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Genetic testing for epithelial ovarian cancer

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A B S T R A C T

As the treatment of epithelial ovarian cancer (OC) moves further into personalised medicine, the importance of determining the presence or absence of inherited mutations in cancer susceptibility genes has grown. It is now becoming routine to test for germline mutations in the BRCA1 and BRCA2 genes, which are responsible for a significant proportion of hereditary epithelial OC and are established predictive biomarkers of potential benefit from poly ADP ribose polymerase (PARP) inhibitors. The identification of patients with hereditary OC allows the patient to benefit from personalised treatment, while allowing family members to undergo cascade testing, where identification of unaffected carriers can allow early detection, risk-reduction or prevention for both breast and OC, and ultimately improve long-term outcomes. Other susceptibility genes, include the Lynch Syndrome (mismatch repair) genes and several other genes involved in the homologous recombination pathway (HRD genes), are implicated in OC genesis, and are also becoming of increasing interest as therapeutic options grow for these patients. This review will highlight the importance of the early detection of a germline gene pathogenic variant, which informs on the clinical course of disease in a particular patient, and therefore, guides therapeutic management including risk reducing and personalised treatment.

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Introduction

Ovarian cancer (OC) is the sixth most common cancer in women in the UK, and is the leading cause of death amongst women with gynaecological cancers. Patients often present at an advanced stage and frequently relapse after first-line treatment. Traditionally, the choice of an appropriate chemotherapy agent was based on the time since the patient last received platinum-based treatment, with patients deemed either platinum resistant or sensitive. However, the last 10 years have brought a growing evidence base for the use of BRCA mutation status as a biomarker for treatment response, prognostic outcome and suitability for targeted agents, such as PARP inhibitors. BRCA mutation testing is now becoming a routine part of optimal management of patients with epithelial OC, and the wider interest in genomic stratification has also led to the increased use of testing for the mismatch repair (MMR) genes to guide immunotherapy choice, and homologous recombination status as deficient (HRD) genes to identify other patients who would particularly benefit from PARP inhibitors. In this review, we will discuss the implications of this testing, both for the patient and the wider family.

BRCA1 and BRCA2 - background

BRCA1 and BRCA2 are the most commonly mutated cancer susceptibility genes in OC (OC) patients and are also the most clinically relevant, with a number of management implications. This is due to the resultant different biological behaviour within the cancer in those with a pathogenic variant, manifesting as different patterns of disease, earlier average age of onset, varying responses to several commonly used systemic anticancer therapies and divergent prognoses [1–3]. BRCA mutations have also been shown to be important in predicting response and susceptibility to targeted agents, particularly poly ADP ribose polymerase (PARP) inhibitors [4]. In addition to the clinical management implications, BRCA mutation testing in OC patients may also have implications for their family members, as it allows family members of those with a pathogenic variant to undergo testing themselves and identify their own future risk of cancer, and consider risk-reducing strategies to modify that risk. BRCA mutation testing, therefore, has an important role to play in the context of prevention and reduction of future overall burden of cancers associated with these pathogenic variants.

Prevalence of BRCA1 and BRCA2 pathogenic variants in OC

The majority of women with epithelial OC do not have a BRCA pathogenic variant, with inherited BRCA pathogenic variants responsible for a significant 12–14% of all OC [5,6]. BRCA1/BRCA2 pathogenic variants are more prevalent in those with strong family history of breast and/or OC, a younger age at diagnosis of OC, those with high-grade serous histology; and in certain populations such as those with Ashkenazi Jewish ancestry, where 1:40 people carry one of three specific BRCA pathogenic variants [7].

The lifetime risk of OC ranges significantly by study and population, with earlier studies, carried out in high-risk families with multiple cases of early-onset ovarian and breast cancer generally suggesting higher lifetime risks; while risk estimates from studies of populations unselected for family history suggest lower lifetime risks. More recent estimates of large cohorts suggest the lifetime risk of OC is 39%–46% for those with a BRCA1 pathogenic variant and 12%–20% for those with a BRCA2 pathogenic variant [8,9], as shown in Table 1.

Phenotype of tumours

Epithelial OC is a heterogeneous disease with differing molecular and clinical characteristics of each histological subtype (high-grade serous, low-grade serous, clear cell, endometrioid and mucinous). High-grade serous OC is associated with the highest likelihood of germline BRCA1 and BRCA2 pathogenic variants (8–18%), with particular enrichment in those diagnosed <50 years of age [5]. BRCA1 and BRCA2 pathogenic variants also occur in those with endometrioid or clear-cell histologies (5–15%) and, less commonly in those with carcinosarcoma [10–12]. Pathogenic variants are rare in those with mucinous or low-grade serous OC, reflecting the different biological drivers in these types of tumours. It should, however, be noted that many of the older studies have a higher proportion of BRCA carriers

Table 1
Risk and features associated with ovarian cancer susceptibility genes.

