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ARTICLE

Mainstreaming germline *BRCA1/2* testing in non-mucinous epithelial ovarian cancer in the North West of England

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Abstract

Poly(ADP-ribose) polymerase (PARP) inhibitors improve survival in *BRCA*-mutant high-grade serous ovarian carcinoma. As a result, germline and somatic *BRCA1/2* testing has become standard practice in women diagnosed with ovarian cancer. We outline changes in testing and detection rates of germline *BRCA1/2* pathogenic variants (PVs) in cases of non-mucinous epithelial ovarian cancer diagnosed during three eras, spanning 12 years, within the North West of England, and compare the uptake of cascade testing in families identified by oncology-led mainstreaming versus regional genetics clinics. Eras included: Period 1 (20% risk threshold for testing): between January 2007 and May 2013; Period 2 (10% risk threshold for testing): between June 2013 and October 2017 and; Period 3 (mainstream testing): between November 2017 and November 2019. A total of 1081 women underwent germline *BRCA1/2* testing between January 2007 and November 2019 and 222 (20.5%) were found to have a PV. The monthly testing rate increased by 3.3-fold and 2.5-fold between Periods 1–2 and Periods 2–3, respectively. A similar incidence of germline *BRCA1/2* PVs were detected in Period 2 (17.2%) and Period 3 (18.5%). Uptake of cascade testing from first-degree relatives was significantly lower in those women undergoing mainstream testing compared with those tested in regional genetics clinics (31.6% versus 47.3%, $P = 0.038$). Mainstream testing allows timely detection of germline *BRCA1/2* status to select patients for PARP inhibitors, but shortfalls in the uptake of cascade testing in first-degree relatives requires optimisation to broaden benefits within families.

Introduction

Ovarian cancer is the commonest cause of gynaecological-related cancer death in Europe and North America [1]. Epithelial ovarian cancer (EOC) is histologically classified into five main subtypes, including: high-grade serous (HGS), low-grade serous, endometrioid, clear cell and mucinous [2]. High-grade serous carcinoma accounts for approximately 80% of all EOC and 70% of all ovarian tumours. These highly aggressive tumours have an insidious clinical prodrome and commonly present with advanced stage disease in which cure is unlikely [3]. For advanced stage HGS carcinoma, standard therapy includes cytoreductive surgery and platinum/taxane-based

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chemotherapy plus maintenance bevacizumab or poly (ADP-ribose) polymerase (PARP) inhibitors [4, 5]. Randomised trials have demonstrated that PARP inhibitors significantly improve survival in HGS carcinoma, with the greatest benefits in *BRCA*-mutant tumours [6–14]. It is estimated that approximately 20% of all HGS carcinomas contain a germline or somatic *BRCA1/2* pathogenic variant (PV) [15, 16].

The incorporation of PARP inhibitors in standard therapy for HGS carcinoma has significantly increased demand for germline and somatic *BRCA1/2* testing [17–23]. As a result, mainstream (oncology-led) germline *BRCA1/2* testing has been implemented in unselected populations of women diagnosed with ovarian cancer. Although mainstream testing has increased the absolute number of *BRCA1/2* variants detected, it is not known how this new model of testing impacts on cascade testing of first-degree relatives. In this study, we outline how the rate of germline *BRCA1/2* testing in cases of non-mucinous EOC, diagnosed in the North West of England, has varied across three eras of testing, spanning 12 years. Moreover, we report the uptake of pre-symptomatic testing in families in whom the germline *BRCA1/2* PV was identified through mainstreaming versus standard regional genetics testing.

