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ARTICLE

# High detection rate from genetic testing in *BRCA*-negative women with familial epithelial ovarian cancer

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ABSTRACT

**Purpose:** Epithelial ovarian cancer (EOC) is associated with pathogenic variants (PVs) in homologous recombination and/or mismatch repair genes. We aimed to review the testing of women with familial EOC at our center.

**Methods:** Women with familial EOC (≥2 EOC in family, including index case) referred to our center between 1993 and 2021 were included. Genetic testing (*BRCA*/Lynch syndrome screening, exome sequencing, panel testing, 100,000 Genome Project, and NIHR BioResource genome sequencing) and clinical demographic, diagnosis, and survival data were reviewed.

**Results:** Of 277, 128 (46.2%) women were *BRCA* heterozygotes (*BRCA1*: 89, *BRCA2*: 39). The detection rate in *BRCA*-negative women was 21.8%; the most commonly affected gene was *BRIP1* (5.9%). The non-*BRCA* detection rate was significantly higher in families with 2 affected members with EOC only (22.4%) than the families with ≥3 (11.1%) affected members (odds ratio = 9.9, 95% CI = 1.6-105.2, $P$ = .0075). Overall, 112 different PVs in 12 homologous recombination/mismatch repair genes were detected in 150 of 277 (54.2%) unrelated women.

**Conclusion:** This is the largest report of women with familial EOC undergoing wider testing to date. One-fifth of *BRCA*-negative women were heterozygous for a PV in a potentially actionable gene. Wider genetic testing of women with familial EOC is essential to optimize their treatment and prevention of disease in family members.



## Introduction

One of the most relevant risk factors for epithelial ovarian cancer (EOC) is a family history of breast and/or EOC (hereditary breast and ovarian cancer). Approximately 10% to 15% of ovarian cancer is thought to be hereditary, although, estimates vary.[1-4] There is a 3-fold increase in ovarian cancer risk in women with a first-degree relative with ovarian cancer.[3] Pathogenic variants (PVs) in high risk genes, *BRCA1* and *BRCA2*, moderate risk genes, such as *RAD51C/D*, and genes involved in mismatch repair (MMR) contribute to approximately 20% to 25% of all EOCs.[1,5] Genes identified as associated with an increased ovarian cancer risk include those involved in homologous

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recombination (HR) (*BRCA1*, *BRCA2*, *RAD51C*, *RAD51D*, *BRIP1*, and *PALB2)* and MMR *(MSH2*, *MSH6*, *MLH1*, and *PMS2)*. The prevalence of MMR-deficiency or microsatellite instability in familial ovarian cancer has been estimated to be 10% to 20%.[6,7]

A number of different genetic testing strategies are available to investigate the cause of familial ovarian cancer, and these have evolved over time as techniques such as next-generation sequencing (NGS) were developed. They are also adapted for family history, such as a *BRCA1*/*BRCA2* screen for a family history of breast and ovarian cancer or a screen for Lynch syndrome for women with a family history of ovarian and endometrial and/or colorectal cancer. After the discovery of a number of high and moderate susceptibility genes associated with increased risk of EOC, the use of cancer gene panels began to be introduced in the United Kingdom, initially predominantly through private or research testing. There was some controversy regarding the use of these panels. To address this, in 2018, the UK Cancer Genetics Group supported by the UK Genetic Testing Network (UKGTN) published a consensus statement to be adopted by the UK National Health Service.[8] It was recommended with majority (>75%) vote that ovarian cancer panels include *BRCA1*, *BRCA2*, *MLH1*, *MSH2*, *MSH6*, *PMS2*, *RAD51C*, and *RAD51D*, and the inclusion of *BRIP1* was made after expert presentation and discussion due to contributing sufficient risk of ovarian cancer that risk-reduction salpingo-oophorectomy could be considered.[8]

Because our historical familial EOC cases had not been tested with the full recommended genetic screen and to assess the effect of ovarian cancer panel testing conducted through research or the NHS clinical services, we aimed to review testing outcomes of women with familial ovarian cancer known to our center.

