Gynecologic Oncology xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Frequency of mutations in a large series of clinically ascertained ovarian cancer cases tested on multi-gene panels compared to reference controls

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HIGHLIGHTS

- Ovarian cancer risks for mutations in hereditary cancer panel genes were assessed.
- Mutations by gene from 7768 ovarian cancer cases and reference controls were compared.
- BRCA1, BRCA2, BRIP1, MSH2, MSH6, RAD51C, and RAD51D were confirmed as high-risk genes.
- ATM was identified as a moderate risk ovarian cancer gene.
- The results will inform clinical management of women with mutations these genes.

ARTICLE INFO

Article history:
Received 26 June 2017
Received in revised form 29 August 2017
Accepted 30 August 2017
Available online xxxx

Keywords:
Ovarian cancer
Multi-gene panel testing

ABSTRACT

Objectives. Given the lack of adequate screening modalities, knowledge of ovarian cancer risks for carriers of pathogenic alterations in predisposition genes is important for decisions about risk-reduction by salpingo-oophorectomy. We sought to determine which genes assayed on multi-gene panels are associated with ovarian cancer, the magnitude of the associations, and for which clinically meaningful associations could be ruled out.

Methods. 7768 adult ovarian cancer cases of European ancestry referred to a single clinical testing laboratory underwent multi-gene panel testing for detection of pathogenic alterations in known or suspected ovarian cancer susceptibility genes. A targeted capture approach was employed to assay each of 19 genes for the presence of pathogenic or likely pathogenic alterations. Mutation frequencies in ovarian cancer cases were compared to mutation frequencies in individuals from the Exome Aggregation Consortium (ExAC). Analyses stratified by family and personal history of other cancers and age at diagnosis were also performed.

Results. Significant associations ($p < 0.001$) were identified between alterations in 11 genes and ovarian cancer, with eight of these displaying ≥5-fold increased risk (*BRCA1*, *BRCA2*, *BRIP1*, *MSH2*, *MSH6*, *RAD51C*, *RAD51D*). Relative risks of ovarian cancer greater than two-fold were also observed for *ATM*, but could reliably be ruled out for *RAD50* and *CHEK2*.

Conclusions. These results will inform clinical management of women found to carry pathogenic alterations in genes tested on multi-gene panels. The knowledge that some genes are not associated with OC can reduce concerns of women found to carry pathogenic alterations in those genes.

1. Introduction

Ovarian cancer (OC) is the fifth leading cause of cancer death in U.S. women [1]. Because of the difficulties inherent in pre-symptomatic screening for OC, it is critically important to identify women at high risk of this disease who can be offered risk-reducing salpingo-oophorectomy (RRSO). Genetic screening is an important prevention tool for OC as RRSO in *BRCA1* and *BRCA2* mutation carriers is proven to reduce mortality [2]. Due to the large hereditary component of OC, multi-gene panel testing is commonly offered to women diagnosed with this form of cancer [3–6]. Equally important, relatives of women with OC who test negative for a pathogenic alteration can have some measure of assurance of lower personal risk.

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http://dx.doi.org/10.1016/j.ygyno.2017.08.030
0090-8258/

Pathogenic mutations in *BRCA1* and *BRCA2* are found in 10–15% of unselected OC cases and account for up to 40% of heritable OC cases [7–11]. Several other genes have been associated with OC risk, such as *BRIP1*, *RAD51C*, and *RAD51D* [10,12–16]. However, the magnitude of the associations for these OC susceptibility genes is less well defined. In addition, it has been suggested that *PALB2* and *BARD1* confer increased risk of OC [12,13], but these findings need further evaluation. OC is also a well-established feature of Lynch syndrome that is associated with pathogenic alterations in the mismatch repair (MMR) pathway (*MLH1*, *MSH2*, *MSH6*, *PMS2*), but the gene-specific risks for OC with each of the MMR genes are not well defined [6,12,17,18]. Earlier studies of cancer predisposition genes involved in OC have been characterized by small sample sizes, a limited number of genes examined, or both. For example, Ramus et al. analyzed ~3200 cases and ~3400 controls but only examined four genes (*BRIP1*, *NBN*, *PALB2*, and *BARD1*) [13]. In contrast, Norquist et al. examined a larger set of genes in 1915 cases and reference controls [12].

In this study, we sought to determine the frequency of pathogenic alterations in a large series of OC cases referred for clinical testing and to provide estimates of OC risk associated with pathogenic alterations in genes commonly tested on multi-gene cancer panels.

2. Materials and methods

2.1. Study population

The data analyzed in this study were based on 10,203 adult (age at diagnosis ≥ 21) women with OC selected from 140,449 individuals referred to Ambry Genetics (Aliso Viejo, CA) for hereditary cancer multigene panel testing between March 15, 2012 and June 30, 2016. Test requisition forms were provided by the ordering clinician and, for the majority of individuals, included details on patient demographics and clinical history including personal and family history of cancer, ages at diagnoses, along with tumor pathology for a subset of women.

