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Hereditary predisposition to ovarian cancer, looking beyond *BRCA1/BRCA2*

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HIGHLIGHTS

- We demonstrated the diagnostic yield of multi-gene panel testing in HBOC
- We examined the contribution of 19 genes in a BRCA1/2 negative population
- Genetic panel testing increases diagnostic yield

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ABSTRACT

Objective. Genetic predisposition to ovarian cancer is well documented. With the advent of next generation sequencing, hereditary panel testing provides an efficient method for evaluating multiple genes simultaneously. Therefore, we sought to investigate the contribution of 19 genes identified in the literature as increasing the risk of hereditary breast and ovarian cancer (HBOC) in a *BRCA1* and *BRCA2* negative population of patients with a personal history of breast and/or ovarian cancer by means of a hereditary cancer panel.

Methods. Subjects were referred for multi-gene panel testing between February 2012 and March 2014. Clinical data was ascertained from requisition forms. The incidence of pathogenic mutations (including likely pathogenic), and variant of unknown significance were then calculated for each gene and/or patient cohort.

Results. In this cohort of 911 subjects, panel testing identified 67 mutations. With 7.4% of subjects harboring a mutation on this multi-gene panel, the diagnostic yield was increased, compared to testing for *BRCA1* and *BRCA2* mutations alone. In the ovarian cancer probands, the most frequently mutated genes were *BRIP1* (n = 8; 1.72%) and *MSH6* (n = 6; 1.29%). In the breast cancer probands, mutations were most commonly observed in *CHEK2* (n = 9; 2.54%), *ATM* (n = 3; 0.85%), and *TP53* (n = 3; 0.85%).

Conclusions. Although further studies are needed to clarify the exact management of patients with a mutation in each gene, this study highlights information that can be captured with panel testing and provides support for incorporation of panel testing into clinical practice.

Introduction

Ovarian carcinoma is notable for a lack of effective screening, ambiguous symptoms, and highest mortality among gynecologic malignancies. The late-stages of presentation have hampered modern efforts to improve morbidity and mortality associated with this disease [1,2]. However, insight into genetic susceptibility for ovarian cancer has revolutionized care by providing an opportunity for risk-reducing surgery in women with a known predisposition to disease [3,4].

Familial ovarian carcinoma has been described in the context of a hereditary breast and ovarian cancer (HBOC) syndrome. Inherited in an autosomal dominant fashion, HBOC is characterized by a young age of onset, multiple primaries, bilateral breast cancer, and family history of 1st- or 2nd-degree kin with similar diagnoses [5]. Current estimates predict the incidence of ovarian and breast carcinoma in United States to be 21,980 and 232,670, respectively [6]. Of these ovarian cases, about 20–25% are thought to be a result of a hereditary predisposition, while 5–7% of breast carcinoma is thought to be hereditary [7,8]. Historically, known genetic causes of HBOC have largely been explained by germline mutations in the *BRCA1* and *BRCA2* (*BRCA1/2*) genes, with an estimated risk of ovarian carcinoma from 30–62% in individuals with

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mutations [9–12]. A small contribution of hereditary ovarian carcinoma risk has also been attributed to *MLH1*, *MSH2*, *MSH6*, and *PMS2*, the mismatch repair (MMR) or Lynch genes [13,14].

Literature supports that *BRCA1/2* genes are responsible for the majority of known cause of HBOC and MMR genes are responsible for an additional proportion of hereditary ovarian cancer, while the remaining hereditary risks are accounted for by several other genes as well as proportion that is as yet unexplained [15]. Through the understanding of other genetic hereditary syndromes (including Li–Fraumeni, Cowden, and Peutz–Jegher syndromes), and homologous recombination (HR) pathway genes, in which *BRCA1/2* interact, several additional genes contributing to the HBOC phenotype have been identified and well described in the literature [13–33]. Historically, testing many genes for a single proband has been laborious and cost prohibitive. Thus, testing was commonly restricted to more commonly mutated genes that best fit the patient's clinical history. However, the advent of next generation sequencing technology has made it possible for clinicians to order a single test, evaluating multiple genes simultaneously, in a cost effective and time efficient fashion, enabling a more complete genetic evaluation. Such testing has been clinically available since 2012; however, limited literature exists assessing the value of multi-gene panel testing in cohorts of patients with breast and/or ovarian carcinoma. Therefore, we sought to investigate the contribution of 19 genes identified in the literature as increasing the risk of hereditary breast and/or ovarian cancer in a *BRCA1/2* negative population of patients with a personal history of breast and/or ovarian cancer by means of a next generation sequencing panel.

