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Complete List of Authors:	Morgan, Robert; Christie NHS Foundation Trust, Department of Medical Oncology; University of Manchester , Division of Cancer Sciences, Faculty of Biology, Medicine and Health Burghel, George; Manchester University NHS Foundation Trust, Manchester Centre for Genomic Medicine Flaum, Nicola; Christie NHS Foundation Trust, Department of Medical Oncology; University of Manchester , Division of Cancer Sciences, Faculty of Biology, Medicine and Health Bulman, Michael; Manchester University NHS Foundation Trust, Manchester Centre for Genomic Medicine Clamp, Andrew; Christie NHS Foundation Trust, Department of Medical Oncology Hasan, Jurjees; Christie NHS Foundation Trust, Department of Medical Oncology Mitchell, Claire; Christie NHS Foundation Trust, Department of Medical Oncology Schlecht, Helene; Central Manchester University Hospitals NHS Foundation Trust, North West Genomic Laboratory Hub woodward, emma; Manchester University NHS Foundation Trust, Department of Clinical Genetics Lallo, Fiona; Manchester University NHS Foundation Trust, Department of Clinical Genetics Crosbie, Emma; Manchester University NHS Foundation Trust, Department of Gynaecological Oncology; University of Manchester , Division of Cancer Sciences, Faculty of Biology, Medicine and Health Edmondson, Richard; Manchester University NHS Foundation Trust, Department of Gynaecological Oncology; University of Manchester , Division of Cancer Sciences, Faculty of Biology, Medicine and Health Wallace, Andrew ; Manchester University NHS Foundation Trust, Manchester Centre for Genomic Medicine Jayson, Gordon; Christie NHS Foundation Trust, Department of Medical Oncology; University of Manchester , Division of Cancer Sciences, Faculty of Biology, Medicine and Health Evans, D Gareth; Manchester University NHS Foundation Trust, Department of Clinical Genetics; University of Manchester , Division of Evolution and Genomic Sciences, Faculty of Biology, Medicine and Health
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Prevalence of germline pathogenic *BRCA1/2* variants in sequential epithelial ovarian cancer cases

Robert D. Morgan[1,2], George J. Burghel[3], Nicola Flaum[1,2], Michael Bulman[3], Andrew R. Clamp[1], Jurjees Hasan[1], Claire Mitchell[1], Helen Schlecht[3], Emma R. Woodward[3], Fiona Lalloo[3], Emma J. Crosbie[2,4], Richard J. Edmondson[2,4], Andrew J. Wallace[3], Gordon C. Jayson[1,2], D. Gareth R. Evans[3,5]*

Affiliations:

1. Department of Medical Oncology, The Christie NHS Foundation Trust, Manchester, United Kingdom
2. Division of Cancer Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, United Kingdom
3. Manchester Centre for Genomic Medicine, St Mary's Hospital, Manchester University NHS Foundation Trust, Manchester, United Kingdom
4. Department of Obstetrics and Gynaecology, St Mary's Hospital, Manchester University NHS Foundation Trust, Manchester, United Kingdom
5. Division of Evolution and Genomic Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, United Kingdom

***Corresponding author:**

Professor D. Gareth R. Evans, MD, FRCP

Division of Evolution and Genomic Sciences,

Faculty of Biology, Medicine and Health,

University of Manchester,

Manchester, United Kingdom

Email: Gareth.Evans@mft.nhs.uk

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ABSTRACT

Introduction: Poly(ADP-ribose) polymerase inhibitors significantly improve progression-free survival in platinum-sensitive high-grade serous and endometrioid ovarian carcinoma, with greatest benefits observed in women with a pathogenic *BRCA1/2* variant. Consequently, the demand for germline *BRCA1/2* testing in ovarian cancer has increased substantially, leading to screening of unselected populations of patients. We aimed to determine the prevalence of pathogenic germline *BRCA1/2* variants in women diagnosed with epithelial ovarian cancer, categorised according to the established risk factors for hereditary breast and ovarian cancer syndrome and the Manchester BRCA Score, in order to inform risk stratification.

Methods: A cohort of sequential epithelial ovarian cancer cases recruited between June 2013 and September 2018 underwent germline *BRCA1/2* testing by next-generation sequencing and multiplex ligation-dependent probe amplification.

Results: Five hundred and fifty-seven patients were screened. Of these, 18% had inherited a pathogenic *BRCA1/2* variant. The prevalence of pathogenic *BRCA1/2* variants was >10% in women diagnosed with ovarian cancer earlier than 60 years old (21%) and those diagnosed later than 60 years old with a family history of breast and/or ovarian cancer (17%) or a past medical history of breast cancer (34%). The prevalence of pathogenic *BRCA1/2* variants was also >10% in women with a Manchester BRCA Score of ≥15 points (14%).

Discussion: Our study suggests that age at diagnosis, family history of breast and/or ovarian cancer, past medical history of breast cancer or a Manchester BRCA Score of ≥15 points are associated with a >10% prevalence of germline pathogenic *BRCA1/2* variants in epithelial ovarian cancer.

INTRODUCTION

Ovarian cancer is the eighth most common cancer occurring in women and the second commonest cause of gynaecological-related cancer death worldwide [1]. Standard of care treatments include cytoreductive surgery and platinum- and taxane-based chemotherapy [2, 3]. Molecularly targeted agents offer the promise of anti-cancer treatments that specifically target biological vulnerabilities within tumour cells, thereby offering alternative therapies to traditional cytotoxic agents. To date, pathogenic *BRCA1/2* variants are the only predictive biomarkers validated in ovarian cancer [4]. Several phase 2/3 trials have shown that poly(ADP-ribose) polymerase (PARP) inhibitors significantly improve progression-free survival (PFS) in platinum-sensitive high-grade serous and endometrioid ovarian cancer, with the greatest benefit achieved in women with a pathogenic *BRCA1/2* variant [5-10]. Indeed, a recently reported randomised, double-blinded, placebo-controlled, phase 3 trial, SOLO1, demonstrated that 24 months of olaparib maintenance therapy following a partial/complete response to cytoreductive surgery and platinum-based chemotherapy in FIGO stage 3/4 *BRCA*-mutant high-grade serous or endometrioid ovarian carcinoma reduced the risk of disease progression or death at 3 years with a hazard ratio 0.28 (95% confidence interval 0.20-0.39, P<0.001) [9].

