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One in 10 ovarian cancer patients carry germ line *BRCA1* or *BRCA2* mutations: results of a prospective study in Southern Sweden

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Abstract

At least 10% of all ovarian cancers are estimated to have a hereditary background. Hereditary breast–ovarian cancer (HBOC) due to mutations in the BRCA genes is a major cause of hereditary ovarian cancer, although its frequency and relationship to age and family history in unselected series of ovarian cancers is not completely known. We report here the results of a full mutational screening analysis for germ line *BRCA1* and *BRCA2* mutations in 161 patients with invasive epithelial ovarian carcinomas. Age at diagnosis ranged from 22 to 82 years (mean 59 years). Deleterious (frame-shift, nonsense and missense) mutations were detected in 13/161 (8%) of the patients and affected *BRCA1* in 12 cases and *BRCA2* in one case. Four additional missense variants (one in *BRCA1* and three in *BRCA2*) with a possible association with an increased risk ovarian cancer were revealed, resulting in a total frequency of BRCA gene alterations of 17/161 (11%). The 13 patients with deleterious mutations had a mean age of 57 years (range 41–76 years) and only three of these patients were below 50 years of age. A family history of at least one breast cancer and/or ovarian cancer was reported in all but 1 of the patients with BRCA mutations compared with only 24% of patients without mutations. Our findings in this prospective study confirm approximately 1 in 10 patients with ovarian cancer carry a germ line BRCA gene mutation associated with HBOC, and also indicate that a large number of these patients are over 50 years of age at diagnosis.

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1. Introduction

Ovarian cancer represents the seventh most common cancer in the world [1,2] with a high incidence being found in Scandinavia, where ovarian cancer affects approximately 2/100 females at a median age of 64 years, with 80% of the patients being above 50 years of age at diagnosis [3,4]. A family history of ovarian cancer is a strong and consistent risk factor for the disease and, consequently, first-degree relatives of patients with ovarian cancer have a 2- to 4-fold increased risk of the disease [5,6]. Familial ovarian cancer is associated with tumour development about 10 years earlier than its sporadic counterpart and may occur in different contexts; site-specific ovarian cancer for which no genetic defect has yet been identified, hereditary breast–ovarian cancer (HBOC), and as part of hereditary non-polyposis colorectal cancer (HNPCC, Lynch syndrome) [7–10]. Among families in which disease-causing mutations have been detected, HBOC and HNPCC have been estimated to account for 85–90% and 10–15% of cases, respectively [9,11]. Among the HBOC patients, *BRCA1* accounts for 70–80% of the mutations and is estimated to confer a 40–60% life-time risk for ovarian cancer at a mean age of 50–55 years, whereas *BRCA2* accounts for 10–20% of the mutations identified and yields a 10–20% risk of ovarian cancer at a mean age 55–65

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years [7,12–15]. The BRCA proteins are multifunctional with important control functions in homologous recombination, DNA double-strand break repair and early cellular response to DNA damage. BRCA1 also has a transcriptional activator or repressor function and possesses a central role in chromatin remodelling and centrosome regulation. Hence, BRCA1 and BRCA2 function as tumour suppressors and, presumably, cause tumour development through a caretaker defect with an increased genetic instability due to alterations in chromosome structure [16].

As an extension of a previous retrospective pilot study of 37 invasive epithelial ovarian carcinomas, which indicated a high frequency (16%) of germ line BRCA gene mutations [17] we have screened the *BRCA1* and *BRCA2* genes for germ line mutations in a prospective series of ovarian cancer patients. Recognition of patients with HBOC among ovarian cancer patients is important in order to suggest adequate treatment and follow-up for the patients and to offer genetic counselling followed by predictive testing and possible risk-reducing prophylactic surgery to relatives at risk.