Materials & methods

Participants

Subjects were referred for multi-gene panel testing with OvaNext (Ambry Genetics, Aliso Viejo, CA) between February 2012 and March 2014. For this retrospective cohort study, all identifying information was removed from the database and the subjects were assigned codes. This study was conducted in accordance with all regulations set for by the Institutional Review Board of Dignity Health at St. Joseph's Hospital and Medical Center, Phoenix, AZ. Inclusion criteria included: personal history of breast and/or ovarian, fallopian, or primary peritoneal cancer. Exclusion criteria included: male gender or mutation(s) or likely pathogenic variant(s) identified in *BRCA1/2* gene(s). From test requisition forms self-identified demographic data, and self-identified personal and family histories of cancer were extracted.

Genetic panel

The following 21 genes were analyzed and included for all subjects: *ATM*, *BARD1*, *BRCA1*, *BRCA2*, *BRIP1*, *CDH1*, *CHEK2*, *EPCAM*, *MLH1*, *MRE11A*, *MSH2*, *MSH6*, *MUTYH*, *NBN*, *PALB2*, *PMS2*, *PTEN*, *RAD50*, *RAD51C*, *STK11*, and *TP53* [13–33].

Mutation identification and analysis

Genetic testing was completed in Ambry's laboratory using the following protocol. Genomic deoxyribonucleic acid was isolated from subject's whole blood or saliva samples using the QIAsymphony instrument (Qiagen, Valencia, CA) according to manufacturer's protocols. Deoxyribonucleic acid was quantified using a spectrophotometer (Nanodrop, Thermoscientific, Pittsburgh, PA or Infinite F200, TECAN, San Jose, CA). Genomic deoxyribonucleic acid was then combined with primer pairs in micro-droplets designed to the specified targets for each gene to complete sequence enrichment (Raindance Thunderstorm Technologies, Billerica, MA). Using Illumina HiSeq technology (Illumina, San Diego, CA) enriched libraries were applied to the solid surface flow cell for clonal amplification and sequencing. Sanger sequencing was performed for any region with insufficient depth of coverage, defined as 50×. Additionally, bi-directional Sanger sequencing was performed to confirm all variant calls, other than known previously defined benign and likely benign alterations. To detect large deletions and duplications, a targeted chromosomal microarray with increased probe density in regions of interest was completed (Aglient, Santa Clara, CA).

Initial data processing and base calling, including extraction of cluster intensities, was done using RTA 1.12.4 (HiSeq Control Software 1.4.5). Sequence quality filtering was executed with the Illumina CASAVA software (ver 1.8.2 Illumina, Hayward, CA). Sequence fragments were aligned to the reference human genome (GRCh37) and variant calls were generated using CASAVA. A minimum quality threshold of Q30 was applied which translates to an accuracy of >99.9% for called bases and mean coverage was >300×.

Annotated variants were then analyzed to determine the likelihood of pathogenicity and classified into five tiers based on the recommendations of the American College of Medical Genetics and Genomics [34]. Alterations were classified in the following categories: pathogenic mutation, variant likely pathogenic, variant of unknown significance (VUS), variant likely benign, and benign based on a previously described multifactorial algorithm [35].

Data analysis

The incidence of pathogenic mutations (including likely pathogenic), and VUS were then calculated for each gene. The incidence of mutations and VUS were compared across cohorts based on demographic and clinical history information.