The prevalence of germline pathogenic *BRCA1/2* variants in ovarian cancer is estimated at between 10 and 15%, with the majority of heterozygotes diagnosed with high-grade serous ovarian carcinoma [11-15]. High-grade serous carcinoma is the commonest histological subtype, accounting for approximately 70% of all cases of ovarian cancer [16, 17]. At present, access to PARP inhibitors as maintenance therapy in Europe and North America is restricted by morphological subtype (serous or endometrioid), *BRCA1/2* status (germline or somatic) and/or platinum sensitivity (complete or partial response to the latest platinum-based

therapy). It is not surprising therefore that clinical demand for *BRCA1/2* testing has increased significantly as oncologists and patients seek to access these drugs [18-23]. As a result, germline *BRCA1/2* testing is increasingly prevalent in unselected populations of women with ovarian cancer, resembling routine tumour testing for somatic mutations in other tumour types e.g. *BRAF* (melanoma), *RAS* (colorectal cancer), *EGFR* (lung cancer) and *PDGFRA* and *KIT* (gastrointestinal tumours). Unlike routine tumour testing for somatic variants, testing for germline *BRCA1/2* variants could be stratified according to risk factors associated with hereditary breast and ovarian cancer syndrome [24].

In this study, we report the prevalence of germline pathogenic *BRCA1/2* variants in a large cohort of women diagnosed with epithelial ovarian cancer in the North West of England, correlating the prevalence of germline pathogenic *BRCA1/2* variants with risk factors associated with hereditary breast and ovarian cancer syndrome. Our aim is to inform risk stratification for germline *BRCA1/2* testing in epithelial ovarian cancer when conducted in an oncology clinic rather than a specialised genetics department.

METHODS

Patient selection

Women diagnosed with epithelial cancer of the ovary, fallopian tube or peritoneum (FIGO stage 1 to 4 [25]) who underwent germline *BRCA1* and *BRCA2* testing between 1st June 2013 and 1st September 2018 were included. Germline *BRCA1/2* testing took place in the oncology clinics at the Christie NHS Foundation Trust, Manchester or the genetics clinics at St Mary's Hospital, Manchester. Only women treated for ovarian cancer at The Christie Hospital or St Mary's Hospital were included in the study. Pathogenic (class 5) or likely pathogenic (class

4) *BRCA1* and *BRCA2* variants were included and will be referred to collectively as "pathogenic *BRCA1/2* variants" throughout this manuscript, whilst variants of unknown clinical significance (class 3) were excluded [26]. Cases of non-epithelial ovarian cancer were excluded. Women from a Jewish ancestry were excluded because across the North West of England this group undergo founder mutation testing first, and the Manchester BRCA Scoring System is not designed to assess risk in this population.

A family history was defined as any index case of epithelial ovarian cancer and a first-degree or second-degree relative with breast and/or ovarian cancer. An index case was diagnosed with sporadic ovarian cancer if she had no first-degree or second-degree relative with breast and/or ovarian cancer. All demographic data were extracted from case notes and/or electronic patient records.

Survival bias

In order to account for survival bias we performed a subgroup analysis according to the year the index case was diagnosed with ovarian cancer (pre versus post 2012). This strategy was adopted because the prevalence of pathogenic *BRCA1/2* variants detected in women diagnosed with ovarian cancer before 2012 may have been biased by long-term survivors [27, 28]. In women diagnosed with ovarian cancer before 2012, the minimum time from the diagnosis of ovarian cancer to subsequent germline *BRCA1/2* testing was 18 months (January 2012 to June 2013); an interval that approximates to half the median overall survival for ovarian cancer [17].

Germline *BRCA1/2* testing

Germline *BRCA1* and *BRCA2* variants were detected by testing DNA extracted from peripheral circulating lymphocytes. Next generation sequencing (NGS) was used to detect

variants throughout the whole coding sequence of *BRCA1* and *BRCA2*, including at least 15 base pairs beyond each exon-intron junction. Enrichment occurred using a custom designed long range PCR based approach followed by a normalisation step using SequalPrep normalisation plates and library preparation using the Illumina Nextera DNA Library Preparation Kit. NGS analysis was on an Illumina MiSeq using v2 2×150 base pair sequencing chemistry. Single nucleotide variants and small deletions, duplications, insertions and insertion/deletions (<40 base pairs) were called using a bioinformatic pipeline validated to detect heterozygous and mosaic variants in NGS data to an allele fraction of ≥4%. The bioinformatic pipeline was developed for use across a broad range of inherited cancer syndromes, some of which have a high frequency of somatic mosaicism e.g. neurofibromatosis type 2. An allele fraction cut off of ≥4% for variant detection was determined following clinical validation, as this was the lowest allele fraction limit of detection where both sensitivity and specificity remained high.

Testing for large genomic rearrangements/copy number variation (e.g. whole exon or whole gene deletions/duplications) in *BRCA1* and *BRCA2* was performed by multiplex ligation-dependent probe amplification (MLPA) [29]. The MLPA MRC Holland probe kits P002-D1 (BRCA1) and P045-C1 (BRCA2) were used to analyse germline DNA. Amplified ligation products were subject to fragment analysis using an ABI 3130xl Genetic Analyser and size called using GeneMapper v2.0 (Applied Biosystems). Copy number status calling 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 analysis assays were performed in duplicate for confirmation of results.