Of the 10,203 OC cases, 7768 were Caucasian, including 7349 that self-identified as Caucasian and 419 that self-identified as Ashkenazi Jewish. The 7768 Caucasian individuals were tested on at least one of nine cancer panels offered at Ambry Genetics that include the majority of genes of potential relevance to OC (Supplemental Table 1).

The characteristics of the 7768 cases of OC included in the primary analyses are shown in Table 1. The median (range) age at diagnosis was 57.5 (21–90) years. Personal history of cancer other than ovarian was reported in 1992 (26%) cases, with breast cancer being the most frequent. The majority of women in the analyses (n = 6710 (86.4%)) also reported at least one first- or second-degree family member with a history of any cancer, with breast and colorectal cancers being the most common.

Table 1
Characteristics of Caucasian individuals included in risk analyses.

		Caucasian Only analysis subset	
		n	%
Total patients		7768	
Ovarian cancer–age at diagnosis			
<40		748	9.6
40–49		1192	15.3
50–59		2192	28.2
60–69		2163	27.8
70–79		1128	14.5
≥80		273	3.5
Unknown		72	0.9
Histopathology		1746	22.5
Carcinosarcoma		24	1.4
Germ cell		13	0.7
Sex cord		54	3.1
Other		20	1.1
Epithelial		1637	93.8
Serous		953	58.2
Endometroid		208	12.7
Clear cell		152	9.3
Mucinous		94	5.7
Mixed		80	4.9
Transitional cell		3	0.2
Other		148	9.0
Personal history of other (non-ovarian) cancers[a]			
Breast	No	6666	86.1
	Yes	1073	13.9
Colorectal	No	7595	98.2
	Yes	142	1.8
Pancreatic	No	7703	99.6
	Yes	34	0.4
Endometrial	No	7232	93.5
	Yes	504	6.5
Family history of cancer (1st & 2nd degree only)[a]			
Ovarian	No	5911	84.9
	Yes	1049	15.1
Breast	No	3557	55.1
	Yes	2903	44.9
Colorectal	No	4878	70.1
	Yes	2082	29.9
Pancreatic	No	6274	90.1
	Yes	686	9.9
Endometrial	No	6391	91.8
	Yes	569	8.2

[a] Categories are not mutually exclusive.

2.2. Multigene panel testing and sequence variant classification

Each of the multi-gene panels utilized in this cohort evaluates germline mutations using targeted custom capture and sequencing along with targeted chromosomal microarray analysis for copy number variant analysis as previously described [19]. In this study, germ-line genetic testing results were evaluated for 19 OC susceptibility genes. All variants identified were evaluated by a five-tier variant classification system by Ambry Genetics as previously described [20]. In primary analyses, we included variants classified as pathogenic or likely pathogenic (Supplemental Table 7). Ambry Genetics routinely submits variants and their classifications to the ClinVar database.

As previously shown in ovarian [12], breast [20] and prostate [21] cancer studies, the Exome Aggregation Consortium [22] (ExAC) dataset is an effective control dataset for the estimation of the gene-specific frequencies of pathogenic alterations. In this study OC cases were compared to non-Finnish European (NFE) controls from the ExAC dataset. Importantly, the dataset excluded germ-line variants found in exomes from The Cancer Genome Atlas (TCGA) to ensure to the extent possible that the 'controls' were cancer-free. Variants reported as PASS and non-PASS in ExAC were initially included in the dataset. Several of the non-PASS variants were observed in the OC cases, validated by Ambry Genetics, and classified as pathogenic or likely pathogenic. Therefore, restricting to PASS-only variants in the ExAC dataset was expected to inflate risk estimates for each gene. Variants reported as non-PASS were reviewed, and were excluded when observed at significantly different frequencies in other populations, genotyped in <20,000 controls, or called as multiallelic variants at the same position.

Variants in ExAC that were also observed by Ambry Genetics were classified based on the laboratory classification system. All other nonsense, frameshift, consensus dinucleotide splice site (±1 or 2) were classified as pathogenic or likely pathogenic and were included in analyses. The remaining missense, splice site (±3+ positions), synonymous, or intronic variants were classified as pathogenic/likely pathogenic when reported as pathogenic or likely pathogenic by at least one clinical laboratory in ClinVar [23], with no conflicting reports (Benign/Likely Benign).

Following variant classification, some additional exclusions were applied. Variants with minor allele frequency (MAF) > 0.3% (except common founder mutations) in OC cases or ExAC controls were excluded from the study. Additionally, three individual variants associated with

Please cite this article as: J. Lilyquist, et al., Frequency of mutations in a large series of clinically ascertained ovarian cancer cases tested on multigene panels compared to re..., Gynecol Oncol (2017), http://dx.doi.org/10.1016/j.ygyno.2017.08.030