Genes were grouped in four categories: HR pathway/moderately penetrant (*ATM*, *BARD1*, *CHEK2*, *MRE11A*, *NBN*, *PALB2*, *RAD50*, *RAD51C*), Lynch syndrome genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM*), other highly penetrant (*CDH1*, *PTEN*, *STK11*, *TP53*), and other moderate risk (*MUTYH*). Monoallelic *MUTYH* mutation carriers were not included in the mutation positive cohort.

Results

A total of 1187 subjects were referred for multi-gene testing. After applying exclusion criteria the cohort included 911 subjects. The following subjects were excluded from data analysis: 10 males, 26 females with an identified *BRCA1*/2 mutation and 240 females without a personal history of breast or ovarian cancer. Demographic details for the 911 subjects are provided in Table 1. There were a total of 466 subjects with a personal history of ovarian cancer, 353 subjects had a personal history of breast cancer, and 92 had personal histories of both breast and ovarian cancer.

Mutations identified in this cohort

Of the 911 subjects negative for a *BRCA1/2* mutation, 7.4% (n = 67) had a pathogenic or likely pathogenic mutation (collectively referred to as mutation herein) in a gene other than *BRCA1/2* (Table 3, Fig. 1). Thirty subjects (6.44%) with ovarian cancer had a mutation, 26 (7.37%) subjects with breast cancer had a mutation, and 11 (11.96%) subjects

Table 1
Subject Demographics: Ethnicity of distribution of subjects.

Ethnicity	N	%
White	688	75.52
Unknown	90	9.88
Ashkenazi Jewish	54	5.93
Hispanic	24	2.63
African American/Black	18	1.98
Mixed Ethnicity	17	1.87
Asian	15	1.65
Middle Eastern	5	0.55
Total	911	100

Table 2
Subject age at time of malignancy diagnoses.

	Breast cancer cohort			Ovarian cancer cohort			Breast & ovarian cancer cohort					
	Pos N = 26	Neg N = 237	Total cohort N = 353	Pos N = 30	Neg N = 292	Total cohort N = 466	Pos N = 11	Neg N = 56	Total cohort N = 92	Pos N = 11	Neg N = 56	Total cohort N = 92
	Breast cancer age at Dx			Ovarian cancer age at Dx			Breast cancer age at Dx			Ovarian cancer age at Dx		
Mean	48	52	55	53	55	55	57	55	55	58	57	57
Median	46	51	53	50	56	56	54	53	54	59	58	61
Age range	27–75	22–92	22–92	26–78	–85	17–85	45–75	36–76	28–76	41–72	27–87	26–87
Std dev	13.3	12.1	9.98	15.1	13.6	14	9.9	10.7	10.4	9.5	14.4	13.7
Age at Dx provided (total N)	26	230	340	29	282	447	10	53	88	11	53	88

Dx = diagnosis, Std dev = standard deviation, Pos = positive mutation status, Neg = negative mutation status.

with both breast and ovarian cancer had a mutation. One individual with ovarian cancer harbored both an *ATM* and *MSH6* mutation. One individual with breast cancer had 2 *CHEK2* mutations. Mutations were identified in all genes on the panel with the exceptions of *MSH2* and *STK11* in this cohort.

In the ovarian cancer cohort (n = 466), among the HR pathway/moderately penetrant genes, mutations were seen most frequently in *BRIP1* (n = 8; 1.71%), *ATM* (n = 4; 0.86%), and *RAD51C* (n = 3; 0.64%). Mutations were also identified in *CHEK2* in 2 individuals and a *NBN*, *PALB2*, *RAD50*, and *MRE11A* mutation was identified in 1 individual per gene. Among Lynch syndromes mismatch repair genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM*), 8 mutations were identified (1.72% of the cohort) with *MSH6* mutations being the most common, accounting for 75% (n = 6) of all Lynch mutations in the ovarian cancer only cohort. Among the other highly penetrant genes, 2 individuals with ovarian cancer had *PTEN* alterations while no alterations were identified in *TP53*, *CDH1*, or *STK11*.

Table 3
Mutations observed by personal cancer history. Positives by gene & cancer history.