Manchester BRCA Scoring System

The Manchester Scoring System is a simple-to-use, paper-based model that can be used to determine the combined *BRCA1* and *BRCA2* carrier probability of an index case with a relevant cancer (Table 1) [30]. The development of the Manchester Scoring System was based on empirical data gathered from the Manchester mutation-screening programme [31]. Each individual, from one side of the family, is scored for each gene separately, *BRCA1* and *BRCA2* (Table 1). For index cases of breast cancer or any index case or unaffected relative of an index case of ovarian cancer (<60 years) the *BRCA1* and *BRCA2* scores are adjusted according to pathology [30]. The pathology adjustment takes into account the higher prevalence of germline pathogenic *BRCA1/2* variants in triple-negative breast cancer and high-grade serous ovarian carcinoma [32]. A Manchester Score of 15-19 points equates to a combined *BRCA1* and *BRCA2* probability of 10%, and 20 points to a 20% probability [30].

RESULTS

Five hundred and fifty-seven women of non-Jewish ancestry underwent germline *BRCA1* and *BRCA2* testing following a diagnosis of epithelial ovarian cancer (Table 2). A total of 103 women (18%) had a pathogenic *BRCA1/2* variant (68 *BRCA1*, 35 *BRCA2*) (Table 2). The mean age at which ovarian cancer was diagnosed differed in patients with pathogenic *BRCA1* (51.9 years [range 36-76]) and *BRCA2* (59.4 years [range 33-86]) variants. The types of pathogenic *BRCA1/2* variants detected are reported in Table 3. Twenty-three *BRCA1/2* variants of unknown clinical significance (class 3) were detected.

Pathogenic *BRCA1/2* variants were most commonly detected in women diagnosed with high-grade serous ovarian cancer, although women diagnosed with this histological subtype were most frequently screened (Table 2). All women diagnosed with germline *BRCA*-mutant

endometrioid ovarian cancer had poorly differentiated (high-grade) tumours. No *BRCA1/2* heterozygotes had low-grade serous, low-grade endometrioid, undifferentiated or mucinous ovarian cancer (Table 2) [16]. One woman diagnosed with FIGO stage 3C carcinosarcoma of the ovary had inherited a germline *BRCA1* variant, although the epithelial histological component of her invasive tumour was high-grade serous. Eighty-four women (15%) had been diagnosed with breast cancer and 268 (48%) had a first-degree or second-degree relative with breast and/or ovarian cancer (Table 2).

The prevalence of pathogenic *BRCA1/2* variants was >10% in women diagnosed with ovarian cancer under the age of 60 years (21%) (Table 4). Also, the prevalence of pathogenic *BRCA1/2* variants was >10% in women diagnosed at 60 years or older with a family history of breast and/or ovarian cancer (17%) or a past medical history of breast cancer (34%) (Table 4). In women diagnosed with sporadic ovarian cancer at 60 years or older the prevalence of pathogenic *BRCA1/2* variants almost reached 10% (7/76) (Table 4). However, in women diagnosed with sporadic ovarian cancer at 60 years or older without a past medical history of breast cancer, the prevalence of pathogenic *BRCA1/2* variants fell below 5% (2/46).

Survival bias may have affected the prevalence of pathogenic *BRCA1/2* variants detected in the subgroup of women diagnosed with ovarian cancer at 60 years or older with a family history of breast and/or ovarian cancer, prior 2012, although the difference was not statistically significant (24% versus 15%; Fisher's exact test P=0.21) (Table 5).

The prevalence of pathogenic *BRCA1/2* variants was >10% (101/463) in all women with a Manchester BRCA Score of ≥15 points, and there was a stepwise increase in prevalence as the Manchester Score increased (Table 6). In contrast, in women with a Manchester Score of <15 points the prevalence of pathogenic *BRCA1/2* variants was substantially <10% (2/94) (Table 6).

Risk stratification by age alone confirmed women diagnosed with epithelial ovarian cancer under the age of 30 years were unlikely to have a germline pathogenic *BRCA1/2* variant (Table 7).

DISCUSSION

By testing germline DNA in women diagnosed with epithelial ovarian cancer across North West England we found the overall prevalence of pathogenic *BRCA1/2* variants exceeded 10% (103/557) (Table 2). Furthermore, by separating groups according to established risk factors for hereditary breast and/or ovarian cancer syndrome we found the prevalence of pathogenic *BRCA1/2* variants was consistently >10% in those women diagnosed with ovarian cancer under the age of 60 years and in those diagnosed over 60 years old with either a family history of breast and/or ovarian cancer or a past medical history of breast cancer (Table 2).

A number of studies have also assessed the prevalence of germline pathogenic *BRCA1/2* variants in ovarian cancer. In an East of England series (GTEOC study), the prevalence of germline pathogenic *BRCA1/2* variants amongst all high-grade serous and endometrioid ovarian cancer cases was 8% (18/232) and increased to 12% (17/146) in women diagnosed <70 years, but fell to 1% (1/86) in those aged ≥70 years [19]. Similarly, in a Scottish series the prevalence of pathogenic *BRCA1/2* variants amongst unselected non-mucinous epithelial ovarian cancer was 13.1% (31/236), but fell to 8.2% (13/159) in women diagnosed >70 years old [20]. In an unselected series from Europe (AGO-TR-1 trial), the prevalence of pathogenic *BRCA1/2* variants in epithelial ovarian cancer was 20.8% (109/523) and fell to 10.6% in women diagnosed ≥60 years old, but increased to 31.9% (71/109) in women with a family history of breast or ovarian cancer [33]. Moreover, in a large Australian study, the prevalence

of pathogenic *BRCA1/2* variants in non-mucinous ovarian cancer was 14.1% (141/1,001), but fell to 8.3% (38/457) in women diagnosed ≥61 years old, 11.2% (103/738) in women without a personal history of breast and 8.3% (62/749) in women without a family history of breast and/or ovarian cancer [11]. The data from these series and our study therefore suggests that, three clinical features could be used to risk stratify for testing for germline *BRCA1/2* variants in women diagnosed with ovarian cancer, including age at diagnosis, family history of breast and/or ovarian cancer and past medical history of breast cancer. This is important if criteria for selecting which patients to tests are used by funding bodies.