		Cumulative lifetime risk of Ovarian cancer	Average age at diagnosis (years)	Predominant histological subtype
BRCA genes	BRCA1	44–59%	51.3	High-grade serous
	BRCA2	11–37%	61.4	High-grade serous
Lynch genes	MLH1	10–20%	45	Non-serous
	MSH2	17–24%	43	Non-serous
	MSH6	8–13%	46	Non-serous
HRD genes	RAD51C	5–9%	40–49	High-grade serous
	RAD51D	6–12%	40–49	High-grade serous
	BRIP1	6–11%	61	High-grade serous

with non-serous OC, and this may reflect the changes in histological classification over the last 20 years, with many of the cancers previously classified as clear cell or mixed clear/cell serous now reclassified as serous.

The behaviour of tumours with a BRCA mutation also differs from those without, with several studies demonstrating differing patterns of metastatic spread. Women with OC and a germline BRCA1 or BRCA2 mutation are more likely to develop visceral metastases to the liver, the lung, the spleen or the brain than those who are BRCA wild type [3]. In one small retrospective series, visceral metastases were noted in 74% of women with a pathogenic variant in BRCA, compared to 16% of BRCA wild-type women. Of note, the study demonstrated that the presence of visceral metastases did not impact on overall outcome in the BRCA carriers, as it does in those without a pathogenic variant, exemplifying the clinical significance of the biological distinction of BRCA-deficient OC.

Average age at diagnosis/age of onset/age-specific OC risks for BRCA mutation carriers

The presence of a germline BRCA pathogenic variant is associated with earlier disease onset, of both breast and OC [13]. This is an important consideration when deciding the most appropriate age to recommend risk-reducing bilateral salpingo-oophorectomy (RRBSO). A large cohort study reported a mean age of OC diagnosis of 51.3 years in BRCA1 carriers and 61.4 years in BRCA2 carriers, with lifetime risks of OC by the age of 80 of 48.8% in BRCA1 and 21.23% in BRCA2 [14]. Kuchenbaeker et al. used the international BRCA1 and BRCA2 Cohort Consortium, which included a cohort of 5066 carriers (2905 BRCA1 and 2161 BRCA2) that were suitable for assessment of OC risk. In the median follow-up period of 4 years, there were 109 OC diagnoses (85 in BRCA1 and 24 in BRCA2), with median ages of diagnosis of 54 and 59.5 years, respectively. There were 10 OCs diagnosed between 40 and 49 years (11.7%) in BRCA1 carriers, but only one case diagnosed between 50 and 55 years in the BRCA2 carriers. The cumulative lifetime risks of OC at 80 years of age were 44% for BRCA1 carriers and 17% for BRCA2 carriers [8]. This current evidence, reflected in the international guidelines issued by ESMO, SGO and the NCCN suggest that it is reasonable for BRCA2 carriers to undergo RRBSO at 40–45 years of age, and for BRCA1 carriers to have surgery from 35 to 40 years, below which the risk is small. The long-term risks of early menopause and the completion of family are important considerations when advising on the age of RRBSO.

There is clear evidence that much of the BRCA-related OC actually originates in the distal fallopian tube, rather than on the surface of the ovary [15]. This had led to the suggestion that it may be possible to undertake a bilateral salpingectomy alone at a younger age, and leave the ovaries in-situ until menopause [16]. These would then be excised in a second procedure, removing the need for hormone replacement therapy, and preventing the premature menopausal symptoms that can dissuade some women from undergoing RRBSO. Trials are currently underway assessing the degree of cancer protection, and long-term outcome from this approach, but the results will not be available for some years. RRBSO was also initially reported to have a substantial benefit in reducing subsequent breast cancer risk, both in those without any history of breast cancer, or in contralateral breast cancer risk [17–19]. However, more recent studies have suggested that this risk may have been overstated, or may only exist for BRCA2 carriers, who predominantly develop oestrogen receptor-positive breast cancer [20,21].

The benefit in reducing breast cancer in those with a BRCA1 pathogenic variant, where the majority of the breast cancer is ER negative is less clear. This area remains to be of great interest, and further studies are awaited.

Differences in prognosis

There is a clear differentiation in the outcome, with improved short-term survival reported in BRCA mutation carriers at 5 years, although the benefit at 10 years is less clear [22,23]. It should be noted that the studies on which these studies were based pre-date the routine use of drugs, such as pegylated liposomal doxorubicin, and PARP inhibitors, for which there is clear evidence for improved response in those with a pathogenic variant in BRCA, and it is likely that further studies will show a greater differentiation. There is also variance by gene, with median survival being longer in BRCA2 mutation carriers than in BRCA1 mutation carriers followed by BRCA wild type [1,22,24–26]. The improved prognosis in BRCA carriers may be explained by greater platinum-based chemosensitivity in these individuals and the longer disease-free intervals noted in these patients [27].