## Materials and Methods

### Study participants

We reviewed the genetic test results and clinical data of 277 women referred to the Manchester Centre for Genomic Medicine (MCGM) between 1993 and 2021 with a diagnosis of EOC and at least 1 additional first- or second-degree relative with a diagnosis of EOC. Patients were retrospectively identified from within the patient database of women known to MCGM. Clinical information regarding testing results, date of EOC diagnosis, date of sample test, other cancer diagnoses, age at EOC diagnosis, EOC histology, stage at diagnosis, survival data, family history, and Manchester score (MS) were obtained from the MCGM laboratory database; Manchester University NHS Foundation Trust clinical records software, and the Christie NHS Foundation Trust. The MS is a model developed to determine the probability of an index case being a *BRCA1/2* heterozygote using the data on an individual's breast, ovarian, pancreatic, and prostate cancer history and family history.[9]

### Genetic testing

Genetic testing from 1996 onward included *BRCA1/2* screening. Panel testing was introduced in 2016, therefore, most samples received retrospective testing. All women with EOC up to 2017 were tested after referral to genetics, and a minority of women with familial EOC were tested as part of mainstreaming by medical oncologists only for *BRCA1/2* and *PALB2*.

Screening of *BRCA1* and *BRCA2* was performed using Sanger sequencing or sequencing using long range polymerase chain reaction, Nextera XT library preparation and MiSeq sequencing and dosage analysis using MRC-Holland multiple ligation-dependent probe amplification (MLPA) probe kits, P002 for *BRCA1* and P045 for *BRCA2* (MRC-Holland). Testing for each individual is described in Supplemental Table 1. If a *BRCA1/2* PV was detected, no further testing was carried out, and it was considered the causal factor in this woman's familial EOC. If a *CHEK2* deletion or duplication was detected using P045, it was further analyzed using probe kit P190. PVs were confirmed using Sanger sequencing. NGS was performed for the Inherited Cancer Panel using the Illumina SureSelect XT next-generation sequencing (NextSeq) (Illumina) using the Agilent BRAVO robot (Agilent). The Inherited Cancer Panel for ovarian cancer included *BRCA1*, *BRCA2*, *BRIP1*, *MLH1*, *MSH2*, *MSH6*, *RAD50*, *RAD51C*, and *RAD51D*.

The Lynch syndrome screening involved screening for *MLH1*, *MSH2*, and *MSH6* performed through sequencing using long range polymerase chain reaction, Nextera XT library preparation, and MiSeq sequencing. Variants were confirmed using Sanger sequencing. Dosage analysis was performed using MRC-Holland MLPA probe kits, P003 for *MLH1* and *MSH2* and P072 for *MSH6* (MRC-Holland). *MSH6* exon 1 was screened using Sanger sequencing owing to poor coverage of this region through NGS technology. *PMS2* was tested using a bespoke sequencing and MLPA kit if suspected.

Exome sequencing was performed by BGI using the BGISEQ-500 sequencer (BGI). FASTQ files were received from BGI already demultiplexed and mapped against HG19 using Burrows-Wheeler Aligner. Sequence Alignment Map files were converted to Binary Alignment Map files using SAMTools. Duplicates were marked on the Binary Alignment Map file using Picard software. Base recalibration and base quality score recalibration were performed using the Genome Analysis Toolkit. Variants were called using HaplotypeCaller within Genome Analysis Toolkit. Variant quality score recalibration to flag likely false positives and realignment were performed on the variant call format files. For logistic reasons, the samples were processed individually, and joint calling was not performed. Variants were processed on VarSeq (Golden Helix Inc) and exported to Microsoft Excel per sample. Variants with read depths of <30 were excluded. Common variants with an allele frequency of >1% were also excluded.

