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14. ABSTRACT

Ovarian, peritoneal and fallopian tube carcinomas (OC) are the most deadly of the gynecological cancers. Our data indicate that at least 20% of unselected OC is hereditary and that 20-25% of inherited mutations occur in genes other than BRCA1 and BRCA2. The large fraction of OC associated with inherited mutations in a variety of genes provides an important opportunity to reduce OC mortality. Maximizing the benefit from OC risk assessment and prevention requires an improved understanding of the penetrance of OC genes beyond BRCA1/2. Furthermore, minimal data exist regarding the hereditary component of OC, including BRCA1/2, in non-white populations. Our study defined the genetic causes of hereditary OC in African Americans (AA) as well as the spectrum of cancers, the age of onset, and the relative risk associated with mutations in non-BRCA1/2 genes.

15. SUBJECT TERMS
Ovarian cancer, drug resistance, rucaparib, phase 2, DNA repair, homologous recombination, nonhomologous end-joining (NHEJ), poly(ADP-ribose)polymerase, BRCA1, BRCA2, PARP1

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1. INTRODUCTION

Ovarian, peritoneal and fallopian tube carcinomas (OC) are the most deadly of the gynecological cancers and can be considered together as one entity. While women with early stage OC have an excellent chance of cure, attempts to improve early detection have been largely ineffective. In contrast to surveillance, surgical prophylaxis with risk-reducing salpingo-oophorectomy (RRSO) reduces OC mortality in high risk women. Inherited mutations in *BRCA1* and *BRCA2* (*BRCA1/2*) account for about 15% of OC. Inherited loss of function mutations in other related genes account for another 5-6% of cases, but less is understood about the OC risk associated with mutations in these genes. Furthermore, there are other OC genes that have not yet been discovered. Our hypothesis is that rare, inherited, damaging mutations in genes other than *BRCA1/2* confer a relatively high cancer risk that would warrant age appropriate surgical prophylaxis. A better understanding of the etiologic contribution from, and penetrance of, genes other than *BRCA1/2* to hereditary OC is needed to guide clinical decision-making and to optimize recommendations for OC prevention. Our overall goal is to refine the understanding of inherited OC susceptibility, emphasizing genetic variation in diverse racial populations and genes other than *BRCA1* and *BRCA2*. We achieved these objectives through two specific aims:
Aim 1: Identify rare variants in OC susceptibility genes other than *BRCA1/2* in women with ovarian, fallopian tube or peritoneal carcinoma who have an increased likelihood of genetic risk.
Aim 2: Identify the genetic contribution of many genes to OC susceptibility among African American women with ovarian, fallopian tube or peritoneal carcinoma.

2. KEY WORDS

Ovarian cancer, BRCA1, BRCA2, cancer susceptibility, RAD51C, RAD51D, PALB2, BRIP1, BARD1, African-American, familial, hereditary

3. ACCOMPLISHMENTS

The first goal was to enroll and BROCA sequence 200 high risk probands in years 1 and 2, and that goal was exceeded. Our BROCA targeted sequencing assay includes 11 known OC genes: *BRCA1, BRCA2 (FANCD1), BARD1, BRIP1 (FANCJ), RAD51C (FANCO), RAD51D, PALB2 (FANCO),MSH2, MLH1, MSH6, PMS2,* 9 other known breast cancer genes: *ATM, CHEK2, FAM175A (abraxas), FAMCM, NBN, PTEN, RECQL, TP53* and *XRCC2*) and 25 other candidate genes in the Fanconi anemia-BRCA pathway: *ATR, BABAM1, BAP1, BLM, BRCC3, BRE, CHEK1, ERCC1, ERCC4 (FANCQ), FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG (XRCCC9), FANCI, FANCL, GEN1, MRE11A, RAD50, RAD51, RBBP8 (CtIP), SLX4 (FANCP), UIMC1 (RAP80).* All damaging mutations were confirmed with Sanger Sequencing.

Patients were selected and recruited for the study who had ovarian, peritoneal or fallopian tube carcinoma (collectively termed OC) and either 1. a second non-skin invasive cancer. 2. a relative with OC or 3. a mutation in a non BRCA gene. All probands were consented to detailed genetic analyses and detailed pedigrees were constructed and pathology reports obtained to confirm diagnoses. When possible archived tumor tissue was obtained. If a mutation was known or discovered in the family, other relatives were consented, enrolled and tested for the family mutation. Non-affected relatives were tested for the mutation while relatives with breast or ovarian cancer were tested with full BROCA sequencing.