Materials and methods

Patient selection

Women diagnosed with non-mucinous epithelial cancer of the ovary, fallopian tube or peritoneum who underwent germline *BRCA1* and *BRCA2* testing between 1st January 2007 and 1st November 2019 were included. Index cases with a diagnosis of carcinosarcoma were also included. Germline *BRCA1/2* testing took place primarily in the genetics clinics at St Mary's Hospital, Manchester. In the latter 24 months, mainstream testing was carried out by oncologists or surgeons in gynaecological oncology clinics in the North West England including: The Christie Hospital (Manchester), Royal Preston Hospital (Preston) and Blackpool Victoria Hospital (Blackpool). Pathogenic (ACMG Class 5) or likely pathogenic (ACMG Class 4) *BRCA1* and *BRCA2* variants are included and will be referred to collectively as *BRCA1/2* PVs throughout this paper [24]. Variants of unknown clinical significance (ACMG Class 3) were excluded. Index cases of mucinous ovarian carcinoma or non-EOC were excluded.

All women from an Ashkenazi Jewish ancestry found to have a *BRCA1/2* founder mutation were excluded because the Manchester BRCA Scoring System was not designed to assess risk in this population [25]. In the North West of England, all women from an Ashkenazi Jewish ancestry undergo founder testing for the three common germline PVs (*BRCA1*:c.68_69del, *BRCA1*:c.5266dup and *BRCA2*:c.5946del) before full gene sequencing.

A family history was defined as any index case of non-mucinous EOC with a first-degree or second-degree relative with breast, ovarian, prostate or pancreatic cancer. An index case was categorised as 'sporadic appearing' non-mucinous EOC if there was no first-degree or second-degree relative with breast, ovarian, prostate or pancreatic cancer.

All demographic data were extracted from clinical case notes and electronic patient records.

Germline BRCA1/2 testing

Germline *BRCA1/2* variants were detected by testing DNA extracted from peripheral circulating lymphocytes. Next generation sequencing was used to detect variants throughout the whole coding sequence of *BRCA1/2*, including at least 15 base pairs beyond each exon-intron junction. Single nucleotide variants and small deletions, duplications, insertions and insertion-deletions variants (<40 base pairs) were called using an inhouse bioinformatic pipeline that has been validated to detect heterozygous and mosaic variants to an allele fraction ≥0.04.

Testing for large genomic rearrangements (e.g., whole exon/gene deletions or duplications) in *BRCA1/2* was performed by multiplex ligation-dependent probe amplification (MLPA) [26]. The MLPA probe kits P002-D1 (*BRCA1* P002-D1 used from August 2015; prior to this, earlier versions of P002 probe kit were used) and P045-C1 (*BRCA2* P045-C1 used from December 2016; prior to this, earlier versions of P045 probe kits were used) (MRC Holland, Amsterdam, Netherlands) were used. Amplified ligation products were subject to fragment analysis using an ABI 3130xl Genetic Analyser and size called using GeneMapper 2.0 (Applied Biosystems®, Thermo Fisher Scientific, MA, USA). Calling of copy-number status was performed using data exported from GeneMapper using custom developed MLPA spreadsheets that report relative dosage quotient for each probe compared to five reference control samples. All MLPA assays were performed in duplicate for confirmation of results.

Pre-symptomatic testing for germline *BRCA1/2* PVs was performed using bi-directional Sanger sequencing using BigDye v3.1 chemistry and analysis was performed using Mutation Surveyor. Polymerase chain reaction primers for pre-symptomatic testing were designed to avoid hybridising to the sites of common polymorphic variants (minor allele frequency > 0.01). Pre-symptomatic testing for pathogenic copy-number variants was carried out using MLPA using the MRC Holland probe-sets outlined above. Familial PV positive controls were run alongside all pre-symptomatic tests.

Table 1 The Manchester Scoring System with pathology adjustment.