Patients recruited through the NIHR BioResource study underwent genome sequencing and cancer panel screening

(Illumina TruSight Cancer panel). This is described in detail elsewhere.[10]

## Variant classification

Multiple in silico prediction tools were used to assess potential variant effect prediction. Clinical prediction software Alamut Visual version 2.15 (SOPHiA GENETICS) was used, which incorporates scores from splicing prediction tools MaxEntScan, NNSPLICE, GeneSplicer, Exonic Splicing Enhancer (ESE) tools, and missense prediction tools Sorting Intolerant From Tolerant,[11] MutationTaster, Polymorphism Phenotyping v2,[12] and data from ClinVar,[13] database for Nonsynonymous SNPs' Functional Predictions,[14] and Catalog of Somatic Mutations in Cancer (COSMIC). Missense variants were further assessed using the in silico tool Rare Exome Variant Ensemble Learner (REVEL).[15] Variant classification according to the American College of Medical Genetics and Genomics/Association for Molecular Pathology criteria was checked and confirmed.

## Statistical analysis

Independent *t* test, Fisher exact test, and odds ratios were calculated using GraphPad Prism 8.4.3.

# Results

## Demographics

A total of 277 unrelated women with familial EOC were identified, and they underwent testing as shown in Figure 1. Most of these women had high grade serous ovarian cancer (HGSOC) or what was historically classified as poorly differentiated cystadenocarcinoma, the majority of which would likely be classified as HGSOC under modern histology assessment (Table 1). Data regarding age at diagnosis were available for 270 women, and mean age was 56 (range: 27-86) years.

In total, 47 (17.0%) individuals also had breast cancer (BC) in addition to EOC; 38 (80.9%) of these were in women with clinically significant PVs, and there were 23 other cancer diagnoses in 21 women (Supplemental Table 1). Of the 47 women with BC, mean age at BC diagnosis was 48.5 (range: 32-68) years, 47.8% had ER-positive tumors, and 9 women (19.1%) had triple-negative BCs. A PV was detected in 37 (78.7%) of these women, and 35 (74.5%) were detected in *BRCA1/BRCA2*. Detection rate by personal and family history is summarized in Table 2.

## Detection rates

PVs were detected in 128 of 277 (46.2%) women (*BRCA1*: 89, *BRCA2*: 39). Of the remaining 149 *BRCA*-negative women, further testing was carried out in 101 women. All testing by individual is described in Supplemental Table 2. This included a Lynch syndrome screen or ovarian cancer panel screen through the NHS or testing via research consisting of exome sequencing of HR/MMR genes, genome sequencing through the 100,000 Genome Project, or genome sequencing through the NIHR Bioresource MPS study (Figure 1).

In total, 48 *BRCA*-negative women were unable to undergo further testing owing to absent or poor quality DNA samples. There was no significant difference between the average age at diagnosis and death of women who underwent further testing and those who did not (57.6 years vs

