ELSEVIER

European Journal of Cancer 41 (2005) 143–150

European Journal of Cancer

www.ejconline.com

Prevalence and clinical correlations of *BRCA1*/*BRCA2* unclassified variant carriers among unselected primary ovarian cancer cases – preliminary report

Ewa J. Majdak [a,b,*], Geertruida H. De Bock [c,1], Izabela Brozek [d], Magdalena Perkowska [d], Karolina Ochman [d], Jaroslaw Debniak [a], Tomasz Milczek [a], Cees J. Cornelisse [e], J. Jassem [f], Janusz Emerich [a], Janusz Limon [d], Peter Devilee [b,*]

[a] *Department of Gynecology, Medical University of Gdansk, ul. Kliniczna 1A, Gdnask, Poland*
[b] *Human and Clinical Genetics Department, Leiden University Medical Center, P.O. Box 9503, 2300 RA Leiden, The Netherlands*
[c] *Department of Medical Decision Making, Leiden University Medical Center, The Netherlands*
[d] *Department of Biology and Clinical Genetics, Medical University of Gdansk, Poland*
[e] *Pathology Department, Leiden University Medical Center, The Netherlands*
[f] *Department of Oncology and Radiotherapy, Medical University of Gdansk, Poland*

Received 28 June 2004; received in revised form 10 September 2004; accepted 12 October 2004

Abstract

The objective of this study was to determine the prevalence of *BRCA1* and *BRCA2* gene mutations in unselected ovarian cancer patients, and to analyse clinical and pathological features of ovarian cancer unclassified variant mutation carriers in comparison with *BRCA1* pathogenic mutation carriers and sporadic cases. A consecutive sample of 205 women with primary ovarian cancer was screened for mutations in the *BRCA1* and *BRCA2* genes using a direct test for small deletions and insertions, conformational sensitive gel electrophoresis and direct sequencing. Data regarding medical and familial history were collected using questionnaires. Clinical and pathological data were extracted from medical records. Unclassified variants and polymorphic mutations accounted for 8% ($n = 16$) and 6% ($n = 13$) of all cases, respectively. *BRCA1* pathogenic mutations were found in 18 (9%) patients. None were found in *BRCA2*. The mean age of onset for *BRCA1*–associated tumours was 43.1 years (standard deviation (SD: 7.3) whereas in the patients with an unclassified variant, polymorphism, or no detectable gene changes, the mean age of onset ranged from 49.5–56.4 years. The most significant predictors for pathogenic or unclassified variant changes in *BRCA1* in ovarian cancer patients were a younger age of onset and a history of hyperthyroidism and infertility. Except for infertility and hyperthyroidism, unclassified variant-linked ovarian tumours share features with sporadic tumours rather than with *BRCA1* pathogenic mutations.

Keywords: Hereditary ovarian carcinoma; *BRCA1*, *BRCA2*; Unclassified variants; Clinical correlations

1. Introduction

Ovarian cancer is the leading cause of cancer death among gynaecological malignancies. Because the onset of this tumour is generally asymptomatic, most patients present with advanced stages (III/IV). The 5-year

* Corresponding authors. Tel.: +48 58 349 34 41; fax: +48 58 341 80 03; Tel.: +31 71 5276117.
E-mail addresses: ewamajdak@yahoo.com (E.J. Majdak), P.Devilee@LUMC.NL (P. Devilee).
[1] Present address: Department of Epidemiology, University of Groningen, The Netherlands.

0959-8049/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ejca.2004.10.011

survival rates for cases with these stages are estimated to be approximately 20–25% [1].