	Total positive	Total positive with ovarian cancer	Total positive with breast cancer	Total positive with breast and ovarian cancer
BRCA Pathway/Moderate Risk				
BRIP1	10	8	1	1
CHEK2	16	2	9	5[b]
ATM	7	4[a]	3	0
RAD51C	3	3	0	0
NBN	4	1	2	1
PALB2	4	1	2	1
RAD50	2	1	1	0
MRE11A	1	1	0	0
BARD1	2	0	2	0
Total	49	21	20	8
Lynch Genes				
MSH6	10	6[a]	2	2
MLH1	2	2	0	0
PMS2	1	0	0	1
MSH2	0	0	0	0
Total	13	8[a]	2	3
Other Highly Penetrant				
PTEN	2	2	0	0
TP53	3	0	3	0
CDH1	1	0	1	0
STK11	0	0	0	0
Total	6	2	4	0
Total Individuals	67[a]	30[a]	26	11
Other Moderate Risk				
Individuals with a monoallelic *MUTYH* mutation	16	**9**	**4**	**3**[b]

[a] 1 individual has both an *MSH6* and *ATM* mutation.
[b] 1 individual has 2 *CHEK2* mutations and a *MUTYH* mutation, counted once in *CHEK2* count.

In the breast cancer only cohort (n = 353), among the *BRCA* pathway/moderately penetrant genes, mutations were most commonly observed in *CHEK2* (n = 9; 2.55%), and *ATM* (n = 3; 0.85%). Interestingly, only 1 individual with breast cancer only carried a *BRIP1* mutation, despite the high frequency of *BRIP1* mutations in the ovarian cancer only cohort. Mutations were also identified in *NBN* (n = 2), *PALB2* (n = 2), *BARD1* (n = 2), and *RAD50* (n = 1). Of note, 2 individuals with breast cancer only had a *BARD1* mutation while no individuals from the ovarian only cohort had a *BARD1* mutation. Among Lynch syndromes mismatch repair genes only 2 mutations were identified in the breast only cohort, both of which were in *MSH6*. Among the other highly penetrant genes, 3 individuals with breast cancer had *TP53* alterations and 1 had a *CDH1* mutation, while no alterations were identified in *PTEN* or *STK11*.

Among probands with breast and ovarian cancer, at least 1 in 7 of those *BRCA1/2* negative patients had a mutation in another breast and ovarian cancer susceptibility gene. Eleven (11.96%) subjects in this cohort had a mutation, including 5 with a mutation in *CHEK2*, 1 with a *BRIP1* mutation, 1 with a *NBN* mutation, 1 with a *PALB2* mutation, 2 with a *MSH6* mutation, and 1 with a *PMS2* mutation.

Variants of unknown significance

At least one VUS was identified in more than 1 in every 4 subjects (259/911; 28.43%) (Fig. 2). This includes 12 individuals who also carried a mutation. Thus, 12/67 (17.91%) of individuals with a positive test result also carried at least one VUS. Of the 911 subjects, 43 carried two or more VUS (4.72%), including 2 individuals with three VUS, and 1 individual with four VUS. When a VUS was present, the vast majority (84.77%; 217/256) had just one VUS.

When considering ethnicity, VUS rates are highest among Middle Eastern probands (80% with one or more VUS) followed by Asian probands (53.3% with one or more VUS) and Black probands (44.4% with one or more VUS). Rates of more than one VUS were highest among Middle Easterners and Blacks (40.00% and 22.2% with two or more VUS, respectively). VUS rates were lowest among Whites (27.18%) and those with ethnicity unknown/not reported (22.22%).