147 patients with OC and a second primary cancer were enrolled. 29 patients had a non-breast second cancer. Of these 29 patients, pathogenic mutations were identified in 3 (10.3%), which

included two *BRCA1* mutations, and 1 *MSH2* mutation. This mutation rate is not significantly different than we have found in the unselected OC population. In contrast, the mutation rate in the 118 patients who had breast cancer (before or after their OC diagnosis) was 48.3% (N=57), which included mutations in the following genes: *BRCA1* (N=32, 27.1%), *BRCA2* (N=11, 9.3%), *PALB2* (N=3, 2.5%), *BRIP1* (N=3, 2.5%) and one each (0.8%) in *BLM, CHEK2, RAD51C,* and *RAD51D.* The mutation rate in OC patients with breast cancer was significantly higher than in those with a non-breast second cancer (P=0.0001). These data are currently being collated with the family data in preparation for publication.

We recruited 145 subjects from 95 families with a proband with OC and a relative with OC on either side of the family. Many of these families had undergone previous genetic testing prior to referral and were referred specifically because they no mutations had been found. Therefore, these families do not represent the mutation rate that might be found in families not previously evaluated for genetic mutations. Sequencing revealed pathogenic mutations in 25 families (26.3%) including 10 *BRCA1*, 6 *BRCA2*, 2 *BRIP1*, 2 *NBN*, 2 *MSH6*, 1 *ATM*, 1 *BARD1*, and 1 *FANCA.* Of these results, we believe the *FANCA* mutation is incidental and not causative to the cancer in the family based on our previous case control data (Norquist et al, JAMA Oncology, 2016, PMCID: PMC4845939). Furthermore, the *FANCA* mutation does not segregate with other cancers in the family including 2 early onset breast cancer. *NBN* and *ATM* are two genes that may be moderate penetrant OC genes based on our case control data. The three families with *NBN* and *ATM* mutations could not be adequately evaluated for segregation, because while the probanc with OC carried the mutation, other OC affected family members were decreased and without relatives that allowed reconstruction of the genetic inheritance. Unfortunately, these families demonstrate the difficulty of studying highly affected OC families in contrast to breast cancer families, which have more surviving affected family members.

We also recruited or identified families with mutations in non-*BRCA* OC genes or suspected OC genes including 10 families with ATM mutations, 2 with ATR mutations, 1 *BAP1*, 12 *BARD1*, 2 *BLM*, 26 *BRIP1*, 5 *CHEK1*, 1 *FAM175A*, 1 *FANCA*, 4 *NBN*, 23 *PALB2*, 10 *PMS2*, 1 *POLE*, 16 *RAD51C*, 12 *RAD51D*. These families do overlap with the severely affected OC families and patients with double primaries just described. In these families, we are enrolling and testing relatives in an ongoing manner in order to pool data from multiple families and calculate penetrance. We are also obtaining and testing tumor tissue when available for loss of heterozygosity (LOH). Interestingly, we noted that *BRIP1* and *RAD51C* mutations were always paired with LOH in tumor DNA. However, the presence of LOH in OC associated with *RAD51D* and *PALB2* mutations was variable and we never identified *BARD1* LOH. *BARD1* heterodimerizes with *BRCA1* in its tumor suppressor function. Therefore, *BARD1* mutation may act in a dominant negative manner, not requiring loss of the wildtype allele. Therefore, even those all of these genes are consider “tumor supressors”, the presence or lack of LOH should not be used a defining characteristic for causation.

A second goal was to enroll and exome sequence 30 BROCA negative families. As we attempted to recruit families severely affected by ovarian cancer, it became evident that the patients with ovarian cancer were frequently deceased. We hoped to overcome this by obtaining archived tissues on deceased relatives, but frequently tissue was not available as the 10 year mandatory storage time was exceeded or subjects had surgery at unknown locations. Therefore, in order to sequence two or more relatives from these families, we included patients with breast cancer (usually early onset) in place of one or more of the deceased relatives with ovarian cancer. The

first 20 families were sequenced using whole exome sequencing. The latter 10 families were sequenced with whole genome sequencing (WGS) in order to evaluate families for structural rearrangements as well as deep intronic variants. Data analysis is very complex and for WGS is currently ongoing, but to date we have not identified any clearly causative genetic alterations. We are continuing to recruit severely affected OC families.

