criteria for Lynch syndrome based on revised Amsterdam criteria (Fig. 1).

In contrast to the young cases enrolled at diagnosis without previous genetic testing, 8 of the 9 outside referrals had been previously tested for *BRCA1* and *BRCA2* mutations. No mutations were found in the previously untested patient. Only one *BRCA2* mutation was found. It had been previously detected in that patient. This mutation (*BRCA2* L2653P), initially classified as a variant of unknown significance (VUS), was reclassified to "favor deleterious" by Myriad Genetics at the time of study entry. Loss of the wildtype allele was identified in her primary tumor consistent with the deleterious reclassification. Notably, a reversion to wildtype sequence was identified in her recurrent tumor, at which time she demonstrated progression during treatment with a PARP inhibitor.

In addition to mutations in one of the 11 OC genes, one damaging mutation was identified in a known breast cancer gene, *CHEK2* (*CHEK2* c.758_761del ACTG), in a patient with endometrioid OC, and a damaging mutation in the breast cancer gene *NBN* (*NBN* c.1550_1551insA) was identified in another patient with endometrioid OC. These were considered to be incidental mutations as these genes do