Negative mutation findings and age of onset

Five hundred and eighty-six subjects (64.32%) did not have any significant or inconclusive genetic test results, including 237 probands with breast cancer, 293 probands with ovarian cancer, and 56 probands with breast and ovarian cancer. The age of onset among subjects negative for a reportable finding was slightly older compared to those with a mutation, though there was no significant difference in age of onset among any of the subgroups. In the breast cancer only cohort, the average age of diagnosis among negative probands was 52 compared to 48 for positive probands (p = 0.07). In the ovarian cancer only cohort the average age of ovarian cancer diagnosis was 55 among negative probands and 53 among positive probands (p = 0.37). In the breast and ovarian cancer cohorts,

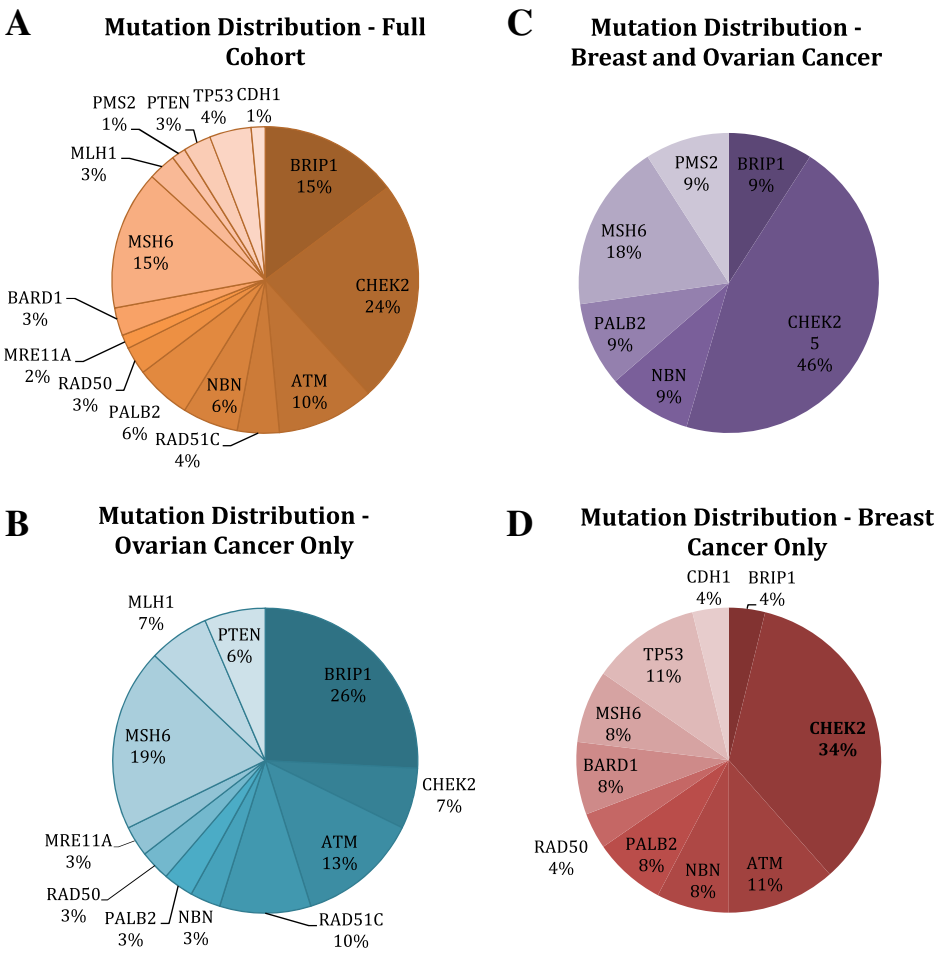

Fig. 1. Mutation distribution: A. Full Cohort, B. Ovarian Cohort, C. Breast and Ovarian Cohort, and D. Breast Cohort.

however, age of onset was slightly older among positive individuals compared to those negative for a mutation, though the total number of subjects available to compare in this group is small ($p = 0.72$ for breast cancer age of onset, $p = 0.97$ for ovarian cancer age of onset) (Table 2).

Family history

Consistent across all cohorts, >90% of probands reported at least one relative with cancer, with 93% of negative probands reporting a family history of cancer, and 98% of positive probands reporting a family history of cancer in at least one relative ($p = 0.19$). Among these relatives, 77.5% of positive probands reported cancer of any kind in at least one 1st degree relative, and 72.7% of negative probands reported cancer in at least one 1st- degree relative. Thus, the vast majority of individuals with breast or ovarian cancer referred for OvaNext testing had a family history of cancer, the majority of which included cancer in a 1st- degree relative (Table 4A).