	BRCA1	*BRCA2*
Cancer, age at diagnosis		
FBC, <30	6	5
FBC, 30–39	4	4
FBC, 40–49	3	3
FBC, 50–59	2	2
FBC, >59	1	1
MBC, <60	5	8
MBC, >59	5	5
Ovarian cancer, <60	8	5
Ovarian cancer, >59	5	5
Pancreatic cancer	0	1
Prostate cancer, <60	0	2
Prostate cancer, >59	0	1
Pathology adjustment		
Breast cancer (index case only)		
Grade 3	+2	0
Grade 2	0	0
Grade 1	−2	0
ER positive	−1	0
ER negative	+1	0
Triple-negative[a]	+4	0
HER2 amplified[b]	−6	0
Ductal carcinoma in situ	−2	0
Lobular	−2	0
Ovarian cancer (any case in family[c])		
Mucinous, germ cell or borderline tumours	0	0
High-grade serous, <60	+2	0
Adopted (no known status in blood relatives)	+2	+2

Each individual and family relevant tumour (from one side of the family only) is given a numerical weight and these are summated to provide a score for each of the two genes, *BRCA1* and *BRCA2* [23]. Score 'Cancer, age at diagnosis' first and then adjust score through 'Pathology adjustment'.

FBC female breast cancer, *MBC* male breast cancer, *ER* oestrogen receptor.

[a]Also score grade in addition to triple-negative.

[b]Also score grade and ER status in addition to HER2 status.

[c]Only if the relative is not related to index case through more than one unaffected woman aged > 60 years.

Manchester BRCA Scoring System

The Manchester BRCA Scoring System is a paper-based model that can be utilised to determine the combined *BRCA1* and *BRCA2* carrier probability of an index case (Table 1) [27]. The development of the Manchester BRCA Scoring System was based on empirical data gathered from the Manchester genetic variant screening programme [28]. Each individual, from one side of the family, is scored for *BRCA1* and *BRCA2* separately. For an index case of breast cancer or ovarian cancer, or, an unaffected relative of an index case of ovarian cancer (<60 years old), the *BRCA1* and *BRCA2* scores are adjusted according to pathology. A Manchester Score of 15–19 equates to a combined *BRCA1/2* probability of 10%, and 20 points a probability of 20%.

Eras assessed

Three time periods were assessed. In 2004, NICE published familial breast cancer guidelines indicating that testing should use a 20% likelihood threshold for implementing germline testing for *BRCA1*/2 variants. This changed to a 10% threshold in May 2013. In November 2017, after training of oncologists in obtaining informed consent for germline *BRCA1/2* testing, all non-mucinous EOC cases could be offered testing for germline *BRCA1/2* PVs in oncology clinics, although clinicians were encouraged to refer any index case with a family history to their regional genetics centre. We therefore used an initial time period of 77 months from January 2007 to May 2013 when a 20% threshold applied (Period 1). The second period was a 53-month period from June 2013 to October 2017 when a 10% threshold pertained (Period 2). The final 24 months from November 2017 to November 2019 was the mainstreaming period (Period 3).

Uptake of pre-symptomatic testing was assessed by Kaplan–Meier statistics. Pre-symptomatic testing involved a targeted search for a germline *BRCA1/2* PV that had been found in an index case with non-mucinous EOC within the family.

Results

One thousand and eighty-one women of non-Ashkenazi Jewish ancestry underwent germline *BRCA1/2* testing following a diagnosis of non-mucinous EOC. In total, 222 women (20.5%) had a germline *BRCA1/2* PV (*BRCA1*: 131, *BRCA2*: 91).

The mean age at diagnosis of EOC was lower in *BRCA1* (52.1 years, range: 33–75) than *BRCA2* heterozygotes (59.5 years, range: 33–86) and germline wild type (58.15 years, range: 20–87). This was similar during Period 3 (mainstreaming): *BRCA1*: 53.1 years (range: 33–67), *BRCA2*: 60.5 years (range: 47–82) and germline wild type: 60.5 years (range: 23–85).

The monthly rate of germline *BRCA1/2* sample testing rose 3.3-fold from Period 1 to Period 2 and 8.4-fold from Period 1 to Period 3 (Table 2). Despite lowering of (Period 2) and subsequent removal of the testing thresholds (Period 3) there was an equivalent incidence of germline *BRCA1/2*

Table 2 Monthly sampling and detection rates of germline *BRCA1/2* PVs over three eras in the North West of England.