Pathogenic mutations in the *BRCA1* or *BRCA2* genes are found in approximately 10% of primary epithelial ovarian tumours. Half of the reported mutations in breast cancer information core (BIC) database are pathogenic mutations. These are frame-shifting, nonsense or splice-site alterations that lead to premature truncation of the protein upon translation and approximately 60% are unique to each family (http://research.nhgri.nih.gov/bic/). The other half accounts for missense alterations and intronic variants with unknown disease relevance. They are classified as benign polymorphisms or variants of uncertain clinical significance (unclassified variants; UV). However, recent studies have demonstrated that some of these mutations might affect mRNA processing (by changing the consensus splice and branch sites), or alter the function of the encoded protein product due to unfolding or extrusion caused by a single amino acid change [2–9].

Estimates of the lifetime ovarian carcinoma risk in women who carry *BRCA* mutations vary from 10% to 60%, as opposed to estimated rates of 1–2% in the general population [10,11]. The factors that affect penetrance appear to be both clinical and molecular in nature. It has been shown that *BRCA1*-related ovarian carcinomas have slightly different clinical and pathological characteristics to sporadic cases [12–22]. The goal of our study was to investigate the prevalence and clinical features of *BRCA1*/2 unclassified variant carriers in comparison with *BRCA1* pathogenic mutations and sporadic tumours.

2. Patients and methods

Between 1994 and 2002, we evaluated a total of 207 consecutive cases of primary epithelial ovarian cancer patients at the Department of Gynecology at the Medical University of Gdansk. The treating physician was asked to give a detailed explanation of the purpose of the study. Two patients did not wish to participate in the study and one did not wish to know the results of genetic testing. Consequently, 205 patients agreed to participate in the study. The protocol was approved by the Medical Ethical Committee.

All patients with invasive tumours were treated with primary radical or debulking surgery via laparotomy from a midline incision, followed by chemotherapy. The primary tumour site (ovary or peritoneal) was confirmed by a review of the pathological reports. The surgical stage was classified according to the International Federation of Gynecology and Obstetrics (FIGO) standards and was determined from the patient's medical history records, surgical and pathological reports [23]. All patients who participated in the study underwent a "second-look" operation (SLO). Complete pathological remission (CPR) was defined on the basis of negative histological findings on SLO. Patients who had residual disease on SLO underwent second-line chemotherapy. If the diameter of residual changes was smaller than 1 cm, patients were eligible for intraperitoneal chemotherapy. If residual changes were bigger than 1 cm, patients received intravenous (i.v.) chemotherapy. Response to second-line chemotherapy was defined by negative third-look surgery, regression of measurable disease, or a decrease in the CA 125 level (<35 U/ml) on two consecutive tests. Tumour recurrence was defined as an elevation of the CA 125 level (>35 U/ml) on two consecutive tests, the appearance of measurable lesions on clinical examination or radiological imaging, or pathological evidence of recurrent disease.

Clinical and pathological data were extracted from the medical records. Patients with regular menstrual cycles at their diagnosis of ovarian cancer were classified as premenopausal. Postmenopausal women were those whose periods had ended naturally before the diagnosis was established. In cases of surgical menopause, the age of menopause was defined by the date of the ovarian resection. A family history of cancer in patients with ovarian cancer comprised relatives with breast, ovarian, prostate, colorectal, uterus and fallopian tube cancers. Ovarian tumours in patients with no family history of cancer and no *BRCA* mutation were defined as sporadic. Data regarding patients' medical and family history of cancer were assessed using a questionnaire. Patients with unknown data with regard to their family history of cancer due to loss of contact with their families or adoption or descendence from families with a small number of relatives (no siblings) were defined as patients with an unknown family history of cancer.