In our study, across the North West of England, selection criteria for germline *BRCA1/2* testing was mostly based upon an individual's pathology adjusted Manchester Score of ≥15 points, with 17% (94/557) falling below the 15-point threshold [30]. This scoring system provides an alternative method for determining whether an individual's combined *BRCA1* and *BRCA2* carrier probability is ≥10% (Table 1). In our series, a Manchester Score of ≥15 points was associated with a >10% prevalence of pathogenic *BRCA1/2* variants, whereas a Manchester Score of <15 points was associated with a prevalence substantially <10%. Furthermore, one of the *BRCA2* heterozygotes with a Manchester Score <15 had a strong family history of prostate cancer with two first-degree relatives diagnosed at <60 years old, giving Manchester Score of 14 (ovarian cancer<60 [5+5], 2 x prostate cancer<60 [+2, +2]). Overall therefore, the Manchester Score provides a better trade off of sensitivity and specificity than simply excluding women with sporadic ovarian cancer diagnosed after the age of 60 years old.

Although this study is unlikely to unduly influence the debate regarding universal germline *BRCA1/2* testing in unselected populations of women diagnosed with ovarian cancer versus those at higher-risk of inheriting a variant, we consider a number of potential problems with

unselected screening beyond the obvious financial burden. Firstly, pathogenic *BRCA1/2* variants occur much less frequently in non-high-grade non-serous ovarian carcinoma [11, 19, 20]. Indeed, somatic mutations in other genes are more commonly found in non-high-grade non-serous epithelial subtypes, including *PIK3CA, PTEN, KRAS*, *BRAF*, *ERBB2* and *ARID1A* [34-38]. Moreover, at present PARP inhibitors are only licensed in high-grade serous and endometrioid subtypes. Therefore, there does not seem to be a biological rationale or therapeutic incentive for unselected germline *BRCA1/2* testing in non-high-grade non-serous/endometrioid subtypes.

Secondly, if unselected germline *BRCA1/2* testing becomes the prerogative of oncologists, the additional clinical expertise provided by geneticists may be lost [39-42]. No *BRCA1/2* test is 100% accurate for all variants, and therefore accepting a diagnosis of *BRCA1/2* wild-type or variant of unknown clinical significance in an index case with a strong family history of cancer may be naive. Many NGS-based assays in use will identify variants in the coding regions of *BRCA1/2* +/- 5-10 base pairs either side of the intron-exon junction, but these assays would not detect rarer pathogenic variants such as deep intronic variants or those located in 5'-untranslated regions [43-45]. Furthermore, initially reported variants of unknown significance can be reclassified following further investigations such as segregation analysis, RNA sequencing or additional data from case-control analyses [39]. This level of genetic scrutiny only occurs in specialist genetics departments. There is therefore some concern that women diagnosed with epithelial ovarian cancer whom have a strong family history of cancer, may evade further necessary diagnostic investigations that would be performed by geneticists, if they are labelled as *BRCA1/2* wild-type or variant of unknown clinical significance by oncologists alone.

Finally, by only screening for germline *BRCA1/2* variants there is a risk of missing other moderate-to-low penetrance actionable cancer-predisposition genes, such as *RAD51C/D, BRIP-1, MLH1, MSH2/6* and *PMS2* [24]. The prevalence of each individual cancer-predisposition gene is too low in ovarian cancer to warrant screening in an unselected population [46-48], however there is a risk that by focusing testing solely on *BRCA1* and *BRCA2*, other cancer-predisposition genes will remain undetected. In the North West of England, if a woman diagnosed with *BRCA1/2* wild-type ovarian cancer has a Manchester Score of ≥20 points, she is offered extended panel testing for alternative germline variants. We would therefore recommend that any patient diagnosed with ovarian cancer and a family history of cancer should be referred to the local genetic department irrespective of their *BRCA1/2* status.

There are some limitations with the study. Our study was biased by including mostly women with high-grade serous ovarian cancer and established risk factors for hereditary breast and/or ovarian cancer syndrome. Although we are confident that our series represents an almost comprehensive investigation of patients with high-grade serous ovarian cancer diagnosed under the age of 60 years, we acknowledge that a comparably smaller number of women diagnosed with ovarian cancer later than 60 years old were tested, especially those without risk factors for hereditary breast and ovarian syndrome. Consequently, the overall prevalence of germline pathogenic *BRCA1/2* variants reported in our study should be interpreted in the context of a selected population of women diagnosed with epithelial ovarian cancer.

In conclusion, the findings from our study suggest that if a 10% pre-test probability threshold is required prior to germline *BRCA1/2* testing in ovarian cancer then using age at diagnosis, a family history of breast and/or ovarian cancer, a past medical history of breast cancer or a Manchester Score of ≥15 should provide appropriate risk prediction.