Chemosensitivity – role of chemotherapy for BRCA carriers

BRCA1 and BRCA2 are key players in the process of double-strand DNA repair by homologous recombination (HR). This explains why BRCA carriers are more likely to respond to platinum-based chemotherapy upfront or as a subsequent line, as platinum works by affecting DNA cross-linkage, and thereby disrupting DNA synthesis. BRCA pathogenic variant carriers are also more likely to retain platinum sensitivity for longer when compared to BRCA wild-type patients, with many able to be re-treated with platinum-based chemotherapy on multiple occasions [5]. BRCA pathogenic variant carriers with recurrent OC also have higher overall response rates when compared to BRCA wild-type patients when treated with some non-platinum-based chemotherapy, such as pegylated liposomal doxorubicin. Several studies have demonstrated higher response rates for BRCA pathogenic variant carriers treated with this drug, with objective response rates of 56.5% versus 19.5% [2,28].

Targeted agents – PARP inhibitors including location of pathogenic variants and outcome

Most women with newly diagnosed advanced OC have a relapse within 3 years post standard management with surgery and platinum-based chemotherapy. The benefit of PARP inhibitors in OC has been established in multiple studies, and at multiple time points through treatment. The sensitivity of BRCA1 and BRCA2 mutation carriers to PARP inhibitors is explained through synthetic lethality, with tumours in those with BRCA mutations unable to use the HR pathway to repair double-stranded DNA breaks. This means damage must be repaired by the base excision repair pathway, which requires PARP for the pathway. By blocking this pathway with a PARP inhibitor, cells cannot repair DNA damage and undergo apoptosis. Further mechanisms of PARP activity have subsequently been identified, including PARP trapping [29]. The clinical relevance of PARP inhibition was first reported in a phase I clinical trial of olaparib, which showed a response rate of 47% in patients with a germline BRCA1 or BRCA2 pathogenic variant and breast, prostate or OC [4]. This was the original proof of concept publication, which sparked multiple clinical trials including the pivotal Study 19 (olaparib), NOVA (niraparib), SOLO-2 (olaparib) and ARIEL-3 (rucaparib), all of which support improved outcomes in patients with platinum-sensitive relapsed OC and a BRCA1/2 pathogenic variant with PARP inhibitors [30–34].

The phase II trial Study 19 recruited 265 patients with platinum-sensitive, recurrent, high-grade serous OC patients who had responded to their latest platinum-based regimen, and randomised them to receive olaparib or placebo until progression. Recruitment was irrespective of BRCA status, but all patients had to provide blood and/or tumour for centralised BRCA testing, and the study cohort was enriched for BRCA carriers. A significant improvement was observed in Progression Free Survival (PFS) with those treated with olaparib (8.4 months) compared with placebo (4.8 months), $P < 0.001$ [30]. A pre-planned retrospective analysis in the subjects with BRCA mutations revealed an even larger improvement in PFS with olaparib (11.2 months) when compared with placebo (4.3 months), $P < 0.0001$ [31].

Several studies then assessed the use of PARP inhibitors in those with a first platinum-sensitive relapse (second line treatment). The NOVA trial included patients with high-grade serous, platinum-sensitive OC treated with 2 prior lines of platinum-based chemotherapy, who had a complete or partial response, and randomised them to receive niraparib or placebo until disease progression. Patients were categorised according to the presence or absence of a germline BRCA mutation, and also by HR status (deficient or proficient). Those treated with Niraparib had a significantly longer PFS compared with placebo in both the germline BRCA-mutated cohort (21 months compared with 5.5 months, HR 0.27, $P < 0.001$) and non-germline BRCA-mutated cohort (9.3 months compared with 3.9 months, HR 0.45, $P < 0.001$) [32].

In the assessment of maintenance therapy in a platinum-sensitive cohort of relapsed high-grade OC patients with germline BRCA pathogenic variants, the phase III ARIEL-3 trial also included subjects with somatic BRCA pathogenic variants as well as BRCA wild-type patients. BRCA wild-type subjects were further stratified by HR status as deficient or proficient (HRP). From a total of 564 patients, 196 were recruited. The BRCA-mutated cohort included 130 germline pathogenic variant carriers and 56 somatic pathogenic variant carriers with 10 unknown germline/somatic pathogenic variant carriers. Median PFS increased from 5.4 months to 16.6 months with rucaparib (HR 0.23, $P < 0.0001$. A significant benefit was observed with rucaparib in time to first subsequent therapy (HR 0.28, $P < 0.0001$); time to second progression/death (HR 0.50, $P < 0.0002$) as well as time to second subsequent therapy/death (HR 0.37, $P < 0.0001$) [34].