3. DNA extraction and mutation analysis

All patients were eligible for genetic testing for *BRCA1* and *BRCA2* mutations. High molecular weight DNA was extracted using the Promega Wizard Kit from the whole blood of 137 patients. The lysis of erythrocytes was performed by absorbing to ammonium ions in red-blood-cell lysis buffer. The white blood cells were isolated and digested by buffer (consisting of NaCl and ethylene diamine tetraacetic acid (EDTA)), 100 μl of 20 mg/ml proteinase K, 4 μl of 1 U/ml ribonuclease A, and 250 μl of 20% sodium dodecyl sulphate (SDS). DNA from 68 patients was isolated from 50 μm sections of frozen ovarian tumour tissue using the Promega Wizard kit. 600 μl of NLS solution and 17.5 μl of 20 mg/ml proteinase K were added to each sample and samples were incubated overnight at 55 °C. On the next day, 3 μl of RNase solution was added to the nuclear lysate and mixed gently by inverting the tube and incubating it for 15–30 min at 37 °C. At the room temperature, 200 μl of protein precip-

itation solution was then added and the tubes centrifuged. The precipitated protein formed a tight white pellet. The supernatant containing the DNA was carefully removed and added to 600 μl of isopropanol kept at room temperature. After centrifuging at 13 000–16 000*g*, supernatant was removed and 600 μl of 70% ethanol kept at room-temperature was added to the white DNA pellet. The solution was then centrifuged and the ethanol was aspirated away. 100 μl of DNA dehydration solution was added to the DNA and the samples were incubated at 65 °C for 1 h.

Mutation scanning methods included detection of small deletions and insertions (DSDI) and fluorescent conformation-sensitive gel electrophoresis (F-CSGE). DSDI comprises 17 amplimers in the coding regions of both genes – 10 in *BRCA1* and 9 in *BRCA2* (Table 1). All 17 fragments were amplified by standard polymerase chain reaction (PCR) amplification protocols, and PCR products were size-fractionated using an ABI 3700 machine. The analysis was performed using Genescan and Genotyper software, resulting in a peak at the position of the expected fragment-size. Deletions and insertions were identified by the presence of an additional peak. Altered fragments were reamplified from the genomic DNA, using primers with a M13 tail on the forward and reverse ends, and sequenced on an ABI PRISM Big Dye Terminator Cycle.

F-CSGE resolves the various conformations that double-stranded DNA can adopt in mildly denaturating polyacrylamide gel matrices [4]. The gel matrix consists of 0.5× MDE and 15% deionised formamide. After filtration through a 0.22 μm nitrocellulose filter (Millipore) and degasing for 10 min, 125 μl 10% APS and 17.5 μl TEMED were added. Polymerisation was allowed to occur overnight at 4 °C.

The entire coding region of *BRCA1*, *BRCA2* and 15–50 bp of each flanking intron was subdivided into 83 segments – 33 for *BRCA1* and 50 for *BRCA2*. All primer sequences were selected outside of the Alu-repeat sequences, which are particularly abundant in the introns of *BRCA1*. The "forward" primers were fluorescently labelled with FAM, HEX or TET. The 83 fragments were amplified in 48 mono- or duplexPCRs (1–2 amplimers each). Using a 96-well microtitre plate, a 14 μl reaction mixture was prepared in each well, containing 10 pmol primers, 1× PCR buffer (50 mM KCL, 10 mM TRIS–HCl pH 8.4, 2.5 mM $MgCl_2$, 0.2 mg/ml bovine serum albumin (BSA), 0.2 mM deoxyadenesine triphosphates (dNTPs)) and 0.1 U Goldstar Taq-polymerase. Subsequently, 1 μl of each DNA sample (50 ng/μl) was added to the reaction mixtures. PCR was performed for 40 cycles consisting of 30 s at 94 °C, 30 s at 58 °C, and 30 s at 72 °C. After PCR, reaction mixtures were pooled per DNA-sample into a 96-well microtitre plate in a HEX:FAM:TET ratio of 3:2:2 (end volume of 24 μl), in a total of 8 pools for each DNA sample. To allow gel loading, 7 μl of this mixture was sampled into a fresh plate and heat/air-dried by exposing it to 45 °C for 1 h. The fragments were dissolved in 2.5 μl of pink loading dye, to which 0.25 μl GeneScan – 500 TAMRA size-standard and 0.25 μl loading buffer were added for 15 min on ice. Using an 8-channel loading device, 1.5 μl of this mixture was loaded onto a F-CSGE gel, which was pre-run for 15 min. The samples were subjected to electrophoresis through these gels for 4.5 h at 1680 V at 30 °C. Gels were analysed using GeneScan and Genotyper software. Upon detection of a CSGE-variant, the segment of the gene was reamplified from the DNA-sample with the M13 tail on both the reverse and forward ends (FWD: 5′-TTG TAA AAC GAC GGC CAG T; REV: 5′-GGA AAC AGC TAT GAC CAT G). Sequencing was performed using universal primers and an ABI PRISM Big Dye Terminator Cycle.