2. Patients and methods

2.1. Patients

The aim of our study was to survey all new ovarian cancer patients diagnosed within the Southern Swedish health care region (currently approximately 1.5 million inhabitants) during a 2-year time period, June 1998 to June 2000. The study was approved by the Lund University medical ethics committee. All patients gave their written informed consent to participate in the study. The population-based Swedish cancer registry, which is estimated to include 98% of all cases diagnosed, contained 325 epithelial ovarian malignancies, excluding borderline tumours, during this period. Most patients in our study ($n=299$) were referred to the Department of Gynecologic Oncology, Lund University Hospital, for clinical–pathological review and treatment recommendations, while 26 patients were lost to inclusion ($n=21$) or had non-ovarian cancer ($n=5$). 25 patients were not included in the study because they were too ill to give informed consent or died before inclusion. Another 28 cases were subsequently excluded because of non-epithelial ovarian cancer, peritoneal carcinomas of unknown origin or borderline tumours. Accordingly, 246 patients with invasive epithelial ovarian cancer, stages I–IV, were eligible for our study. 71 patients rejected participation and 14 were excluded because of difficulties in communicating the implications of genetic analysis. Hence, a total of 161 (65%) of 246 eligible patients were included in the study. All 161 patients gave their written informed consent to participate and provided a blood sample for BRCA gene mutation screening. Clinicopathological data are summarised in Table 1. The histopathological diagnosis was based on the original histopathological records and the re-evaluation by a gynaecological pathologist at our centre. The dominating histological types among the participating cases were serous adenocarcinomas (65%), endometroid carcinomas (14%) and mucinous carcinomas (10%), followed by clear cell carcinomas and serous surface papillary carcinomas (SSPC). Tumour stage was I in 25%, II in 10%, III in 52% and IV in 12% of the cases. Family history was obtained via a written questionnaire from 156 participating patients and was classified as any breast and/or ovarian cancer, in a second degree relative as one or two first-degree relatives with breast and/or ovarian cancer, or as HBOC (at least 3 cases of breast and/or ovarian cancer in the family with one individual <50 years at diagnosis).

2.2. Mutation screening

Genomic DNA was extracted from the peripheral blood using the Wizard genomic DNA purification kit (Promega, Madison, WI, USA). Polymerase chain reactions (PCR) were performed using AmpliTaq Gold

Table 1
Summary of clinical data

	All participants *n* (%)	Patients with BRCA mutations *n* (%)
Age (years)	($n=161$)	($n=13$)
Mean (range)	59 (22–82)	57 (41–76)
<50	29 (18)	3 (23)
51–70	97 (60)	8 (62)
>70	35 (22)	2 (15)
Tumour stage		
I	40 (25)	1 (8)
II	16 (10)	3 (23)
III	83 (52)	7 (54)
IV	19 (12)	2 (15)
Unspecified	3 (2)	–
Histology[a]		
Serous	105 (65)	8 (62)
Endometrioid	23 (14)	3 (23)
Mucinous	16 (10)	–
Clear-cell	8 (5)	1 (8)
Papillary, SSPC	7 (4)	1 (8)
Unspecified	2 (1)	–
Family history of breast or ovarian cancer		
None	109 (68)	0 (0)
Any second-degree relatives	22 (14)	2 (15)
1 or 2 first-degree relatives	21 (13)	5 (38)
HBOC	4 (2)	4 (31)
Unknown	5 (3)	2 (15)

HBOC; hereditary breast–ovarian cancer; SSPC, serous surface papillary carcinomas.

[a] Classified according to the dominating histological component.

polymerase from Perkin Elmer-Roche (Branchburg, NJ, USA). All primer sequences as well as the detailed PCR conditions are available from the authors upon request and have also been published in Refs. [18–20]. *BRCA1* exon 11 and *BRCA2* exons 10 and 11 were screened using the protein truncation test (PTT). TNT T7 Quick Coupled Transcription/Translation System kit (Promega) was used for the *in vitro* synthesis and the radiolabelled products were size-separated in 15% sodium dodecyl sulphate/polyacrylamide gel electrophoresis (SDS/PAGE) gels. Exons 7 and 10 in *BRCA1* and exon 3 in *BRCA2* were directly sequenced on an automated sequencer, ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) with the reagents supplied by Perkin Elmer-Roche. Remaining exons, also including the 5′ and 3′ ends of the large exons analysed by PTT, were screened by denaturing high-performance liquid chromatography (DHPLC), using a Wawe™ DNA Fragment Analysis System (Transgenomic, Cheshire, UK). Fragments with chromatograms indicating a variant were further characterised with direct sequencing.

All patients with disease-causing mutations were offered a consultation at the Oncogenetic clinic, Lund University Hospital.

3. Results

Any family history of breast and/or ovarian cancer was reported in 47/156 (30%) patients. 24 (15%) patients had one or two first-degree relatives affected by any of the diseases and 4/156 (3%) patients reported a family history compatible with HBOC (Table 1). Metachronous cancer had developed in 17 patients; 9 breast cancers, 5 cervical cancers, 1 colorectal cancer, 1 carcinoma of the vulva and 1 skin cancer. An additional 3 patients were at the time of diagnosis of the ovarian cancer also found to have synchronous endometrial cancer.