The phase III SOLO-2 trial recruited 295 patients with known germline BRCA1/2 pathogenic variants treated with 2–5 prior lines of platinum-based chemotherapy. Patients were platinum-sensitive on their most recent recurrence with complete or partial response to the last line of platinum-based chemotherapy. They were randomised to receive olaparib or placebo until progression or withdrawal from trial. Following a median follow-up of 22 months, patients treated with olaparib had a significantly longer PFS of 19.2 months vs 5.5 months with placebo (HR 0.30, $P < 0.001$). At the time of reporting, 83/195 participants receiving olaparib were still on treatment compared to 13/99 on placebo [33].

After demonstrating benefit in relapsed disease, PARP inhibitors were then assessed in those with advanced disease undergoing first-line treatment. The first study showing a benefit in PFS from PARP inhibitor maintenance in the first-line treatment of BRCA-mutated OC, SOLO1 has been practice-changing. This international, randomised, double blind, phase III trial showed the benefit of olaparib as maintenance therapy in newly diagnosed advanced OC and a BRCA 1/2 pathogenic variant, with a 70% lower risk of disease progression or death with olaparib than with placebo after a median follow up of 41 months [35]. Overall survival data are awaited, together with further studies in the first line, expected to be published in the next 1–2 years.

Having demonstrated significant improvements in PFS for BRCA pathogenic variant carriers, Olaparib, niraparib and rucaparib have current European and FDA approval for routine use in OC patients with BRCA pathogenic variants. Olaparib and niraparib are available as maintenance therapy in platinum-sensitive disease in the first-line setting (olaparib), second and subsequent line setting (olaparib, niraparib and rucaparib). Rucaparib is also licenced in platinum-resistant OC patients with BRCA pathogenic variants.

Indicators of prolonged PARP response and overall survival

BRCA2 mutations have been associated with a longer duration of response to the PARP inhibitor olaparib, with many of the BRCA2 mutation carriers who have a long-term response to PARP inhibitors having mutations in the RAD51-binding domain or DNA-binding domains within the gene. This suggests that the site of the pathogenic variant itself within the gene may be significant for both the response rate and duration of response [36].

There is a known link between the increased risk of OC (over breast cancer risk) and pathogenic variants in the OC cluster region of the BRCA2 gene. The association of the location of pathogenic variants in BRCA2 with clinical outcome has been investigated in a number of studies, and supports the important role of interactions with other proteins in the HR pathway [37–39].

When considering improved outcome and OC survivorship, with respect to PARP inhibitors, the rare but serious potential side effect of myelodysplasia/acute myeloid leukaemia (AML) must be considered. ARIEL-3 reported three cases (1.1%) of myelodysplastic syndrome/AML with rucaparib (two with germline BRCA mutations and one HRP patient) with no cases reported in the placebo group [34]. However, in SOLO-2, four patients (2%) receiving olaparib and four patients (4%) receiving placebo developed a haematological malignancy [33]. The higher rate of occurrence of haematological malignancy in the placebo arm in SOLO-2 raises the possibility that this side effect is linked to an intrinsic DNA-repair defect in a cohort treated with prior platinum-based chemotherapy. With respect to this potential side effect, careful follow up and monitoring in those treated with PARP inhibitors is essential [40]. The determination of the patients who may be at highest risk, and at which time point of developing these rare potential side effects of PARP inhibitors is yet to be clearly determined, and is an area requiring further research.

Mainstreaming – increase uptake and expanded access screening

With the advent of more targeted treatments, and an expanding evidence base for their use, it is increasingly important to identify the patients who will most benefit from these treatments. The use of PARP inhibitors for those with BRCA mutations (or other forms of HRD) has contributed to the rapid expansion of BRCA testing in OC patients, as there is now a clear stratification of treatment by BRCA pathogenic variant status. Historically, the selection of women for BRCA testing was based on those with a strong personal and/or family history of breast and OC. This was designed to restrict testing at a time when testing was intensely resource-intensive, with testing taking up to a year per gene, and at a very high cost. As the technology developed, testing became faster, cheaper and more widely available, testing began to be undertaken in more women, where it became clear that >40% of OC patients with a BRCA pathogenic variant do not have a relevant family history of cancer [5,29,41,42]. Additionally, age restrictive criteria may miss those with pathogenic variants, as studies of unselected patients show up to a third of mutation carriers are diagnosed over the age of 70 [43]. The current NICE guidelines in the UK suggest any OC patient with a 10% chance of carrying a BRCA pathogenic variant should be offered testing, but these will soon be superseded by the new National test criteria, which will allow testing of any non-mucinous OC patient. This is in keeping with the wider testing recommended by many other guidelines, such as NCCN and ESMO [44–46].