Results were analyzed more specifically regarding breast and ovarian cancer history among 1st and 2nd-degree relatives. Probands positive for a pathogenic alteration and those negative for a reportable finding were compared, subdividing by the probands' histories of breast and ovarian cancer, breast cancer only, or ovarian cancer only (Table 4B).

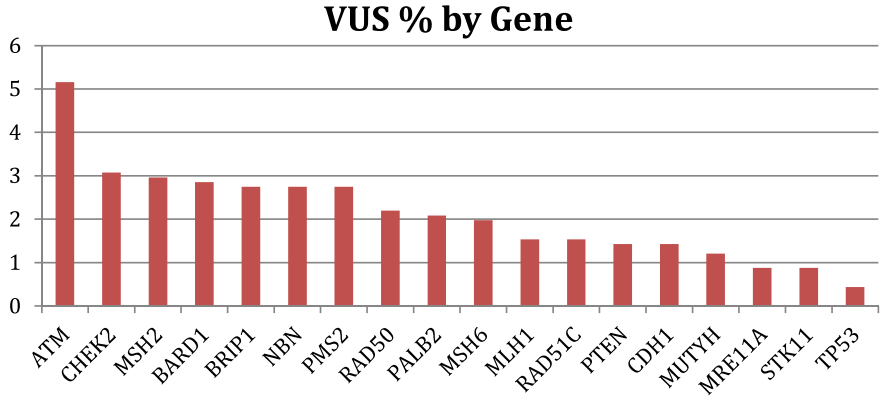

Fig. 2. Variants of unknown significance (VUS) percentage observed by gene.

Table 4A
Family histories: Subjects that report a family history of malignancy and history of affected first-degree family member.

	Mutation positive (n = 67)		Mutation negative (n = 585)	
	n	%	n	%
1 or more 1st degree relative with cancer	54	80.6	426	72.8
1 or more relative affected with cancer	65	97.0	542	92.6

Discussion

Using a hereditary cancer multi-gene panel, we evaluated the contribution of 19 additional genes that increase susceptibility to ovarian cancer among women negative for *BRCA1/2*, with a personal history of breast or ovarian cancer [20]. This yielded the identification of 67 additional mutations not in *BRCA1/2*. With 7.4% of patients harboring a mutation on this multi-gene panel, this increases the diagnostic yield of genetic testing substantially compared to testing for *BRCA1/2* mutations alone.

The majority of mutations (72%) were identified in the HR pathway/moderately penetrant breast and ovarian cancer genes in the HR pathway. Remaining mutations were identified in Lynch syndrome genes and other highly penetrant genes with established National Comprehensive Cancer Network or consortium management guidelines.

The most common mutations identified in subjects with ovarian cancer were in *BRIP1* (n = 9). *BRIP1*, a member of the HR pathway, interacts directly with *BRCA1* and along with several other proteins creating a macro-protein theorized to be involved in DNA repair during replication [19,36]. Rafnar et al., found when evaluating an Icelandic HBOC population that *BRIP1* demonstrates tumor suppressive effects and mutations in the gene increased risk of ovarian cancer with an odds ratio of 8.13. The findings in this cohort of subjects confirm reports of *BRIP1* mutations demonstrated in other series of patients with ovarian carcinoma [15,30]. Reviewing family histories of probands with a *BRIP1* mutation, 4 subjects (44.4%) had a family history of ovarian cancer in a 1st- or 2nd-degree relative, compared to 29.3% of ovarian cancer probands negative for a reportable finding of a family history of ovarian cancer (Tables 4A and 4B). Proband and family history frequencies of ovarian cancer among *BRIP1* mutation carriers suggest that *BRIP1* has a higher penetrance for ovarian cancer compared to other genes on this panel.

Verifying that deficiencies in HR pathway are central in ovarian carcinogenesis, the ovarian cohort demonstrated mutations in other HR genes including *CHEK2*, *ATM*, *NBN*, *PALB2*, *RAD51C*, *RAD50*, and *MRE11A* (n = 29). In fact, the majority of mutations (71.43%) were found in genes in this pathway.