4. Statistical analysis

The mutation status of the 205 patients (i.e., carrier of a proven pathogenic mutation, unclassified variant,

Table 1
The type and location of *BRCA* mutation

Mutation	*BRCA* gene	Exon	Number of cases	Frequency $N = 47$ (100%)	Type of alteration[a]	Predicted defect
5382insC	1	ex20	13	28%	F	Stop1829
3199 A > G	2	ex11D	9	19%	P	Asn > Asp
4956 A > T	1	ex16B	7	15%	UV	Ser > Gly
4486 G > T	2	ex11J	7	15%	UV	Asp > Tyr
3624 A > G	2	ex11F	3	6%	P	Lys > Lys
185insC	1	ex2	2	4%	F	Stop 39
300 T > G	1	ex5	1	2%	M	Cys61Gly
3405 C > T	1	ex11	1	2%	Stop	Gly > stop
IVS 22+5 G > A	1	ex22	1	2%	Splice–site	Stop 1803
3111 G > A	2	ex11D	1	2%	UV	Gln > Gln
5075 A > G	1	ex16B	1	2%	UV	Met > Ile
1186 A > G	1	ex11beta	1	2%	P	Gln > Arg

[a] F, frameshift; M, missense; UV, unclassified variant; P, polymorphism.

known polymorphism, or no mutation detected) was compared with their clinical and pathological characteristics. For categorical variables, Pearson's χ^2 tests were used. For continuously distributed variables, an analysis of variance test (ANOVA) was performed. Differences between patients with a pathogenic mutation, unclassified variant, polymorphism or those without a detected gene change were analysed by univariate analysis, using the following comparisons:

1. Pathogenic mutation carriers versus those with unclassified variants, polymorphic variants or no gene changes.
2. Carriers of unclassified variants versus those with polymorphisms or no gene changes.

To address which clinical and pathological features could characterise carriers of a *BRCA1* mutation, or unclassified variant, a multivariate analysis was performed. Because there were more than two outcomes (*BRCA1*-mutation status, unclassified variant status, polymorphism status, no mutation status) the analysis was performed by using a multinominal regression. In the first step, all clinical and pathological variables from Tables 2 and 3 were included in one model. The variables that contributed significantly at a 5% level in the overall model were included. In the next step, the main effects were estimated using Odds Ratios (OR) including their 95% Confidence Intervals (CI) for all variables for patients with pathogenic mutations, unclassified variants and polymorphisms using patients without any mutation as a reference group. Age of onset was included as a continuous variable.

Because in this model patients with a polymorphism did not differ significantly from patients without any gene changes, a second model was constructed in which carriers of a polymorphism were considered in the same group as patients without a detectable gene change. For the model construction, the same procedure was repeated. Only this model is presented here. Statistical analyses were performed with the statistical package for the social sciences (SPSS)-10 computer program.

5. Results

In total 47 of 205 (23%) of primary ovarian cancer patients carried changes in *BRCA1* or *BRCA2* (Table 1). Eighteen of these represented pathogenic mutations in the *BRCA1* gene and 29 (14%) were defined as either polymorphism ($n = 13$; 6%) or unclassified variant ($n = 16$; 8%). The most frequent change was 5382insC, a well-established Polish founder mutation (Table 1) [25,26], and the second most frequent change present in the BIC database. Two novel pathogenic mutations, not reported in Poland before, were identified – 3405 C > T in exon 11 and IVS 22 + 5 G > A in exon 22 of *BRCA1* gene. Unclassified variants were evenly distributed between the *BRCA1* and *BRCA2* genes (8 cases each).