We identified nine different *BRCA1* and four different *BRCA2* germ line alterations in 17/161 (11%) patients (Table 2). Three *BRCA1* mutations were found in more than one person. Five *BRCA1* and one *BRCA2* mutations represent frame-shift or nonsense alterations predicted to cause protein truncation, and are thus recognised as being disease-associated. The remaining four *BRCA1* and three *BRCA2* mutations were missense mutations that give rise to single amino acid substitutions. One of these, *BRCA1* C61G, is a known deleterious mutation that disrupts the amino-terminal RING domain of the protein, and represents a founder mutation of central European origin. *BRCA1* C1697R and R1699W are located in one of the protein domains to which a transcriptional activation function has been ascribed. Segregation and functional analysis support the deleterious effect of these amino acid substitutions [21,22]. Hence, 13/161 (8%) patients were carriers of deleterious BRCA gene mutations.

Four alterations constituted unclassified variants (Table 2). *BRCA1* M1411T is a novel substitution in exon 13 of unknown functional importance that has not previously been identified in the Swedish population or reported in the Breast Cancer Information Core (BIC) [23]. This variant was found in a 72-year- old patient with a serous ovarian cancer and with a family history of breast cancer and other malignancies. Although it is possible that M1411T maybe associated with an increased risk of breast and ovarian cancer, this unclassified variant should be further characterised by segregation and functional analysis to confirm this association. Two of the *BRCA2* missense variants, Y42C and I3412V, have been reported as unclassified variants in the BIC database on numerous occasions. Y42C is located in the amino-terminal region of *BRCA2*, in a region suggested to possess histone acetyltransferase activity. However, the role of the Tyrosine-42 residue in this respect has not been confirmed [24]. Y42C was found in a 77-year-old ovarian cancer patient who had also developed breast cancer at 74 years of age, but had no family history of these diseases. The missense mutations, I3412V and P3063S, were found in cases without a family history of breast and ovarian cancer. I3412V is located in the carboxy-terminal region of *BRCA2* and downstream of a polymorphic stop codon, K3326X. Since the carboxy-terminal residues of *BRCA2* seem to be dispensable for normal *BRCA2* function, I3412V most likely represents a benign polymorphism. Finally, the *BRCA2* P3063S variant is a new one in the Swedish population and has been reported only once in the BIC [23], being identified in a patient/family of Western European origin. The proline-3063 residue is located in a region of BRCA2 suggested to interact with hBUBR1 in response to microtubuli disruption [25].

Among the 13 patients with deleterious BRCA mutations, 4 reported a family history compatible with HBOC, 5 had breast and/or ovarian cancer in at least one first-degree relative, 2 reported any case of breast and/or ovarian cancer in the family, whereas family data were not available from 2 patients (Table 2). Thus, all BRCA gene mutation carriers, from whom information was available, reported any family history of breast and/or ovarian cancer, compared with 36/144 (25%) of patients without any BRCA mutation ($P < 0.001$, Fisher's exact test) (Tables 1 and 2). Metachronous breast cancer occurred in 9/161 (6%) patients, including 4 of the 13 patients with BRCA mutations (1 of whom had no family history of cancer). The breast cancers among the BRCA mutation carriers had developed 2–12 years after the diagnosis of ovarian cancer.

Table 2
Description of ovarian cancer cases with BRCA gene mutations

Case no.	Age (years)	Stage	Histology	Family history	Other cancer/ age (years)	Gene	Exon	Nucleotide change	Effect	Type	Comment
26	57	III	Serous	Two first-degree		*BRCA1*	5	300 T/G	C61G	M	European founder
233	74	II	Endometrioid	HBOC		*BRCA1*	5	300 T/G	C61G	M	European founder
61	60	III	Serous	Two first-degree		*BRCA1*	11	1201del11	ter 361	F	Swedish founder
53	56	IV	Serous	One first-degree		*BRCA1*	11	1806 C/T	Q563X	N	European founder
231	76	I	Endometrioid	Unknown		*BRCA1*	11	1806 C/T	Q563X	N	European founder
249	58	III	Serous	HBOC	Breast/48	*BRCA1*	11	1806 C/T	Q563X	N	European founder
60	57	II	Serous	Two first-degree	Breast/54	*BRCA1*	11	3172ins5	ter 1025	F	Swedish founder
88	47	II	endometrioid/serous	One first-degree		*BRCA1*	11	3438 G/T	E1107X	N	Recurrent in Sweden
51	55	III	Serous	Unknown	Breast/43	*BRCA1*	11	3829delT	ter 1263	F	Recurrent in Sweden
84	42	II	Serous	HBOC	Breast/44	*BRCA1*	11	3829delT	ter 1263	F	Recurrent in Sweden
80	72	III	Serous	second-degree		*BRCA1*	13	4351 T/C	M1411T	UV	Novel
160	63	III	SSPC	second-degree		*BRCA1*	18	5208 T/C	C1697R	M	Recurrent in Sweden
201	58	III	Clear cell	HBOC		*BRCA1*	18	5214 C/T	R1699W	M	Recurrent in Sweden
282	77	I	Serous	Unknown	Breast/74	*BRCA2*	3	353 A/G	Y42C	UV	Often reported in BIC
108	41	IV	Serous	second-degree		*BRCA2*	15	7786 C/T	R2520X	N	Recurrent in Sweden
264	82	I	Mucinous	Unknown		*BRCA2*	24	9415 C/T	P3063S	UV	Reported in BIC once
74	57	III	Serous/mucinous	Unknown		*BRCA2*	27	10462 A/G	I3412V	UV	Often reported in BIC