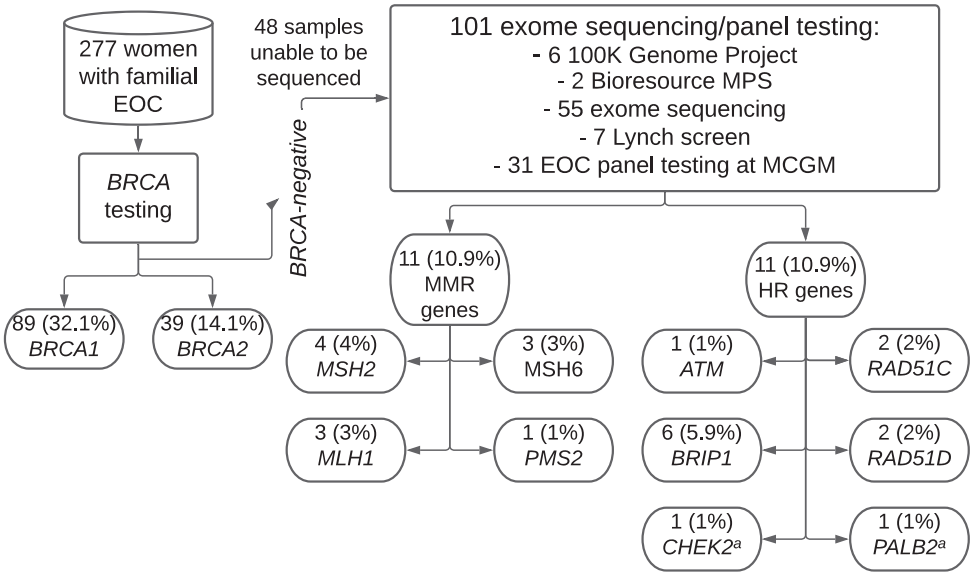


**Figure 1 Flowchart of variants detected through *BRCA* screening and wider testing.** EOC, epithelial ovarian cancer; HR, homologous recombination; MCGM, Manchester Centre for Genomic Medicine; MMR, mismatch repair; MPS, Multiple Primary tumours Study.

**Table 1** Breakdown of cases by histologic subtype

| Histologic Subtype | Number (%) | Heterozygous for PV Detected (%) | Heterozygous for VUS Detected (%) |
|---|---|---|---|
| High grade serous ovarian carcinoma | 156 (56.3) | 86 (55.1) | 10 (6.4) |
| Poorly differentiated cystadenocarcinoma | 42 (15.2) | 31 (73.8) | 5 (11.9) |
| Endometrioid ovarian carcinoma | 31 (11.2) | 18 (58.1) | 1 (3.2) |
| Clear cell ovarian carcinoma | 6 (2.2) | 5 (83.3) | 0 |
| Carcinosarcoma | 6 (2.2) | 2 (33.3) | 0 |
| Low grade serous ovarian carcinoma | 4 (1.4) | 0 | 1 (25) |
| Mixed endometrioid/clear cell ovarian carcinoma | 3 (1.1) | 1 (33.3) | 0 |
| Serous tubal intraepithelial carcinoma | 1 (0.4) | 0 | 0 |
| Borderline | 1 (0.4) | 0 | 0 |
| Mucinous | 1 (0.4) | 0 | 0 |
| Unknown | 26 (9.4) | 7 (26.9) | 2 (7.7) |
| Serous | 200 (72.2) | 113 (56.5) | 16 (8) |
| Total | 277 | 150 (54.1) | 20 (7.2) |

*PV*, pathogenic variant; *VUS*, variant of uncertain significance.

57.7 years, $P = .97$ and 65.5 years vs 64.1 years; $P = .63$, respectively). There was also no significant difference in the proportion of women alive 5 years after diagnosis ($\chi^2_{1,\, n=116} = 3.8$, $P = .051$). However, there was a significant difference in MS, with women who underwent further testing scoring significantly higher (25.7 vs 20.6, $P = .0002$).

In the 101 *BRCA*-negative women who underwent further testing, the detection rate was 22 of 101 (21.8%). The PVs detected were equally split between MMR and HR genes, with *BRIP1* being the most commonly affected gene ($n = 6$; 5.9%).

In total, 52 *BRCA*-negative women were from families with at least 3 family members with EOC. Of these women, 39 (75%) had a *BRCA1*/2 PV (*BRCA1*: 30, *BRCA2*: 9) (Table 3). One woman (1.9%) had a *RAD51D* PV with no PVs in other genes identified in this subset.

The detection rate of variants in non-*BRCA* genes was highest in families with 2 affected members with EOC only (22.4%) and lowest in families with 3 or more affected members with EOC (11.1%) (Table 3). The difference between adjusted detection rates in families with 2 compared with that in families with 3 affected members with EOC was statistically significant (odds ratio = 9.9, 95% CI = 1.6-105.2, $P = .0075$). To see if this was owing to the histology of EOC predominant in each group, we compared the proportions of HGSOC in each group, however, there was no significant differences.

Overall, 153 PVs, 112 different PVs, in 12 genes, including *BRCA1*/2, were detected in 150 of 277 (54.2%) women (Supplemental Table 3). Variants of uncertain significance (VUS) were also detected in 20 women (Supplemental Table 4).