Two patients carried more than one variant: one carried 3624 A > G (polymorphism in *BRCA2* exon 11) together with 4486 G > T (unclassified variant in *BRCA2* exon 11), and the other carried – 4956 A > T (unclassified variant in *BRCA1* exon 16), 3199 A > G (polymorphism in *BRCA2* exon 11) with 3624 A > G (polymorphism in *BRCA2* exon 11).

We subgrouped the patients into those with pathogenic mutation ($N = 18$), those with unclassified variant ($n = 16$), those with either a known polymorphism ($n = 13$) or no detectable changes (''sporadic'' cases) ($n = 158$). We first compared mutation carriers with the latter two groups combined (Table 2). The mean age of onset for tumours in patients carrying a pathogenic *BRCA1* change was 43.1 years (SD: 7.3), whereas in the other 2 groups the mean age of onset ranged from 49.5–56.4 years. The age of onset of ovarian cancer in pathogenic *BRCA1* carriers was more often below 50 years ($P < 0.001$) (Table 2), compared with non-carriers. Ovarian cancer in *BRCA1* pathogenic mutation carriers occurred more often before the menopause ($P = 0.002$), and was also more often of grade 3 compared with unclassified variant carriers or sporadic cases ($P < 0.001$).

Finally, a history of hyperthyroidism, infertility, and a family history of breast or ovarian cancer were all significantly more frequent among those carrying pathogenic *BRCA1* changes versus those that did not (Table 2). In contrast, the occurrence of a family history of breast and/or ovarian cancer in those carrying an unclassified variant was not significantly different from sporadic cases (Table 3). Infertility and hyperthyroidism were the only variables that remained associated with the unclassified variant status compared with patient polymorphisms and sporadic cases. Univariate analysis did not reveal any differences in FIGO stage, histology, residual changes on SLO present, and pathological remission status between pathogenic mutation carriers and others. Only the unclassified variant group showed a tendency to present without residual disease on SLO in comparison to sporadic cases ($P = 0.027$) (Table 3).

In the multivariate model of clinical factors studied, age of onset, hyperthyroidism, and infertility were characteristics related to pathogenic mutation and UV status, using patients without a mutation or with polymorphism as a reference group (see Table 4). Grading of the tumour was related to pathogenic mutation status, but not to unclassified variant status. The wide CIs may be due to small numbers involved.

Table 2
Clinical, pathological and epidemiological characteristics of *BRCA1* pathogenic mutation carriers