BIC, breast cancer information core; M, missense; F, frame-shift; N, nonsense; UV, unclassified variant; HBOC, hereditary breast–ovarian cancer = at least three first-degree relatives with breast and/or ovarian cancer, and at least one aged below 50 years at diagnosis; SSPC, serous surface papillary carcinoma.

The mean age at diagnosis for all patients diagnosed with invasive epithelial ovarian cancer in the regional cancer registry during this period was 68 years (range 22–93 years). Among the patients included in the study, the mean age at diagnosis was similar in patients without detectable alterations (59 years) and among the 13 cases with a deleterious mutation (57 years). However, a higher mean age (71 years) was found in the 85 patients who were not included in the study. The 4 cases with unclassified BRCA variants were all postmenopausal (57, 72, 77 and 82 years of age, respectively). None of the younger cases (<40 years) carried any BRCA gene mutation, and only 3 of the BRCA gene mutation carriers were below 50 years of age at diagnosis. Tumour histology in the 13 patients with BRCA mutations was serous adenocarcinomas in 8, endometrioid adenocarcinomas in 3 and clear cell carcinoma and SSPC in 1 each (Table 1). Thus, the different histopathological tumour types showed a similar distribution in the BRCA gene mutation carriers and non-carriers.

4. Discussion

Studies aiming at determining the frequency of BRCA mutations in ovarian cancer have reported germ line mutations in *BRCA1* in 2–12% and in *BRCA2* in 2–4% of ovarian cancer patients [8,14,26–29]. However, several of these studies have been small, have specifically studied the mutation rates in early-onset patients, in specific ethnic groups or among patients with familial ovarian cancer, and have sometimes been restricted to analysis of founder mutations in the *BRCA1* and *BRCA2* genes. We performed a full *BRCA1* and *BRCA2* mutation screening using PTT for exons 11 and DHPLC or direct sequencing for the remaining exons in a prospective series of 161 patients with invasive epithelial ovarian cancer. The patient material investigated herein represents 65% of the invasive epithelial ovarian cancers diagnosed during the time period compared with the population-based Swedish cancer registry.

Overall, 13 different alterations, nine of which are likely to be disease-causing, were identified in 17/161 (11%) patients, with nine alterations affecting *BRCA1* and four affecting *BRCA2*. Taking only recognised deleterious alterations into account, 13 (8%) of the 161 patients carried BRCA mutations, which in all but 1 case affected *BRCA1*. This finding confirms previous studies that report a mutation frequency of 4–12% in invasive ovarian cancers [11,14,27,30]. Although the techniques (PTT, DHPLC and DNA sequencing) used for *BRCA1* and *BRCA2* analysis have a high sensitivity, the estimated mutation frequency still represents a minimal estimate. Neither of these techniques has 100% sensitivity and the screening strategy applied would not detect larger, intragenic deletions, which have been demonstrated to occur at an as- yet unknown frequency, especially affecting *BRCA1*. A family history of breast and/or ovarian cancer was reported in 35/143 (24%) of the patients without detectable *BRCA* mutation, although none of these patients reported a family history suggestive of HBOC, compared with 92% of the BRCA mutation carriers. The presence of a BRCA gene mutation was strongly correlated with a positive family history of breast and/or ovarian cancer in the family and the single deviating case without any family history

of cancer had been diagnosed with a breast cancer. A previous diagnosis of breast cancer was reported in 4% of the BRCA mutation-negative cases compared with 23% of the patients with mutations identified. The histology of the breast cancers that occurred in patients with BRCA mutations was infiltrating ductal cancer in all the cases. One patient was ER/RP-positive.