## Concurrent variants

Two women with *BRCA1*/2 PVs were also heterozygotes for a concurrent PV in another HR gene. In one woman with a *BRCA1* PV, a *PALB2* PV was also detected on a *BRCA1/BRCA2/PALB2* screen. This was requested because *BRCA1*/2 testing on the pathology sample identified the *BRCA1* c.4625_4626delCT variant, but the protocols at the time determined that complete testing of all 3 genes should still be performed on blood samples. The other woman was found to be a heterozygote for a *BRCA2* and concurrent *CHEK2* PVs, detected from the *BRCA1*/2 screen. Dosage analysis of *BRCA1* and *BRCA2* and further analysis with the

**Table 2** Detection rate by personal and family cancer history

| Personal Cancer History | Total (*n*) | BRCA1/2 (%) | Non-BRCA HR Gene PVs (%) | Non-BRCA MMR Gene PVs (%) |
|---|---|---|---|---|
| OC only | 209 | 86 (41.1) | 9 (4.3) | 6 (2.9) |
| OC + BC | 43 | 33 (76.7) | 1 (2.3) | 0 |
| OC + CRC | 5 | 2 (40) | 0 | 2 (40) |
| OC + EN | 7 | 0 | 0 | 2 (28.6) |
| OC + other | 13 | 7 (53.8) | 1 (7.7) | 0 |
| Family cancer history | | | | |
| OC only | 112 | 39 (34.8) | 5 (4.5) | 6 (5.4) |
| OC + BC only | 136 | 79 (58.1) | 6 (4.4) | 1 (0.7) |
| OC + BC + other | 11 | 5 (45.4) | 0 | 1 (9.1) |
| OC + CRC | 10 | 5 (50) | 0 | 2 (20) |
| OC + EN | 3 | 0 | 0 | 1 (33.3) |
| OC + other (non-BC/CRC/EN) | 9 | 2 (22.2) | 0 | 0 |

*BC*, breast cancer; *CRC*, colorectal cancer; *EN*, endometrial carcinoma; *HR*, homologous recombination; *MMR*, mismatch repair; *OC*, ovarian carcinoma; *PV*, pathogenic variant.

**Table 3** Detection rates grouped by family history and testing method

| Affected Relatives | | Total | *BRCA1* | *BRCA2* | *BRCA* Negative | Non-*BRCA* PV Detected/Tested Using Specified Testing Method | Adjusted Prediction of *BRCA* Negative (*n*) | Adjusted Prediction of *BRCA* Negative (%) |
|---|---|---|---|---|---|---|---|---|
| 2 OC only | | 111 | 18 (16.2%) | 11 (9.9%) | 82 | 13/58 (22.4%) | 18.4 | 16.6 |
| Testing performed | *BRCA* screen | 103 | 18 | 11 | 74 | 0 | – | – |
| | Lynch syndrome screen | 10 | 0 | 0 | 10 | 7/10 (70%) | – | – |
| | Ovarian cancer panel | 23 | 0 | 2 | 20 | 3/20 (15%) | – | – |
| | Research exome/genome sequencing | 34 | 0 | 1 | 32 | 6/32 (18.8%) | – | – |
| 2 OC + BC | | 114 | 41 (36.0%) | 19 (16.7%) | 54 | 8/36 (22.2%) | 12.0 | 10.5 |
| Testing performed | *BRCA* screen | 113 | 41 | 19 | 53 | 0 | – | – |
| | Lynch syndrome screen | 4 | 0 | 0 | 3 | 1 (33.3%) | – | – |
| | Ovarian cancer panel | 12 | 0 | 0 | 12 | 3 (25%) | – | – |
| | Research exome/genome sequencing | 8 | 0 | 0 | 6 | 1/6 (16.7%) | – | – |
| 3+ OC | | 52 | 30 (57.7%) | 9 (17.3%) | 13 | 1/9 (11.1%) | 1.4 | 0.7 |
| Testing performed | *BRCA* screen | 51 | 30 | 8 | 13 | 1 | – | – |
| | Lynch syndrome screen | 0 | NA | NA | NA | NA | – | – |
| | Ovarian cancer panel | 5 | 0 | 1 | 4 | 1/4 (25%) | – | – |
| | Research exome/genome sequencing | 5 | 0 | 0 | 5 | 0 | – | – |

*BC*, breast cancer; *NA*, not available; *OC*, ovarian cancer; *PV*, pathogenic variant.