With the advent of different systemic treatments for OC patients based on BRCA pathogenic variant status, there is a need for patients to be able to easily undergo rapid testing. The average waiting time for a Clinical Genetics appointment in the UK is 6 months, with some centres with wait lists over 12 months[47]. This is not conducive to the use of results for clinical management, and this led to the first combined oncogenetic model, developed within the Mainstreaming Cancer Genetics Programme, and pioneered at the Royal Marsden Hospital, London, UK, which is now the standard of care for patients with OC receiving treatment at this institution [10]. Within this model, expansion of more widespread routine testing has been implemented by offering women of any age with non-mucinous OC BRCA testing within their oncology appointment. To facilitate this, oncology clinicians undertook training in testing to become an approved clinician, able to discuss testing with patients, and take informed consent. Those with a pathogenic variant are then reviewed in the cancer genetics clinic, so the wider implications of the result for both the patient and their family can be addressed. This approach was found to be highly acceptable to both patients and clinicians, highly cost-effective, and allowed testing to be performed in a clinically meaningful timeframe [10,48]. This oncogenetic mainstreaming model has been implemented in cancer treatment centres worldwide, and is likely to become more widespread as the need for BRCA testing to inform first-line maintenance treatment becomes more important. This will require appropriate educational programmes and infrastructure to allow the wider selection of clinicians to potentially undertake testing as part of the routine care of these patients.

In addition to the mainstreaming model, several other models have been trialled. The Cambridge model looked at embedding a genetic counsellor into the gynae cancer clinics, to be able to offer patients review and testing at the same time as they attended their oncology clinic appointment. This significantly increased the uptake of testing, but requires a genetic counsellor to be sitting in clinics when there may be few or no patients suitable for consenting and testing [49]. This may not be possible

in many centres, where there is a nationwide shortage of genetic counsellors, and is not possible in centres that do not have a genetics department on site. An alternative for those without genetics on site, or with large geographical regions to cover is telephone counselling. This has been used successfully in a number of countries to provide counselling in such situations, and has also been shown to be highly acceptable to patients and a good way to improve access to testing [50,51]. It does, however, also rely on the presence of genetics clinicians to undertake the testing, and on patients 'attending' the telephone appointment.

The role of somatic BRCA testing is also of increasing interest. In some countries, this is being used as a screening tool to select patients for germline testing, with only those found to have somatic pathogenic variants referred on for further testing. This allows these patients to receive BRCA-directed treatment, such as PARP inhibitors, whilst awaiting the confirmation of whether the detected variant is of germline or somatic origin [35]. However, this approach does also have some drawbacks. It requires sufficient tissue to be available for testing, which is not often possible in those who are undergoing neoadjuvant chemotherapy, who have had only a biopsy for diagnostic purposes. Somatic testing in most laboratories also currently fails to reliably detect large duplications/deletions from Formalin fixed, paraffin embedded (FFPE) samples, which is a particular issue in countries such as the UK, where around 10–15% of pathogenic variants are of this type [52,53]. Further work is required on the best use of germline and somatic testing together for the optimal patient pathway.

Summary – BRCA testing

Stratification of OC by BRCA status for systemic treatment is now considered standard, and in addition, BRCA status provides additional clinical information including the clinical course of the disease and prognosis. The additional benefit to unaffected family members includes providing therapeutic choices such as risk reducing strategies for cancer prevention (appropriate screening, chemoprevention or risk-reducing surgery). Routine BRCA testing should therefore be implemented widely as part of routine, patient-centred, standard care in OC, a disease that often presents at an advanced stage, has high rates of recurrence and considerable mortality.

Lynch Syndrome

Lynch syndrome, previously known as hereditary non-polyposis colorectal cancer (HNPCC), is caused by an inherited alteration in one of four genes (MLH1, MSH2, MSH6 and PMS2) and is associated with an increased susceptibility to multiple cancer types, predominantly colorectal, endometrial and OC [54–56]. Lynch syndrome is the second most common cause of hereditary OC, and due to the earlier age of onset, this may be the presenting cancer in women with Lynch syndrome, or can occur as a second or subsequent cancer [57]. Lynch syndrome-associated ovarian cancer (LSAOC) has distinct pathological features and clinical behaviour compared to sporadic OC and is generally associated with improved survival. Increasingly, there is interest in finding women with these pathogenic variants to stratify treatment options and consider options, such as immunotherapy, as well as to reduce the risk of further primary cancers. The identification of these women may in future change management, but also allows other family members to undergo testing and stratify their own cancer risk. Women with a pathogenic variant then have the option of utilising risk-reducing strategies to manage this risk, and potentially reduce future cancer burden.