Cancer, age at diagnosis	*BRCA1*	*BRCA2*
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*	+4	0
HER2 amplified†	-6	0
Ductal carcinoma *in situ*	-2	0
Lobular	-2	0
Ovarian cancer (any case in family‡)		
Mucinous, germ cell or borderline tumours	0	0
High-grade serous, <60	+2	0
Adopted (no known status in blood relatives)	+2	+2

Table 1. The Manchester Scoring System with pathology adjustment. Each individual and family characteristic (from one side of the family only) is given a numerical weight and these are added to give a score for each of the two genes, *BRCA1* and *BRCA2* [30]. Score "Cancer, age at diagnosis" first and then adjust score based on "Pathology adjustment". Key: * Also score grade in addition to triple-negative; † Also score grade and ER status in addition to HER2 status; ‡ Only if the relative is not related to index case through more than one unaffected woman aged >60 years; FBC, female breast cancer; MBC, male breast cancer; ER, oestrogen receptor. As an example, a 34 year-old woman diagnosed with ER- HER2 amplified grade 3 invasive ductal carcinoma and a first-degree relative with high-grade endometrioid ovarian cancer diagnosed at 63 years old would score 4+4+2+1-6+5+5=15 points.

Demographics	Tested (n=557)	Combined B*RCA1/2* (n=103)
Histology		
Adenocarcinoma, NOS	13	5 (38)
Carcinosarcoma	6	1 (17)
Clear cell	18	2 (11)
Endometrioid	29	5* (17)
Low-grade serous	10	0
High-grade serous	475	90 (19)
Mucinous	4	0
Undifferentiated	2	0
FH_x of BC/OC	268	68 (25)
PMH_x of BC	84	28 (33)

Table 2. Demographic data. Data are reported as number (percentage; the denominator is column 2 "Tested"). Key: BC, breast cancer; FH_x, family history; NOS, not otherwise specified; OC, ovarian cancer; PMH_x, past medical history; *All *BRCA1/2* heterozygotes had poorly differentiated (high-grade) endometrioid ovarian cancer.

***BRCA1/2* variant type**	**Number (%)**
SNV	22 (21)
Insertion	1 (1)
Deletion	55 (53)
Duplication	11 (11)
Indel	1 (1)
Mosaic	1 (1)
LGR	12 (12)
- Deletion	5
- Duplication	7

Table 3. Germline *BRCA1/2* variant types. Key: SNV, single nucleotide variant; indel, insertion/deletion; LGR, large genomic rearrangement.

Risk factors	Tested	*BRCA1*	*BRCA2*	Combined *BRCA1/2*
<60 y/o	352	56	18	74 (21)
<60 y/o sporadic OC	213	19	9	28 (13)
≥60 y/o + FH_x BC/OC	129	9	13	22 (17)
≥60 y/o + PMH_x BC	59	10	10	20 (34)
≥60 y/o + sporadic OC	76*	3	4	7‡ (9)
≥60 y/o + sporadic OC + no PMH_x BC	46	0	2†	2 (4)

Table 4. Risk factors for pathogenic germline *BRCA1/2* variants in epithelial ovarian cancer cohort. Data are reported as number (percentage; the denominator is column 2 "Tested"). Key: BC, breast cancer; FH_x, family history; OC, ovarian cancer; PMH_x, past medical history; y/o, years old; *30/76 (39%) had a PMH_x of breast cancer; † One patient had a Manchester Score of 14 and one patient had a Manchester Score of 10; ‡ 5/7 (71%) had a PMH_x of breast cancer.

Date of screening	Tested	*BRCA1*	*BRCA2*	Combined *BRCA1/2*
Pre-January 2012	*145*			*29 (20)*
<60 y/o	96	16	4	20 (21)
<60 y/o + sporadic OC	47	4	1	5 (11)
≥60 y/o + FH_x BC/OC	33	3	5	8 (24)
≥60 y/o + PMH_x BC	15	2	2	4 (27)
≥60 y/o + sporadic OC	16*	0	1†	1 (6)
≥60 y/o + sporadic OC + no PMH_x BC	8	0	1†	1 (13)
Post-January 2012	*442*			*74 (18)*
<60 y/o	256	40	14	54 (21)
<60 y/o + sporadic OC	166	15	8	23 (14)
≥60 y/o + FH_x BC/OC	96	6	8	14 (15)
≥60 y/o + PMH_x BC	44	8	8	16 (36)
≥60 y/o + sporadic OC	60‡	3	3	6 (10)ǂ
≥60 y/o + sporadic OC + no PMH_x BC	38	0	1^	1 (3)

Table 5. Evaluation of survival bias according to date of ovarian cancer diagnosis. Data are reported as number (percentage; the denominator is column 2 "Tested"). Key: BC, breast cancer; FH_x, family history; OC, ovarian cancer; PMH_x, past medical history; y/o, years old; * 8/16 (50%) had a PMH_x of BC; † the same patient (Manchester Score 14); ‡ 22/60 (37%) had a PMH_x of BC; ǂ 5/6 (83%) had a PMH_x of BC; ǂ 1/6 (17%) had a Manchester Score <15 and 5/6 (83%) had a Manchester Score ≥15; ^ Patient had a Manchester Score of 10. All germline *BRCA1/2* testing took place after 1st June 2013. There was no evidence of survival bias between cohorts (pre- and post-January 2012).

Manchester Score	Tested	*BRCA1*	*BRCA2*	Combined *BRCA1/2*
<15	94	0	2	2 (2)
15-19	298	27	16	43 (14)
20-29	133	25	11	36 (27)
30-40	20	7	5	12 (60)
40+	12	9	1	10 (83)

Table 6. Manchester BRCA Score. The Manchester Score is reported in points. Data are reported as number (percentage; the denominator is column 2 "Tested").