P190-C1 (*CHEK2*) MLPA probe mix showed this copy number variant to extend from *CHEK2* exons 3 to 15. MLPA does not provide the location of the extra copy of *CHEK2* exon 3 to exon 15, and it is possible that the *CHEK2* gene was not disrupted. Neither *CHEK2* nor *PALB2* were considered causative PVs in these cases and were not counted in the *BRCA*-negative women analysis.

## PVs

Of the 62 different *BRCA1* and 30 *BRCA2* PVs detected, most were frameshift-causing variants (Supplemental Table 3). Variants that occurred in 3 or more unrelated individuals can be seen in the Supplemental Table 3. Of note was the detection of missense variant *BRIP1* c.1045G>C; p.(Ala349Pro) in 3 unrelated individuals. This variant has been described previously by our group.[16]

## Clinical outcomes

We analyzed differences between age at diagnosis, stage at diagnosis, and survival data in women with different PVs (Table 4). The proportion of women diagnosed at stage 1/2 with a *BRCA1* or MMR PV was higher than the proportion with a *BRCA2* or HR PV. Data were limited for the MMR PV group, however, the finding of an earlier age at diagnosis and an earlier stage at diagnosis was not unusual for this group.[17,18] There was no significant difference in survival at 5 years post-diagnosis between any of the groups categorized by PV.

## MS

We examined differences between MS in different groups. The mean MS in the study population was 28.9 (range: 10-71), and the mean results by variant are shown in Table 4. There was a statistically significant difference between women with and women without a PV (independent *t* test: $P < .0001$) but not between heterozygotes for HR PVs and heterozygotes for MMR PVs ($P = .0505$). The MS is designed to predict the probability of *BRCA* PVs on the basis of personal and family history of EOC and BC. These results are therefore not surprising.

To assess if the MS was helpful at predicting non-*BRCA* PV, we compared the mean MS in women with any *BRCA* PV (34.4) with all other women (24.1) (unpaired *t* test: 2-tailed $P < .0001$). The mean MS of women with any PV, including *BRCA* (33.4), was statistically significantly different from women without a detected PV (23.6) (unpaired *t* test: 2-tailed: $P < .0001$).

## Discussion

In our study, a different HR or MMR PV was detected in 1 in 5 *BRCA*-negative women with EOC. A number of international studies have been undertaken to assess detection

**Table 4** Stage, age at diagnosis, and survival outcomes

| Genes/Affected | Staging Data Available/Total Number | Stage at Diagnosis 1/2 | 3 | 4 | Alive 5 Years After Diagnosis[a] | Mean Age at Diagnosis (Median) | Mean Age at Death (Median)[b] | Mean Manchester Score |
|---|---|---|---|---|---|---|---|---|
| All | 198/277 | 49 (24.7%) | 114 (57.6%) | 35 (17.7%) | 121/216 (56%) | 55.8 (55.5) | 62.5 (62) | 28.9 |
| *BRCA1* | 74/89 | 22 (29.7%) | 39 (52.7%) | 13 (17.6%) | 38/68 (55.9%) | 53.3 (53) | 57 (58) | 36.1 |
| *BRCA2* | 32/39 | 5 (15.6%) | 22 (68.8%) | 5 (15.6%) | 19/32 (59.4%) | 63.2 (65) | 64.8 (63) | 30.6 |
| *BRCA1/BRCA2* | 106/128 | 27 (25.4%) | 61 (57.5%) | 18 (17.0%) | 57/100 (57.0%) | 54.8 (53) | 59.6 (58) | 34.4 |
| HR PV | 115/139 | 29 (25.2%) | 67 (58.3%) | 19 (16.5%) | 62/109 (56.9%) | 55.5 (54) | 60.4 (58.5) | 33.9 |
| MMR PV | 3/11 | 3/3 (100%) | NA | NA | 6/7 (85.7%) | 43.5 (47.5) | 45.5[c] | 27 |
| Any PV | 118/150 | 32 (27.1%) | 67 (56.8%) | 19 (16.1%) | 68/116 (58.6%) | 55.5 (54) | 60 (58.5) | 33.4 |
| No PV | 80/127 | 17 (21.3%) | 47 (58.8%) | 16 (20.0%) | 53/100 (53.0%) | 56.1 (56) | 65.2 (64.5) | 23.6 |