In reviewing the MMR genes in subjects with a personal history of ovarian or breast and ovarian carcinoma, an overall mutation frequency of 1.97% (11/558) was observed. This was even higher than the frequency previously reported (0.5%) [15], with nearly 1 in 50 individuals with ovarian carcinoma in this cohort harboring a mutation in an MMR gene, although a selection bias may exist given that this cohort was referred for multi-gene panel testing that included MMR genes. Furthermore, seven family histories did not meet Amsterdam II or Bethesda criteria for Lynch syndrome testing, yet the proband did harbor a mutation in a MMR gene. These criteria have proven ineffective with gynecologic malignancy, and some patients with MMR mutation may be missed if genetic testing for MMR genes is limited to those individuals who meet an established set of criteria for Lynch syndrome genetic testing. These results suggest that expansion of Lynch syndrome testing criteria should be considered, particularly for probands and families presenting predominantly with ovarian cancer.

Interestingly, an additional 10 patients that did meet Amsterdam II or Bethesda criteria did not harbor a MMR mutation, but in fact had a mutation in another gene on this hereditary cancer panel with mutations found in: *CHEK2*, *MRE11A*, *BRIP1*, *RAD51C*, and (monoallelic) *MUTYH*. The personal and family histories among these additional cases did not contain additional features that would be indicative of a hereditary breast and ovarian cancer susceptibility, which may have led a clinician to consider additional testing for these genes.

These findings demonstrated that MMR genes are implicated in a small fraction of ovarian carcinoma and nearly 1 in 50 referred for a hereditary cancer panel genetic testing. However phenotypic assessment alone does not accurately guide a clinician in ordering gene specific testing, and in some cases testing of additional genes may be warranted even when a family meets clinical criteria for Lynch syndrome. Thus, this data suggests that simultaneous testing with multi-gene panels provides an efficient means of evaluating MMR and other ovarian carcinoma susceptibility genes.

Several individuals with breast cancer only in our cohort also had a mutation in an MMR gene (n = 2), however only one family met criteria for Lynch testing. A proband not meeting criteria had invasive ductal carcinoma diagnosed at age 49 with no suggestion of Lynch syndrome, such as family member with endometrial or colorectal cancer, reported in her family history. There is conflicting evidence regarding the risk for breast cancer in individuals with MMR mutations, with results herein contributing further to the perplexing question regarding breast cancer susceptibility and Lynch syndrome. As more multi-gene panel testing is performed clinically and in research settings, more clarity should surface regarding the correlation between breast cancer risk and Lynch syndrome.

In assessing the mutations identified in other highly penetrant genes with established management guidelines, interestingly 2 individuals with ovarian cancer had a *PTEN* alteration, which are of interest as the risk for ovarian carcinoma is not commonly associated with mutations in *PTEN*. Mutations in *TP53* and *CDH1* were identified in breast cancer probands only, which is consistent with established predispositions for these genes, and no alterations were identified in *STK11* in the entire cohort. This trend has been observed in other panel testing as well, where mutations in *STK11* have been limited to individuals with clinical features suggestive of Peutz–Jegher syndrome, which is in contrast to mutations in *TP53*, *CDH1*, and *PTEN* [39]. Data has been emerging in these latter genes (*TP53*, *CDH1*, *PTEN*) suggesting that, much like Lynch findings in this cohort, individuals with mutations identified on multi-gene panel testing in these genes do not necessarily have expected clinical features of the related genetic syndrome.

While panel testing is more likely to provide a more complete capture of an individual's genetic landscape, there are several unanswered questions and areas of future research. As the number of genes tested increases, the likelihood of detecting VUS increases as well.

Table 4B
Family histories: Subjects that report a family history of malignancy and history of affected first-degree or second-degree family member.