Universal screening for Lynch syndrome

Traditional screening for Lynch syndrome uses clinical criteria based on a patient's clinical and family history of cancer, and then undertaking molecular tests for microsatellite instability (MSI) or immunohistochemistry (IHC) to identify those who should go on to have germline testing. The Amsterdam II criteria and revised Bethesda guidelines are well established clinical criteria but may fail to detect a significant proportion of patients with Lynch syndrome, particularly those with MSH6 or PMS2 pathogenic variants [58]. While current guidelines recommend LS screening for all patients with newly diagnosed colorectal cancer, there is no such guideline for screening patients with OC. It is

possible to undertake routine screening of tumours with either IHC for the four MMR proteins, or testing for microsatellite stability, using a panel of 5–7 microsatellite markers. Both have good concordance for identifying those with an underlying gene pathogenic variant, and are considered to be appropriate options to screen tumours [59,60]. However, IHC has the additional benefit of pointing towards the gene that may have a pathogenic variant, based on the pattern of IHC abnormality. Lynch syndrome only causes about 2–3% of cases of OC, which suggests that universal screening may not be cost-efficient. However, Lynch cases are more common in those with non-serous OC; thereby testing this subgroup of patients may be more appropriate.

Prevalence

It is currently estimated that approximately 0.5–2% of OCs are caused by germline pathogenic variants in the MMR genes, a much smaller proportion than the contribution of the BRCA genes. The lifetime risk of OC in people with Lynch syndrome is around 8–14%, but this varies with the particular MMR gene that is mutated [61,62]. LSAOC is associated with pathogenic variants in the MLH1, MSH2 and MSH6 genes, but not with pathogenic variants in PMS2, where OC risk is similar to population risk [63]. Earlier studies suggested a 20% lifetime risk of OC with MLH1, 24% with MSH2 and a 1% risk for MSH6 pathogenic variant carriers, but more recent studies suggested the risk with these three genes was 10–17% [64,65]. Further details are shown in Table 1.

Phenotype

While high grade serous carcinoma is the main histological type of OC caused by BRCA pathogenic variants, those with Lynch syndrome are more likely to have endometrioid and clear cell histological subtypes. Data from Swedish and Danish Lynch Syndrome families identified 63 cases of epithelial OCs. In this series, endometrioid and clear histologies constituted 35% and 17% of tumours respectively, whereas serous tumours occurred in 28%, mucinous tumours in 5% and undifferentiated histologies were reported in 15% [63]. Ryan et al. reported a predominance of high-grade endometrioid tumours ($n = 19$) constituting 53% of cases. High-grade serous adenocarcinomas and mixed tumours comprised 17% ($n = 6$) and 11% ($n = 4$) respectively, while clear cell carcinoma ($n = 4$) constituted 11% of cases in this series [65].

Age-specific OC risks for Lynch syndrome patients

LSAOC is associated with an earlier age of onset, with a mean age at diagnosis of 48 years (range, 30–79 years), although one series reported 79% of patients diagnosed under the age of 50 [64]. This age is approximately 20 years earlier than those with sporadic OC, and is also earlier than the average age of BRCA-associated OC. The age of onset varies depending on the MMR gene mutated, with an average age of onset of 51 years in families associated with MLH1 mutations and 45 years in families associated with MSH2 mutations [56,66,67].

Implications for prognosis

LSAOC generally presents at an early stage - FIGO stage I or II at diagnosis. Members of the International Collaborative Group on HNPCC collected retrospective data on 80 OC patients between 1936 and 1997. The patients were members of HNPCC families, including 31 known mutation carriers, 35 presumptive carriers (by colorectal/endometrial cancer status), and 14 at-risk family members. The study showed that 85% of cases were FIGO stage I or II at diagnosis, which is significantly different compared to sporadic OC where approximately 30% of cases are stage I/II at diagnosis. A synchronous endometrial cancer was reported in 21.5% of cases in this series [67]. In a more recent study, most cases also presented early with 85% of cases diagnosed at FIGO stage I/II vs 15% of cases at stage III/IV and overall survival was excellent (80%, 5-year survival). Most patients in this study were found to have Lynch syndrome after their OC diagnosis, however, two patients were diagnosed at stage 1c through local surveillance programmes, and three patients were detected with occult disease following surgery

for screen-detected synchronous endometrial pathology [65]. The improved prognosis of LSAOC may be explained by an earlier stage at diagnosis; however, a multicentre, retrospective European study of survival in MMR pathogenic variant carriers showed that 10-year OC-specific survival independent of staging was 80.6%. This compared to survival rates of less than 40% reported both in population-based series and in BRCA pathogenic variant carriers. In this series, 18.5% of the cancers were diagnosed at stage III or IV. Five-year survival for stage III/IV was 59% compared to 28% in the general population, and 10-year survival was 59%, compared to 19% for BRCA pathogenic variant carriers [68,69]. These data suggest that even in advanced stage OC, survival may be better in MMR pathogenic variant carriers compared to BRCA pathogenic variant carriers or the general population.