*HR*, homologous recombination; *MMR*, mismatch repair; *NA*, not available; *PV*, pathogenic variant.
[a]Data available for 241 women, 25 women were diagnosed within 5 years of analysis cutoff date and therefore were ineligible for analysis.
[b]Data available for 240 women, 148 women passed away during the analysis period.
[c]Data only available for 2 women.

rates from cancer gene panels/NGS in women with EOC. These studies predominantly assessed women with hereditary breast and ovarian cancer and included varying levels of data regarding family history. Cancer gene panels/NGS in women with familial ovarian cancer in the United Kingdom have not been previously reported. As can be seen in Supplemental Table 5, study detection rates in *BRCA*-negative ovarian cancer gene panels vary between 0% to 10% internationally. Most of these studies were small and did not report detection rates by family history or reported only in very small numbers, although, this is an easily measurable statistic monitored in both oncology and genetics clinics. We found a detection rate of 21.8% out of 101 *BRCA*-negative women with familial ovarian cancer. This is substantially higher than similar cancer gene panels in *BRCA*-negative patients with BC in whom detection rates range from 1% to 12%.[19] This shows the utility of testing in patients with familial ovarian cancer for HR and MMR genes beyond *BRCA1* and *BRCA2*.

All women in this study came from families with 2 or more relatives with EOC. We are unaware of any previous reports that have concentrated on this relatively rare situation. The PV detection rates in the *BRCA*-negative population were significantly lower in families containing 3 family members with EOC than those with only 2 members with EOC, and it seems that further testing is most important in women with EOC and only 2 affected relatives in the family. Further analysis showed that differences in histology between groups could not explain this difference. This could be because lower and moderate penetrance genes are more likely to cause EOC in women in this group. As such, *BRCA2*, which is associated with a 15% to 30% lifetime ovarian cancer risk, is found in families with 3 ovarian cancer cases, but this is clearly much less likely for the remaining genes with penetrance rates of <15%.

Our study found the highest prevalence of non-*BRCA* PV in *BRIP1* driven by the dominant negative *BRIP1* c.1045G>C missense variant.[16] Identification of this variant alone would justify inclusion of *BRIP1* on ovarian cancer gene panels because the penetrance of this variant is likely to be >10%. The degree of increase in risk for *BRIP1* for all EOCs was estimated from a large case-control study to be 11.22-fold (95% CI = 3.22-34.10). Despite this, the penetrance was estimated to be only 5.8% by age 80 years on the basis of testing in unaffected women from the UK Ovarian Cancer Screening Study. This represents only a 3- to 4-fold risk at the bottom end of 95% CIs of the case-control study.[20] This relatively low lifetime risk meant that *BRIP1* did not reach the 75% consensus for inclusion of *BRIP1* in the United Kingdom ovarian panel, although, it was added later.[8] It is clearly time to revisit this penetrance estimate given that case-control evidence for *BRIP1* is much more in keeping with risks equivalent to *RAD51C* and *RAD51D*, (5- to 6-fold relative risk),[21] and it is arguable whether testing of unaffected women (UK Ovarian Cancer Screening Study) gives a robust estimate.