	Ovarian cancer probands (+/− breast cancer)				Breast cancer probands (+/− ovarian cancer)			
	Mutation positive (N = 41)		Negative (N = 348)		Mutation positive (N = 37)		Negative (N = 293)	
	N	%	N	%	N	%	N	%
1 or more relative with ovarian cancer	12	29.3	71	20.4	9	24.3	96	32.8
1 or more relative with breast cancer	14	34.1	146	42.0	21	56.8	191	65.2
1 or more relative with breast cancer at age <50	6	14.6	45	12.9	6	16.2	104	35.5

Although these results are not negative per se, data is lacking as to the clinical impact. These results may be confusing and unsettling to patients as the implications to the proband and her family remains unclear. Furthermore, if individuals underwent testing based on family history alone, without a personal history of breast or ovarian cancer, it remains to be clarified whether risk-reduction surgery would be appropriate for patients with mutations in all genes on the panel. Data is needed regarding incidence, penetrance, and age-adjusted risks to determine these practice guidelines. To quantify these important measures that will steer practice guidelines, particularly given the rarity of pathogenic mutations in many of these genes, large-scale data sharing between laboratories, clinicians, and researchers is imperative. Until this information is discovered on a population level, clinicians and patients alike will be faced with positive results lacking established management. Both parties maybe prompted to proceed with unnecessary additional evaluation (for example pelvic ultrasound and CA-125 screening for ovarian cancer, and MRI for breast cancer), testing of family members or unwarranted risk-reducing surgery. These steps would add to patient concern and financial cost. Further data will help to support or refute the extent in which additional preventive screening and management strategies are warranted for individuals with mutations in moderate penetrance genes. Furthermore, it will provide clarity regarding actual risks for family members considering testing for a known pathogenic mutation identified in the proband.

Although further studies are needed to clarify management of each mutation, this study highlights the information that can be captured with panel testing and merits further review for incorporation in clinical practice. Next generation sequencing is inarguably a cost-effective and time-efficient strategy for testing many genes simultaneously. As more data emerges regarding penetrance and age-adjusted risks through nationwide and international data sharing initiatives such as those led by PROMPT (The Prospective Registry of MultiPlex Testing) and ENIGMA (Evidence-based Network for the Interpretation of Germline Mutant Alleles), the long term impact on reduction in cost of medical care and morbidity/mortality also can be assessed. Furthermore, age of onset of breast or ovarian cancer, and family history of breast or ovarian cancer did not vary significantly among subjects positive for a mutation and those without any reportable findings. Thus, this data does not support the utility of severity of family history or age of onset of cancer to refer HBOC patients for multi-gene panel testing.

As these questions are being answered in future studies, a more complete genetic understanding is providing opportunities for translation and treatment planning. *BRCA* mutation carriers with ovarian carcinoma are noted to have a better prognosis, and clinical response to platinum therapy is providing patients and families with prognostic information specific to their mutation. Moreover, targeted therapies are underdevelopment that exploit the defect in HR, inhibiting poly(ADP-ribose)-polymerase (PARP), and creating a lethal submission of DNA repair by interrupting base excision repair. *BRCA* mutations confer sensitivity to PARP inhibitors and it has been suggested that this sensitivity may extend to other FA-BRCA (HR) genes as well [37–40].

This population of probands was referred nationwide for testing, making our data set large and generalizable to patient cohorts with a history of breast or ovarian cancer. However, our study has several limitations, including a potential selection bias as all subjects were referred for genetic testing and not recruited at random. Additionally, demographic and clinical history data was limited to reported information on test requisition forms without direct review of patient medical records. Therefore, personal and family histories could be incomplete, or underreported for all affected family members.

Here we have evaluated the contribution of 19 genes in a *BRCA1/2* negative population with a personal history of breast and/or ovarian cancer. Multi-gene panels provide an efficient means of capturing a genetic diagnosis; this study highlights information that can be captured with panel testing and suggests a role for incorporation in clinical practice.

Conflict of interest statement

Dr. Elizabeth Chao, Jill Dolinsky, and Charles Dunlop are employed by Ambry Genetics. Drs. Bradley Monk, Dana Chase and Lindsey Minion have nothing to disclose.

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