Thus, taking both family history and metachronous breast cancer into account, all 12 patients with BRCA mutations who provided a family history of ovarian cancer could be identified.

The mean age of the patients in our study (60 years) was lower than the mean age of 68 years among all patients diagnosed within our healthcare region during this time period and also compared with the median age (64 years) of ovarian cancer patients reported in the Swedish population [4]. Consequently, patients lost from the study were older, with a mean age of 71 years. This difference in age between the groups probably reflects a greater interest for genetic testing among younger patients and may also be caused by the fact that many older patients were seriously ill and were therefore lost to inclusion or declined participation. Survival was, as expected, also worse among the non-tested patients (data not shown). Indeed, none of the 13 patients who died within 4 months of diagnosis had been tested. Young age at onset is among the clinical criteria for the classification of HBOC but, interestingly, only 3/13 patients with BRCA mutations in our study were below the age of 50 years at diagnosis. Although our study confirms previous findings that indicate a high frequency of BRCA mutations among women above the age of 60 years, the highest mutation frequencies, at least for *BRCA1,* were found in patients between the ages of 40 and 50 years. Approximately 10% of the ovarian cancers are estimated to occur before the age of 40 years, but a lower mutation frequency has been observed in the youngest age group, i.e. patients below the age of 40 years [7,14,31]. Approximately 15% of BRCA-associated ovarian cancers have been described to occur in women older than 70 years and, indeed, 3/13 mutation carriers in our study were 70 years or older at diagnosis [7]. These observations argue for the importance of obtaining a personal history of synchronous and metachronous cancer, as well as a family history of cancer in older patients with ovarian cancer as well. This recommendation partly contrasts with the situation, e.g. in breast cancer, where the risk of hereditary cancer decreases with increasing age.

The importance of *BRCA2* mutations in ovarian cancer is still not completely defined and is probably considerably lower than that of *BRCA1* mutations. In the present study, only one of 13 deleterious BRCA mutations was found in *BRCA2*. Several unclassified *BRCA2* variants were identified, possibly representing low-moderate penetrance mutations associated with late-onset ovarian cancer, but further functional studies are needed to evaluate this assumption. Furthermore, a genotype–phenotype correlation affecting the risk of ovarian cancer has been described in *BRCA2* with truncating mutations clustering in exon 11 of *BRCA2* conferring the highest risk of ovarian cancer [32]. However, none of the *BRCA2* alterations identified in the present study was located in the ovarian cancer cluster region.

Tumour stage is the sole most important prognostic factor in ovarian cancer and advanced stage is strongly correlated with poor survival. The BRCA mutation carriers in our study did not differ from non-carriers with respect to disease stage, which confirms previous observations [7,33,34]. A longer disease-free survival has been demonstrated by some investigators for patients with BRCA-associated invasive ovarian carcinomas compared with the sporadic cases [7,34,35]. However, other investigations have not demonstrated any survival advantage for *BRCA1* mutation carriers [36]. Tumour histology is of limited prognostic value in ovarian cancer, except for the stage I tumours in which mucinous cancers have the best and clear cell carcinomas have the worst prognosis. However, the histological tumour type might reflect differences in basic tumour biological mechanisms. In unselected tumour materials, serous carcinomas are the most frequent type (40–55%), followed by endometrioid (15–20%), mucinous (10–15%), clear-cell (5–10%) and other types of rare carcinomas in the remaining cases. In *BRCA1*-associated tumours, the dominant histology is the serous subtype followed by endometrioid and clear cell carcinomas, whereas borderline tumours and mucinous carcinomas are generally not part of the BRCA phenotype [8,10,37]. Our study confirmed these observations, although no difference in tumour histology was observed between the BRCA mutation-positive and -negative tumours.

In summary, this prospective study confirms that approximately one in 10 ovarian cancer patients carry germ line mutations in *BRCA1* or *BRCA2*, which represents one of the highest proportions of hereditary cases among our common cancer types. Importantly, the vast majority of BRCA mutation-positive cases can be pinpointed by a positive family history of breast and ovarian cancer possibly combined with information on metachronous breast cancer diagnosis. However, age at diagnosis, stage or histology type were not determinants of a hereditary predisposition. Our findings suggest that a careful family history of cancer should be obtained for all patients with ovarian cancer, irrespective of their age at onset. Identification of one HBOC case for every 10 ovarian cancer patients offers the possibility of preventing further cancer cases in the family through risk-reduction measures, including screening protocols, possible chemoprevention strategies and optional prophylactic oophorectomy for relatives who are at risk.

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