Age	Tested	*BRCA1*	*BRCA2*	Combined *BRCA1/2*
<30	6	0	0	0 (0)
30-39	20	4	1	5 (25)
40-49	113	22	5	27 (24)
50-59	213	30	12	42 (20)
60-69	140*	9	12	21 (15)
≥70	65†	3	5	8 (12)

Table 7. Prevalence of pathogenic germline *BRCA1/2* variants according to age at diagnosis. Data is reported as number (percentage; the denominator is column 2 "Tested"). Age is reported in years. Key: *84/140 (60%) had a family history of breast or ovarian cancer and 37/140 (26%) had a past medical history of breast cancer; † 45/65 (69%) had a family history of breast or ovarian cancer and 22/65 (34%) had a past medical history of breast cancer.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424.

2. National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology: ovarian cancer version 2 [available at https://www.nccn.org/professionals/physician_gls/pdf/ovarian.pdf2018]

3. ESMO Guidelines Working Group. Newly diagnosed and relapsed epithelial ovarian carcinoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2013;24 Suppl 6:vi24-32.

4. Morgan RD, Clamp AR, Evans DGR, Edmondson RJ, Jayson GC. PARP inhibitors in platinum-sensitive high-grade serous ovarian cancer. Cancer Chemother Pharmacol. 2018;81(4):647-58.

5. ARIEL3 investigators. Rucaparib maintenance treatment for recurrent ovarian carcinoma after response to platinum therapy: a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet. 2017;390(10106):1949-61.

6. ENGOT-OV16/NOVA investigators. Niraparib Maintenance Therapy in Platinum-Sensitive,Recurrent Ovarian Cancer. N Engl J Med. 2017;375(22):2154-2164.

7. SOLO2/ENGOT-Ov21 investigators. Olaparib tablets as maintenance therapy in patients with platinum-sensitive, relapsed ovarian cancer and a BRCA1/2 mutation: a double-blind, randomised, placebo-controlled, phase 3 trial. Lancet Oncol. 2017;18(9):1274-84.

8. Ledermann JA, Harter P, Gourley C, Friedlander M, Vergote I, Rustin G, Scott CL, Meier W, Shapira-Frommer R, Safra T, Matei D, Fielding A, Spencer S, Doughtery B, Orr M, Hodgson D, Barrett JC, Matulonis U. Olaparib maintenance therapy in patients with

platinum-sensitive relapsed serous ovarian cancer: a preplanned retrospective analysis of outcomes by BRCA status in a randomised phase 2 trial. Lancet Oncol 2014;15(8):852-61

9. Moore K, Colombo N, Scambia G, Kim BG, Oaknin A, Friedlander M, Lisyanskaya A, Floquet A, Leary A, Sonke GS, Gourley C, Banerjee S, Oza A, González-Martín A, Aghajanian C, Bradley W, Mathews C, Liu J, Lowe ES, Bloomfield R, DiSilvestro P.. Maintenance Olaparib in Patients with Newly Diagnosed Advanced Ovarian Cancer. N Engl J Med. 2018.

10. Swisher EM, Lin KK, Oza AM, Scott CL, Giordano H, Sun J, Konecny GE, Coleman RL, Tinker AV, O'Malley DM, Kristeleit RS, Ma L, Bell-McGuinn KM, Brenton JD, Cragun JM, Oaknin A, Ray-Coquard I, Harrell MI, Mann E, Kaufmann SH, Floquet A, Leary A, Harding TC, Goble S, Maloney L, Isaacson J, Allen AR, Rolfe L, Yelensky R, Raponi M, McNeish IA. Rucaparib in relapsed, platinum-sensitive high-grade ovarian carcinoma: an international, multicentre, open-label, phase 2 trial. Lancet Oncology. 2017;18(1):75-87.

11. Alsop K, Fereday S, Meldrum C, deFazio A, Emmanuel C, George J, Dobrovic A, Birrer MJ, Webb PM, Stewart C, Friedlander M, Fox S, Bowtell D, Mitchell G. BRCA mutation frequency and patterns of treatment response in BRCA mutation-positive women with ovarian cancer: a report from the Australian Ovarian Cancer Study Group. J Clin Oncol. 2012;30(21):2654-63.

12. Norquist BM, Harrell MI, Brady MF, Walsh T, Lee MK, Gulsuner S, Bernards SS, Casadei S, Yi Q, Burger RA, Chan JK, Davidson SA, Mannel RS, DiSilvestro PA, Lankes HA, Ramirez NC, King MC, Swisher EM, Birrer MJ. Inherited Mutations in Women With Ovarian Carcinoma. JAMA Oncol. 2016;2(4):482-90.

13. Zhang S, Royer R, Li S, McLaughlin JR, Rosen B, Risch HA, Fan I, Bradley L, Shaw PA, Narod SA. Frequencies of BRCA1 and BRCA2 mutations among 1,342 unselected patients with invasive ovarian cancer. Gynecol Oncol. 2011;121(2):353-7.

14. Walsh T, Casadei S, Lee MK, Pennil CC, Nord AS, Thornton AM, Roeb W, Agnew KJ, Stray SM, Wickramanayake A, Norquist B, Pennington KP, Garcia RL, King MC, Swisher EM. Mutations in 12 genes for inherited ovarian, fallopian tube, and peritoneal carcinoma identified by massively parallel sequencing. Proc Natl Acad Sci U S A. 2011;108(44):18032-7.

15. Pennington KP, Walsh T, Harrell MI, Lee MK, Pennil CC, Rendi MH, Thornton A, Norquist BM, Casadei S, Nord AS, Agnew KJ, Pritchard CC, Scroggins S, Garcia RL, King MC, Swisher EM. Germline and somatic mutations in homologous recombination genes predict platinum response and survival in ovarian, fallopian tube, and peritoneal carcinomas. Clin Cancer Res. 2014;20(3):764-75.

16. Kurman RJ, Herrington CS, Carcangiu ML. WHO Classification of Tumours of Female Reproductive Organs (IARC WHO Classification of Tumours - Fourth Edition) 2014.