At present, the detection of *BRCA1* or *BRCA2* PVs in germline or somatic testing in a woman with EOC enables the use of PARP inhibitors, which offer a survival advantage.[22-24] The ENGOT-OV26/PRIMA phase III trial described improved survival in women with HR-deficient EOC beyond *BRCA* PVs.[25] In 2017, the US Food and Drug Administration approved the use of PD-1 inhibitor pembrolizumab for the use of microsatellite-high or MMR deficient solid tumors that had progressed with first-line treatment.[26] It is clear that maximizing the use of targeted treatments based on genetic testing is an increasingly used tactic, optimizing EOC management, in particular, treatments targeting HR and MMR pathways.

This study had several limitations. The numbers were small relative to the larger national studies and also geographically limited to patients referred to the MCGM. However, family history taking and confirmation of family history is likely to have been more complete than the studies from gene testing companies. Nevertheless, this is the largest series to our knowledge to assess testing of familial EOC with at least 2 cases in the family. Women reviewed at MCGM are from high risk families, introducing an element of ascertainment bias. There is also the potential for survivor bias because women with very aggressive disease may not have time to be referred for genetic testing. To assess this aspect, we reviewed the time from EOC diagnosis to sample taken. This ranged from –232 months (19.3 years) (as a result of an alternative cancer diagnosis or cascade testing) to +565 months (47 years), with a mean of 40 months and median of 12 months. Our patients therefore seem to reflect a wide range of referral times to clinical genetics. Because these patients were referred over a 28-year time period, a range of genetic analysis techniques were used, and patients were tested for different genes over different periods. The Lynch syndrome screening did not include *PMS2*. However, because newer technologies and clinical studies became available, retrospective samples were eligible for newer testing, therefore, this bias was minimal. The clinical reports from MCGM testing also had a practice of not reporting VUS in routine reports; therefore, Supplemental Table 4 will be an underestimate of the VUS in this group. A total of 7 VUS were also reclassified as benign under current reporting guidelines.

In conclusion, in this article, we describe the largest series of women with familial ovarian cancer undergoing cancer gene panel/NGS screening to date and the only one to our knowledge in the United Kingdom. As expected, almost half of the women tested positive for a *BRCA1*/2 PV, however, 1 in 5 of the remaining women who underwent testing were found to be heterozygotes for another EOC-associated gene. This is a substantial proportion of potentially actionable genes that could affect patient care with the increasing use of targeted therapies in women with HR- or MMR-gene associated EOC as well as use of risk-reduction surgery. Our previous identification of *BRIP1* as the most prevalent gene in this higher penetrance EOC setting should overturn any question relating to its recommended inclusion in EOC panel,[8] and we recommend *BRIP1* testing for all cases of nonmucinous epithelial cancer particularly in *BRCA1/2*-negative women with HR-deficient ovarian cancers and women with a family history of EOC in which an affected relative is not available. Wider genetic testing of women with familial ovarian cancer is essential to both optimize their treatment and enable prevention of disease in family members.

## Data Availability

Data are available from the corresponding author on request.

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## Ethics Declaration

Informed consent was obtained from patients who underwent *BRCA* screening, Lynch syndrome screening, Manchester Centre for Genomic Medicine epithelial ovarian cancer panel sequencing, and exome sequencing to have their samples used in future anonymized research (FH-Risk approved by the NHS North Manchester Research Ethics Committee [08/H1006/77] and the University of Manchester Ethics Committee [08229] and a later substantial amendment to incorporate the study, “Investigation of genetic modifiers in *BRCA1/2* breast cancer and non-*BRCA1/2* high risk families” [Reference 08/H1006/77] was approved by Greater Manchester West [GM West] Research Ethics Committee). Women involved in the 100,000 Genome Project and NIHR BioResource study were consented through their clinical trial protocol.

## Conflict of Interest

The authors declare no conflicts of interest.

## Additional Information

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