There is increasing interest in the genomic profiling of tumours to help direct treatment choice and predict outcome, particularly in those with histological subtypes that are more chemo-resistant [69,70]. Niskakoski and colleagues investigated 107 ovarian tumours (20 from LS and 87 sporadic) and showed that none of the 20 LS-associated ovarian carcinomas had mutations in TP53, KRAS (exon2) or BRAF(V600E) [71]. TP53 somatic mutations are common in endometrioid OC, and almost universal in serous ovarian carcinomas, and are generally associated with both a worse prognosis and more advanced stages at diagnoses [72]. PIK3CA mutations occurred with a frequency of 6/20 (30%), which was comparable to sporadic tumours of endometrioid or clear cell type. Recent data suggest that PIK3CA mutations and the activation of the PI3K/AKT pathway are associated with a favourable prognosis in OC [73]. Niskakoski's study showed that on the molecular level OCs seem to resemble colorectal cancers from LS carriers, which are associated with higher stage-specific survival [74]. It is possible the significant molecular differences observed between LS-associated and sporadic ovarian carcinomas may contribute to the differences in long-term outcome.

Systemic treatment in Lynch syndrome-associated ovarian cancer

There are clear differences in chemotherapy response in those with hereditary OC due to a BRCA pathogenic variant, but these differential responses to chemotherapy have not been found in LSAOC, where there remains a paucity of data [5]. However, over the past few years, there has been progress in the treatment of Lynch syndrome-associated (and other MSI-H, MMRd) cancers. Most notably, monoclonal antibodies that target PD-1 have improved objective response rates and overall disease control rates in patients with advanced MMRd/MSI-H cancers. The first study to examine such agents in the metastatic setting evaluated the clinical activity of pembrolizumab (a monoclonal anti PD 1 antibody) in 41 patients with previously treated, progressive metastatic disease with and without MMR deficiency. In this heavily pre-treated cohort, there were markedly superior response rates and disease control rates in individuals with MMRd/MSI-H cancers compared with those whose cancers were MMR proficient/microsatellite stable. With a median follow-up time of 36 weeks, the median progression-free survival was not reached for either cohort of patients with MMRd/MSI-H cancers [75]. This study was further expanded to evaluate the efficacy of PD-1 blockade in patients with advanced MMR-deficient cancers across 12 different tumour types. Objective responses were observed in 53% of patients, and complete responses were achieved in 21% of patients [75]. Responses were durable, with median progression-free survival and overall survival still not reached. These studies led to the accelerated approval of pembrolizumab by the US Food and Drug Administration to treat advanced, pre-treated MMRd/MSI-H cancer regardless of primary site, in 2017. This is the first tumour-agnostic approval of a drug on the basis of a molecular alteration, but it is likely that others will follow in future.

Wider implications of Lynch-associated ovarian cancer

Gynaecological cancers are often the sentinel cancer in women with Lynch syndrome. In a study of women with Lynch syndrome who developed colon and gynaecological cancer, 50% of cases presented with a gynaecological cancer as their 'sentinel cancer' [76]. Several studies show that the lifetime endometrial cancer risk is approximately 40% for women with MLH1 and MSH2 pathogenic variants, with a median age of 49 years [64,77]. Patients with MSH6 pathogenic variants have a similar risk but are usually diagnosed at a later age [62]. Given the increased risk of both endometrial

and OC in women with Lynch syndrome and the limitations for screening these patients, clinical guidelines state that prophylactic gynaecological surgery should instead be considered in these patients [78]. A hysterectomy and bilateral salpingo-oophorectomy (THBSO) has been endorsed by NCCN as a risk-reducing option that should be considered by women with Lynch syndrome, who have completed their family [78,79]. The timing of THBSO should also be considered, as studies have reported that the cumulative risk to people at the age of 40 did not exceed 2% for endometrial cancer and 1% for OC, therefore prevention efforts should focus on women aged between 40 years and older [64]. For women with Lynch syndrome who need colon surgery, guidelines recommend concomitant THBSO [54].

Other genes involved in ovarian cancer

BRCA1 and BRCA 2 are fundamental components of the HR pathway. This pathway involves a number of other proteins interacting and cooperating with the BRCA1 and BRCA2 proteins in the DNA repair process to maintain genomic stability. Tumours with pathogenic variants in several other genes involved in HR-mediated DNA repair have been associated with OC predisposition [80,81]. These tumours express a BRCA-like phenotype, typified by high-grade serous histology, higher response rates to platinum agents, improved disease-free intervals and overall survival rates. Three HR genes have been associated with an increased susceptibility to OC – RAD51C, RAD51D and BRIP1, which together account for a further 2% of OC cases [82–85].