Era	No. tested	g*BRCA1/2* PV	Monthly sampling rate	Monthly detection rate
January 2007 to May 2013 (20% threshold) Period 1	183	61 (33.3)	2.38	0.79
June 2013 to October 2017 (10% threshold) Period 2	418	72 (17.2)	7.89	1.36
November 2017 to November 2019 (mainstreaming) Period 3	480	89 (18.5)	20	3.71
Total	1081	222 (20.5)	–	–

Data presented as number or number (percentage).

gBRCA1/2 PVs germline BRCA1/2 pathogenic variants.

Table 3 Germline *BRCA1/2* PVs detected in non-mucinous EOC index cases from January 2007 to November 2019 in the North West of England categorised according to Manchester Score.

Manchester Score (pathology adjusted)	No. tested	g*BRCA1/2* PVs
Single ovary only, non-mucinous, ≥60 years old	168	10 (6.0)
Single ovary only, non-mucinous, <60 years old	294	35 (11.9)
Manchester Score <11	186	10 (5.4)
Manchester Score 11–14	126	11 (8.7)
Manchester Score 15–19	451	64 (14.2)
Manchester Score 20–24	131	44 (33.6)
Manchester Score 25–39	160	71 (44.4)
Manchester Score ≥40	27	22 (81.5)
Total	1081	222 (20.5)

Data presented as number or number (percentage).

gBRCA1/2 PVs germline BRCA1/2 pathogenic variants.

PVs detected in Period 2 (17.2%) and Period 3 (18.5%) (Table 2). However, the monthly rate of germline *BRCA1/2* detection rose 2.7-fold from Period 2 to Period 3 (Table 2).

The pathology-adjusted Manchester Score performed well, with scores ≤ 14 points associated with *BRCA1/2* likelihoods < 10% (Table 3). Index cases of non-mucinous EOC with no relevant personal or family history had detection rates of 11.3% if <60 years old and 5.95% if ≥60 years old. Of the 20 *BRCA2* heterozygotes diagnosed at ≥60 years old, 6 (30%) had no relevant personal or family history. In the mainstreaming era, most *BRCA1/2* PVs were detected in women diagnosed with HGS carcinoma, although women with this histological diagnosis were more likely to be tested (Table 4).

During the mainstream testing period, 50 *BRCA1/2* PVs were detected through mainstreaming and 39 from direct referral to clinical genetics, including 18 patients diagnosed with non-mucinous EOC prior to mainstreaming testing. Of

Table 4 Germline *BRCA1/2* PVs detected in non-mucinous EOC index cases from November 2017 to November 2019 in the North West of England categorised according to histology.

Histology	No. tested	g*BRCA1/2* PVs
High-grade serous	427	86 (20.1)
Low-grade serous	8	0
Endometrioid	21	2 (9.5)
Clear cell	14	0
Carcinosarcoma	11	1 (9.1)
Total	481	89 (18.5)

Data presented as number or number (percentage).

gBRCA1/2 PVs germline BRCA1/2 pathogenic variants.

the remaining 71/89 *BRCA1/2* PVs detected, results were obtained between 6 weeks and 3 years following the date of the ovarian cancer diagnosis (mean: 9 months, median: 6.5 months). Of the 50 mainstream *BRCA*-positive cases, 6 were from out-of-region and were referred to their own regional genetics service. Three patients died before an appointment with clinical genetics was possible and two were already known to genomic medicine with a *BRCA1/2* PV, but apparently not to the oncology service and underwent repeat testing. Referrals were received for 35/39 of the remaining *BRCA*-positive cases within 6 weeks of the test result being reported. Three referrals were received 3–5 months after the report was issued. In five *BRCA*-positive cases, reminder letters to oncologists were sent to chase referrals. Patients referred were offered appointments within 10 weeks, but several patients delayed appointments whilst undergoing cancer treatment. Two patients declined appointments, but in one of these families another member had been referred to clinical genetics.