Variable	*BRCA1* pathogenic mutation carriers ($n = 18$; 9%)	UV; polymorphisms; sporadic cases ($n = 187$; 91%)	Statistical significance χ^2
Age of onset (years)			
<50	83.3% (15)	34.8% (65)	**$P = 0.001$**
>50	16.7% (3)	65.2% (122)	
FIGO			
I and II	11.1% (2)	12.8% (24)	$P = 0.834$
III and IV	88.9% (16)	87.2% (163)	
Grading			
G1	50% (9)	90.9% (170)	**$P = 0.001$**
G3	50% (9)	9.1% (17)	
Pathology			
Papillary serous	83.3% (15)	63.1% (118)	$P = 0.86$
mucinous	16.7% (3)	36.9% (69)	
Involvement of the ovaries			$P = 0.183$
Unilateral	11.1% (2)	25.1% (47)	
Bilateral	88.9% (16)	74.9% (140)	
SLO findings			
No residual disease	16.7% (3)	38.7% (72)	$P = 0.125$
Residual disease			
<1 cm	61.1% (11)	50% (93)	
>1 cm	22.2% (4)	50% (93)	
+ Complete pathological remission (CRP)	16.7% (3)	35.8% (67)	$P = 0.095$
Persistent (PR)	83.3% (15)	64.2% (118)	
Premenopausal ovarian cancer	66.7% (12)	31.0% (58)	**$P = 0.002$**
Postmenopausal ovarian cancer	33.3% (6)	69.0% (129)	
+ Infertility			
Yes	66.7% (12)	6.0% (11)	**$P = 0.0001$**
No	33.3% (6)	94.0% (176)	
Hyperthyroidism			
Yes	22.2% (4)	7.5% (14)	**$P = 0.035$**
No	77.8 (14)	92.5% (173)	
Family history of cancer			
Yes	55.6% (10)	20.3% (38)	**$P = 0.001$**
No or unknown	44.4% (8)	79.7% (149)	
Family history of breast cancer			
Yes	44.4% (8)	7.0% (13)	**$P = 0.0001$**
No or unknown	55.6% (10)	93.0% (174)	
Family history of ovarian cancer			
Yes	27.8% (5)	8.0% (15)	**$P = 0.007$**
No or unknown	72.2% (13)	92.0% (172)	

SLO, second-look operation; FIGO, International Federation of Gynecology and Obstetrics.
+, Some data are missing.

6. Discussion

We report the prevalence and spectrum of *BRCA1* pathogenic mutations, unclassified variants and polymorphisms in unselected ovarian cancer series. No study addressing the clinical characteristics of unclassified variants has been performed to date. We characterised a group of ovarian cancer patients with unclassified variants in *BRCA1/2* regarding their clinical, pathological and epidemiological data. Our preliminary findings comprise a relatively small series. Indeed, the small size of two of the three categories studied ($N = 16$ unclassified variant cases and $N = 18$ pathogenic mutation cases) is a limitation in this study. However, this is a preliminary report, and the first to publish data on the clinical correlations of BRCA1/BRCA2 unclassified variant carriers amongst unselected primary ovarian cancer patients, which strengthens the value of this study. Furthermore, molecular methods used for the detection of *BRCA1/BRCA2* alterations had a high sensitivity of up to 95%, therefore the bias of false-negative groupings is likely to be minimal. Further, all of the patients were

Table 3
Clinical, pathological and epidemiological characteristics of unclassified variant carriers

Variable	UV (n = 16; 8%)	Polymorphisms; sporadic cases (n = 171; 83%)	Statistical significance χ^2
Age of onset (years)			
<50	43.8% (7)	33.9% (58)	P = 0.430
>50	56.3% (9)	66.1% (113)	
FIGO			
I and II	18.8% (3)	12.3% (21)	P = 0.459
III and IV	81.3% (13)	87.7% (150)	
Grading			
G1	81.3% (13)	91.8% (157)	P = 0.160
G3	18.8% (3)	8.2% (14)	
Pathology			
Papillary serous	68.8% (11)	62.6% (107)	P = 0.624
Mucinous	31.3% (5)	37.4% (64)	
Involvement of the ovaries			
Unilateral	6.3% (1)	26.9% (46)	P = 0.069
Bilateral	93.8% (15)	73.1% (125)	
SLO findings			
No residual disease	53.3% (8)	37.4% (64)	P = 0.027
Residual disease			
<1 cm	20% (3)	52.6% (90)	
>1 cm	26.7% (4)	9.9% (17)	
Complete pathological remission (CRP)	53.3% (8)	35.1% (60)	P = 0.159
Persistent (PR)	46.7% (7)	64.9% (111)	
Premenopausal ovarian cancer	43.8% (7)	29.8% (51)	P = 0.250
Postmenopausal ovarian cancer	56.3% (9)	70.2% (120)	
+ Infertility			
Yes	25% (4)	4.1% (7)	**P = 0.001**
No	75% (12)	95.9% (164)	
Hyperthyroidism			
Yes	31.3% (5)	5.3% (9)	**P = 0.0001**
NoAC	68.8% (11)	94.7% (162)	
Family history of cancer			
Yes	31.3% (5)	19.3% (33)	P = 0.256
No or unknown	68.8% (11)	80.7% (138)	
Family history of breast cancer			
Yes	6.3% (1)	7.0% (12)	P = 0.908
No or unknown	93.8% (15)	93.0% (159)	
Family history of ovarian cancer			
Yes	12.5% (2)	7.6% (13)	P = 0.490
No or unknown	87.5% (14)	92.4% (158)	