The RAD51 paralogues, RAD51C and RAD51D, have an essential role in DNA repair through the HR pathway [86]. RAD51C pathogenic variants have been identified in up to 2.9% of breast and OC families who previously screened negative for BRCA1/2 pathogenic variants, although there is no strong signal for RAD51C as a breast cancer susceptibility gene [85,87,88]. RAD51D pathogenic variants confer a six-fold increased risk of OC, but do not appear to increase the risk of breast cancer in carriers [84].

A recent case–control study analysed three RAD51 genes in germline DNA in a population of OC patients – RAD51B, RAD51C and RAD51D. The study included 3429 women with invasive epithelial OC and 2772 healthy controls as well as in 2000 unaffected women who were BRCA1/BRCA2 negative from the United Kingdom Familial Ovarian Cancer Screening Study (UK_FOCSS). The study showed that 0.81% of EOC cases in the study had a pathogenic variant in one of these three genes compared with 0.11% in controls. The most frequent pathogenic variants occurred in the RAD51C (n = 14; 0.41%) and RAD51D (n = 12; 0.35%) genes. Two patients had pathogenic variants in the RAD51B gene (0.06%). Pathogenic variants in RAD51C and RAD51D genes had an odds ratio of 5.2 and 12, respectively. For all OC, the estimated cumulative risks of OC in women at the age of 50 were 1.3% (RAD51C) and 3% (RAD51D), rising to 5.2% and 12%, respectively, by the age of 70 years [87]. RAD51B was not associated with an increased risk of OC.

BRIP1 – also known as BACH1 – encodes BRCA1-interacting protein-terminal helicase 1, a DNA helicase that influences the DNA repair ability and tumour-suppressor function of BRCA1 [89]. Germline pathogenic variants in the BRIP1 gene confer a moderate risk for OC. Ramus et al. used next generation sequencing to identify germline pathogenic variants in the coding regions of BRIP1 in 3236 invasive EOC case patients and 3431 control patients of European origin, and in 2000 unaffected high-risk women from a clinical screening trial of OC (UKFOCSS). The study reported a statistically significant difference in the prevalence of deleterious pathogenic variants in BRIP1 between cases and controls (0.92% in cases vs 0.09% in controls, $P = 1 \times 10(-4)$) and estimated a lifetime OC risk by the age of 80 years of 5.8% [83].

Although these genes are separately rare, they together make up a small proportion of patients who may benefit from different systemic treatment options. All were included in the HRD cohort of patients in the PARP inhibitor studies, NOVA and ARIEL 3, investigating the use of niraparib and rucaparib, respectively. This cohort had a clear benefit from these agents, over and above those with HRP tumours, and therefore identifying these patients could in future have significant treatment implications. As testing for hereditary genes in OC patients moves increasingly towards panel testing, it is likely that these patients will be routinely identified, allowing their family members to consider risk-reducing surgery in future.

Summary

Genetic testing in OC patients increasingly has a wide range of implications for patients and their families. There are expanding systemic treatment options that can now be stratified by genetic pathogenic variant status, as well as differences in the biological behaviour of tumours and prognosis. This has led to a rapid adoption of more widespread testing for OC patients – initially for BRCA mutations, but increasingly this is likely to also include the Lynch genes and other HRD genes, particularly as more drugs become licenced for use in these patients. To undertake this, it is likely more clinicians will move towards a mainstream, oncogenetic approach where testing can be undertaken rapidly, and assimilated into the routine management of patients. This will ultimately lead to the identification of more carriers before they develop OC, and the opportunity to undertake risk reduction strategies that will in time reduce the overall cancer burden in the population.

Practice points

- BRCA testing has a wide number of clinical implications for the management of epithelial OC patients, including alterations to chemotherapy and suitability for targeted agents.
- There are 8 genes in which inherited pathogenic variants can cause an increased risk of OC, with the high-risk genes BRCA1 and BRCA2, and moderate-risk genes including MLH1, MSH2, MSH6, BRIP1, RAD51C and RAD51D.
- Most genes cause an increased risk of other tumours; so identifying gene carriers also allows appropriate screening and risk reduction for both OC patients and their family members.

Research agenda

- The introduction of routine panel testing in OC patients.
- The cost-effectiveness of panel testing for OC genes in all OC patients.
- The impact of an MMR gene mutation in response to chemotherapy.

Declaration of Competing Interest

AG has received honoraria from Astra Zeneca, Tesaro and Roche. NA and NC have no conflicts of interest.

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