From the 44 families eligible to be seen by the regional genetics service, 39 have been seen and 98 first-degree relatives were eligible for pre-symptomatic testing (Table 5). To date, 31 (31.6%) have undergone pre-symptomatic testing compared to 44/93 (47.3%) from non-mainstreamed families from the same time period (Table 5). Kaplan–Meier uptake curves of pre-symptomatic testing for unaffected first-degree relatives are shown in Fig. 1. There was a significantly higher uptake in those seen through a standard genetics referral compared to those seen through a mainstreaming approach ($P = 0.038$).

Discussion

Until recently the model for germline DNA testing for cancer predisposition genes was genetics led with clinical geneticists acting as the 'gatekeepers' to access testing of genes such as *BRCA1* and *BRCA2*. With the arrival of PARP inhibitors for the treatment of ovarian and breast cancer, which require

knowledge of germline *BRCA1/2* status prior to prescribing, diagnostic pathways have shifted to curtail specialist genetic counselling for more focused informed consent by oncologists/surgeons [17–22]. The main advantage of this approach is that there is no waiting time for an appointment with clinical genetics. The priority of family screening has therefore been superseded by the drive to establish eligibility for PARP inhibitors in index cases. Now that PARP inhibitors are being used as part of primary therapy in HGS carcinoma, there is an even greater demand for oncology-led germline and somatic *BRCA1/2* testing [6–8].

We have evaluated the effect of mainstreaming (oncology led) testing in non-mucinous EOC index cases compared with two previous eras of testing. Testing rates increased approximately eightfold compared to when a 20% threshold pertained (Period 1) and approximately threefold from the 10% era (Period 2). Despite the lack of a threshold during mainstreaming, germline *BRCA1/2* PV detection rates remained approximately 18%, only slightly higher than when utilising a 10% threshold during Period 2. However, during mainstream testing the germline *BRCA1/2* PV detection rates increased from 0.79 to 3.71 per month, demonstrating the utility of oncology-led testing.

The pathology-adjusted Manchester Score performed as expected with a score of 15 points indicating the 10% threshold and 20 points the 20% threshold. Although likelihood scores are no longer relevant to women diagnosed with ovarian cancer, these scores are still relevant to unaffected relatives where no living relative is available, e.g., a score of 20 points in an affected deceased mother of unknown germline *BRCA1/2* status would make her unaffected children eligible for germline testing at the 10% threshold.

The detection rate of germline *BRCA*1/2 variants in our study was 20.5%; comparable to 22.6% found by Alsop et al. in a similar sized Australian study of 1001 women diagnosed with non-mucinous ovarian cancer [29], but higher than 11.1% described by Song et al. in 2222 cases of EOC [30]. The lower rates of germline *BRCA1/2* PVs detected in non-serous tumours likely reflect the differences in biology of the unique histological and molecular subtypes of EOC. Although the numbers of cases of non-serous tumours tested within our cohort was small (<50 cases), the detection rates of <10% germline *BRCA1/2* PVs reaffirm the necessity to stratify *BRCA1/2* testing by histology.

Whilst the huge upward increment in testing is highly encouraging, our data unlikely represent full uptake. The mean age at testing of around 60 years suggests that older patients are less likely to be tested. This may be because of

Table 5 Pre-symptomatic tests in first-degree relatives of germline *BRCA1/2* pathogenic variant heterozygotes since November 2017 in the North West of England.

Index cases since November 2017	No. of families	Predictive tests	Total unaffected FDR
Standard genetics referral	39	44	93 (47.3)
Mainstreaming	39	31	98 (31.6)

Data presented as number or number (percentage). There was a significantly higher uptake in those with standard genetics referral compared to those with a mainstreaming approach ($P = 0.038$).

FDR first-degree relative.