17. Jayson GC, Kohn EC, Kitchener HC, Ledermann JA. Ovarian cancer. Lancet. 2014;384(9951):1376-88.

18. Rahman B, Lanceley A, Kristeleit RS, Ledermann JA, Lockley M, McCormack M, Mould T, Side L. Mainstreamed genetic testing for women with ovarian cancer: first-year experience. J Med Genet. 2018.

19. Plaskocinska I, Shipman H, Drummond J, Thompson E, Buchanan V, Newcombe B, Hodgkin C, Barter E, Ridley P, Ng R, Miller S, Dann A, Licence V, Webb H, Tan LT, Daly M, Ayers S, Rufford B, Earl H, Parkinson C, Duncan T, Jimenez-Linan M, Sagoo GS, Abbs S, Hulbert-Williams N, Pharoah P, Crawford R, Brenton JD, Tischkowitz M. New paradigms for BRCA1/BRCA2 testing in women with ovarian cancer: results of the Genetic Testing in Epithelial Ovarian Cancer (GTEOC) study. J Med Genet. 2016;53(10):655-61.

20. Rust K, Spiliopoulou P, Tang CY, Bell C, Stirling D, Phang T, Davidson R, Mackean M, Nussey F, Glasspool RM, Reed NS, Sadozye A, Porteous M, McGoldrick T, Ferguson M,

Miedzybrodzka Z, McNeish IA, Gourley C. Routine germline BRCA1 and BRCA2 testing in patients with ovarian carcinoma: analysis of the Scottish real-life experience. BJOG. 2018;125(11):1451-1458.

21. George A, Riddell D, Seal S, Talukdar S, Mahamdallie S, Ruark E, Cloke V, Slade I, Kemp Z, Gore M, Strydom A, Banerjee S, Hanson H, Rahman N. Implementing rapid, robust, cost-effective, patient-centred, routine genetic testing in ovarian cancer patients. Sci Rep. 2016;6:29506.

22. Colombo N, Huang G, Scambia G, Chalas E, Pignata S, Fiorica J, Van Le L, Ghamande S, González-Santiago S, Bover I, Graña Suárez B, Green A, Huot-Marchand P, Bourhis Y, Karve S, Blakeley C. Evaluation of a Streamlined Oncologist-Led BRCA Mutation Testing and Counseling Model for Patients With Ovarian Cancer. J Clin Oncol. 2018;36(13):1300-7.

23. Kentwell M, Dow E, Antill Y, Wrede CD, McNally O, Higgs E, Hamilton A, Ananda S, Lindeman GJ, Scott CL. Mainstreaming cancer genetics: A model integrating germline BRCA testing into routine ovarian cancer clinics. Gynecol Oncol. 2017;145(1):130-6.

24. Rahman N. Realizing the promise of cancer predisposition genes. Nature. 2014;505(7483):302-8.

25. FIGO Committee on Gynecologic Oncology. FIGO's staging classification for cancer of the ovary, fallopian tube, and peritoneum. J Gynecol Oncol. 2015;26(2):87-9.

26. ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405-24.

27. EMBRACE; kConFab Investigators; Cancer Genome Atlas Research Network. Association between BRCA1 and BRCA2 mutations and survival in women with invasive epithelial ovarian cancer. JAMA. 2012;307(4):382-90.

28. McLaughlin JR, Rosen B, Moody J, Pal T, Fan I, Shaw PA, Risch HA, Sellers TA, Sun P, Narod SA. Long-term ovarian cancer survival associated with mutation in BRCA1 or BRCA2. J Natl Cancer Inst. 2013;105(2):141-8.

29. Schouten JP, McElgunn CJ, Waaijer R, Zwijnenburg D, Diepvens F, Pals G. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. Nucleic Acids Res. 2002;30(12):e57.

30. Evans DG, Harkness EF, Plaskocinska I, Wallace AJ, Clancy T, Woodward ER, Howell TA, Tischkowitz M, Lalloo F. Pathology update to the Manchester Scoring System based on testing in over 4000 families. J Med Genet. 2017;54(10):674-81.

31. Evans DG, Eccles DM, Rahman N, Young K, Bulman M, Amir E, Shenton A, Howell A, Lalloo F. A new scoring system for the chances of identifying a BRCA1/2 mutation outperforms existing models including BRCAPRO. J Med Genet. 2004;41(6):474-80.

32. Couch FJ, Hart SN, Sharma P, Toland AE, Wang X, Miron P, Olson JE, Godwin AK, Pankratz VS, Olswold C, Slettedahl S, Hallberg E, Guidugli L, Davila JI, Beckmann MW, Janni W, Rack B, Ekici AB, Slamon DJ, Konstantopoulou I, Fostira F, Vratimos A, Fountzilas G, Pelttari LM, Tapper WJ, Durcan L, Cross SS, Pilarski R, Shapiro CL, Klemp J, Yao S, Garber J, Cox A, Brauch H, Ambrosone C, Nevanlinna H, Yannoukakos D, Slager SL, Vachon CM, Eccles DM, Fasching PA. Inherited mutations in 17 breast cancer susceptibility genes among a large triple-negative breast cancer cohort unselected for family history of breast cancer. J Clin Oncol. 2015;33(4):304-11.

33. Harter P, Hauke J, Heitz F, Reuss A, Kommoss S, Marmé F, Heimbach A, Prieske K, Richters L, Burges A, Neidhardt G, de Gregorio N, El-Balat A, Hilpert F, Meier W, Kimmig

R, Kast K, Sehouli J, Baumann K, Jackisch C, Park-Simon TW, Hanker L, Kröber S, Pfisterer J, Gevensleben H, Schnelzer A, Dietrich D, Neunhöffer T, Krockenberger M, Brucker SY, Nürnberg P, Thiele H, Altmüller J, Lamla J, Elser G, du Bois A, Hahnen E, Schmutzler R. Prevalence of deleterious germline variants in risk genes including BRCA1/2 in consecutive ovarian cancer patients (AGO-TR-1). PLoS One. 2017;12(10):e0186043.