A third goal was to enroll and sequence 100 African-American (AA) women with OC. Enrollment of AA women lagged behind our goal. We therefore collaborated with Dr. Ernst Lengyel at the University of Chicago to access anonymized banked samples from African American patients with OC. We therefore were able to BROCA sequence 21 AA patients from UW and 48 from UC for a total of 69 AA patients with OC. We identified a similar mutation spectrum and frequency to our previous work in a mostly Caucasian population. A total of 10 patients (14.5%, 95% confidence interval 8.1-24.7%) had germline mutations in OC genes, including 6 in *BRCA1* (8.7%), 2 in *BRCA2* (2.9%), and 1 each (1.4%) in *RAD51D* and *PMS2*. Additionally, one patient had an incidental truncating mutation in *CHEK2*.

Opportunities for training and professional development has the project provided?
Nothing to report

Dissemination of Results
Three papers are in currently in preparation: one on the family and double primary data and one on the AA OC population and a third comparing the rates of mutations in fallopian tube versus ovarian and primary peritoneal carcinomas.

4. IMPACT

Impact on the principal discipline
Our data identify equal mutation rates in black versus white American women with OC and a similar diversity in OC genes identified, supporting the recommendation to offer comprehensive genetic testing to all women with OC regardless of race or ethnicity. We have also made progress in determining which non-BRCA genes contribute to OC (i.e. B*ARD1, BRIP1, PALB2, RAD51C, RAD51D, PMS2, MSH6, MLH1, MSH2*, probably *ATM*, and possibly *NBN*) while ruling out the association of some other OC candidate genes including *CHEK2, RAD50, FANCA, MRE11A, FAM175A, FANCM, STK11, a*nd *PTEN.*

Impact on other disciplines
Nothing to report

Impact on technology transfer
Nothing to report

Impact on society
Nothing to report

5. CHANGES/PROBLEMS

Changes in approach
Nothing to report

Problems or delays and plans to resolve them:

We had difficult enrolling our target number of AA OC patients. We resolved that issue by identifying existing banked samples from a collaborator at University of Chicago. We also found it difficult to enroll multiple OC patients from one family, because relatives with OC were usually deceased. We have addressed this by enrolling other women with early onset breast cancer in the family and using living relatives to reconstruct the genetic results of those deceased.

Changes that had a significant impact on expenditures
Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Nothing to report

6. PRODUCTS

Publications, conference papers, and presentations
Nothing to report

Website(s) or other Internet site(s)
Nothing to report

Technologies or techniques
Nothing to report

Inventions, patent applications, and/or licenses
Nothing to report

Other Products
Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

Name:	Elizabeth Swisher MD
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	0000-0003-2331-0434
Nearest person month worked:	1
Contribution to Project:	Dr. Swisher directed all aspects of the project including IRB oversight, recruitment, sequencing analyses, and data interpretation
Name:	Maria Harrell, PhD
Project Role:	Staff scientist
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2
Contribution to Project:	Dr. Harrell oversaw all sequencing including quality control.
Funding Support:	
Name:	Ming Lee PhD
Project Role:	Bioinformaticist
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	Dr. Lee ran the bioinformatics pipeline for the next generation sequencing.
Name:	Kathy Agnew
Project Role:	Staff scientist
Researcher Identifier (e.g. ORCID ID):	

Nearest person month worked:	1
Contribution to Project:	Ms. Agnew managed all incoming samples, keeps the study database, communicates with referring provers and generates result letters.
Funding Support:	
Name:	Marc Radke
Project Role:	Staff scientist
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2
Contribution to Project:	Mr. Radke prepped all sample, extracted DNA and created library preps for DNA sequencing. He performs Sanger sequencing validations. He took over Ms. Agnews duties at her retirement in June, 2018
Funding Support:	

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

No

What other organizations were involved as partners?

Nothing to report

Appendices

None