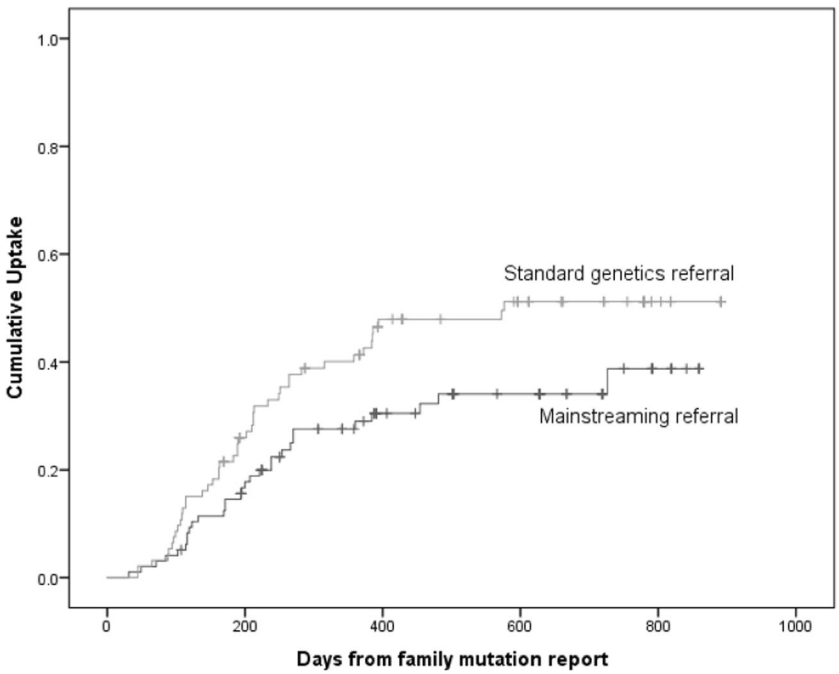

Fig. 1 Kaplan–Meier curve. Figure 1 shows the difference in cumulative uptake of pre-symptomatic germline *BRCA1/2* testing between standard genetics referral (green line) compared to mainstreaming referral (blue line) ($P = 0.038$).

the initial confusion over whether testing was limited in sporadic appearing index cases aged >60 years old who fell below the 10% detection rate. In *BRCA2*-positive cases the mean age of onset was similar to germline wild-type cases across all time periods, although the detection rate dropped from approximately 11% in sporadic appearing cases <60 years old to around 6% in those aged ≥60 years. Indeed, we have recently noted that in certain populations, such as women diagnosed with ovarian cancer >60 years old, the frequency of *BRCA2* PVs is higher than previously thought; a phenomenon uncovered through population-based testing as opposed to risk-based testing [31].

The rate of uptake of testing in first-degree relatives was significantly less in those from mainstream referrals compared with standard genetics referral. This likely reflects differences in counselling offered, sense of urgency and prioritisation by individuals referred. There is a paucity of literature focused on relatives' motivations for accepting genetic testing since mainstream testing begun, although uptake in index cases has been noted to be heavily motivated by the desire to protect and inform family members [32]. As mainstream testing continues, this area will require further research.

There was some delay in index cases accessing genetics services during mainstream testing mainly due to women cancelling appointments. This led to an initial slower uptake of pre-symptomatic testing from the date of the index case's original *BRCA1/2* PV report being issued. However, in our analysis we have not considered that mainstreamed women are likely to have undergone germline testing earlier in their treatment pathway, meaning the difference identified may be non-significant.

Conclusions

Mainstreaming has led to an increase in the absolute number of germline *BRCA1*/2 PVs detected in cases of non-mucinous EOC, demonstrating the clinical utility of this approach to identify patients that may benefit from PARP inhibitors. However, long-term consequences of the impact of germline *BRCA*-positive results within families require further evaluation.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The germline *BRCA1/2* database is approved by North Manchester Research Ethics Committee (08/H1006/77). All women included in this study provided informed verbal and/or written consent to undergo germline *BRCA1/2* testing.

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