34. Kuo KT, Mao TL, Jones S, Veras E, Ayhan A, Wang TL, Glas R, Slamon D, Velculescu VE, Kurman RJ, Shih IeM. Frequent activating mutations of PIK3CA in ovarian clear cell carcinoma. Am J Pathol. 2009;174(5):1597-601.

35. McAlpine JN, Wiegand KC, Vang R, Ronnett BM, Adamiak A, Kobel M, Kalloger SE, Swenerton KD, Huntsman DG, Gilks CB, Miller DM. HER2 overexpression and amplification is present in a subset of ovarian mucinous carcinomas and can be targeted with trastuzumab therapy. BMC Cancer. 2009;9:433.

36. Australian Ovarian Cancer Study Group. Mutational landscape of mucinous ovarian carcinoma and its neoplastic precursors. Genome Med. 2015;7(1):87.

37. Jones S, Wang TL, Shih IeM, Mao TL, Nakayama K, Roden R, Glas R, Slamon D, Diaz LA Jr, Vogelstein B, Kinzler KW, Velculescu VE, Papadopoulos N. Frequent mutations of chromatin remodeling gene ARID1A in ovarian clear cell carcinoma. Science. 2010;330(6001):228-31.

38. Ovarian Cancer Association Consortium. Association between endometriosis and risk of histological subtypes of ovarian cancer: a pooled analysis of case-control studies. Lancet Oncol. 2012;13(4):385-94.

39. Byers H, Wallis Y, van Veen EM, Lalloo F, Reay K, Smith P, Wallace AJ, Bowers N, Newman WG, Evans DG. Sensitivity of BRCA1/2 testing in high-risk breast/ovarian/male breast cancer families: little contribution of comprehensive RNA/NGS panel testing. Eur J Hum Genet. 2016;24(11):1591-7.

40. Wappenschmidt B, Becker AA, Hauke J, Weber U, Engert S, Köhler J, Kast K, Arnold N, Rhiem K, Hahnen E, Meindl A, Schmutzler RK. Analysis of 30 putative BRCA1 splicing mutations in hereditary breast and ovarian cancer families identifies exonic splice site mutations that escape in silico prediction. PLoS One. 2012;7(12):e50800.

41. Montalban G, Bonache S, Moles-Fernández A, Gisbert-Beamud A, Tenés A, Bach V, Carrasco E, López-Fernández A, Stjepanovic N, Balmaña J, Diez O, Gutiérrez-Enríquez S. Screening of BRCA1/2 deep intronic regions by targeted gene sequencing identifies the first germline BRCA1 variant causing pseudoexon activation in a patient with breast/ovarian cancer. J Med Genet. 2018.

42. ENIGMA consortium. Assessment of the functional impact of germline BRCA1/2 variants located in non-coding regions in families with breast and/or ovarian cancer predisposition. Breast Cancer Res Treat. 2018;168(2):311-25.

43. Evans DGR, van Veen EM, Byers HJ, Wallace AJ, Ellingford JM, Beaman G, Santoyo-Lopez J, Aitman TJ, Eccles DM, Lalloo FI, Smith MJ, Newman W. A Dominantly Inherited 5' UTR Variant Causing Methylation-Associated Silencing of BRCA1 as a Cause of Breast and Ovarian Cancer. Am J Hum Genet. 2018;103(2):213-20.

44. Anczukow O, Buisson M, Leone M, Coutanson C, Lasset C, Calender A, Sinilnikova OM, Mazoyer S. BRCA2 deep intronic mutation causing activation of a cryptic exon: opening toward a new preventive therapeutic strategy. Clin Cancer Res. 2012;18(18):4903-9.

45. kConFab Investigators. Splicing and multifactorial analysis of intronic BRCA1 and BRCA2 sequence variants identifies clinically significant splicing aberrations up to 12 nucleotides from the intron/exon boundary. Hum Mutat. 2011;32(6):678-87.

46. Ovarian Cancer Association Consortium. Germline Mutations in the BRIP1, BARD1, PALB2, and NBN Genes in Women With Ovarian Cancer. J Natl Cancer Inst. 2015;107(11).

47. Song H, Dicks E, Ramus SJ, Tyrer JP, Intermaggio MP, Hayward J, Edlund CK, Conti D, Harrington P, Fraser L, Philpott S, Anderson C, Rosenthal A, Gentry-Maharaj A, Bowtell DD, Alsop K, Cicek MS, Cunningham JM, Fridley BL, Alsop J, Jimenez-Linan M, Høgdall E, Høgdall CK, Jensen A, Kjaer SK, Lubiński J, Huzarski T, Jakubowska A, Gronwald J, Poblete S, Lele S, Sucheston-Campbell L, Moysich KB, Odunsi K, Goode EL, Menon U, Jacobs IJ, Gayther SA, Pharoah PD. Contribution of Germline Mutations in the RAD51B, RAD51C, and RAD51D Genes to Ovarian Cancer in the Population. J Clin Oncol. 2015;33(26):2901-7.

48. Norquist BM, Brady MF, Harrell MI, Walsh T, Lee MK, Gulsuner S, Bernards SS, Casadei S, Burger RA, Tewari KS, Backes F, Mannel RS, Glaser G, Bailey C, Rubin S, Soper J, Lankes HA, Ramirez NC, King MC, Birrer MJ, Swisher EM. Mutations in Homologous Recombination Genes and Outcomes in Ovarian Carcinoma Patients in GOG 218: An NRG Oncology/Gynecologic Oncology Group Study. Clin Cancer Res. 2018;24(4):777-83.