+, Some data are missing.

treated in a single institution, with standardised treatments based on one protocol. Therefore, there is no bias in estimating the prognostic value of the treatment methods.

The relative contributions of *BRCA1* and *BRCA2* appeared to be unequal. All pathogenic mutations (18 cases) were found in *BRCA1*. The spectrum of pathogenic mutations in *BRCA1* is consistent with other studies [13,14]. The unclassified variants were evenly distributed between both *BRCA* genes, while the vast majority (84%) of polymorphic variants were found in *BRCA2*. This suggests that *BRCA2* mutations are less likely to cause a substantial proportion of ovarian cancer cases in Poland, in keeping with the low reported cumulative ovarian cancer risks for *BRCA2*.

Our data regarding clinical and pathological features of *BRCA1*-associated ovarian tumours supports the hypothesis that *BRCA1*-linked hereditary ovarian cancers have a distinct clinical behavior. Unclassified variant-linked ovarian tumours did not differ significantly from sporadic cases and benign polymorphisms. Age at diagnosis for the hereditary cases has important implications with regard to genetic counselling. In our series, the age of onset of *BRCA1*-linked ovarian tumours was approximately 11 years younger than sporadic cases and 9 years younger than unclassified

Table 4
Multivariate model comparing patients with pathogenic mutations and unclassified variants using patients without any mutations as a reference group

Variable	Pathogenic mutation ($n = 18$)	Unclassified variant ($n = 16$)	No mutation ($n = 171$)
Age of onset: mean (SD)	OR= 0.85; 0.78–0.93	OR = 0.93; 0.87–0.99	1
Hyperthyroidism	OR = 8.77; 1.31–58.61	OR = 8.02; 2.04–31.49	1
Grading	OR = 4.28; 1.81–10.12	OR = 2.01; 0.93–4.34	1
Infertility	OR = 41.43; 8.21–209.1	OR = 6.39; 1.41–28.88	1

SD, standard deviation; OR, Odd Ratio; 95% confidence interval (CI).

variants. Younger age of onset in *BRCA1*-linked ovarian tumours is consistent with other studies [12,27–32]. In our study, the age of onset in unclassified variants was 2 years younger compared with sporadic cases. Our study confirms, other findings with regard to *BRCA1*-linked ovarian tumours, namely high grade (G3), bilateral ovarian involvement and family history of breast or ovarian cancer [12]. Most hereditary ovarian tumours comprise primary invasive adenocarcinomas of the serous type [24,27–30].

Interestingly, in our series, women with *BRCA1* pathogenic mutations and unclassified variants reported hyperthyroidism and infertility in anamnesis more often than sporadic cases. It has been shown that hyperthyroidism is associated with an increased ovarian cancer risk in the general population [33,34]. Skjoldebrand and colleagues [34] have shown that ultrasound images of the ovaries of patients with hyperthyroidism resembled those of patients with polycystic ovary syndrome. Underlying causes of these findings might include direct influence of thyroid hormones on the ovaries, impaired androgen action on the ovarian microenvironment resulting from an elevation in steroid hormone binding protein (SHBG) in hyperthyroid patients, or both. *In vitro* studies showed that thyroid hormones had an stimulating influence on ovarian and breast epithelium [34]. Thyroid hormone receptors are located in the nucleus. Upon binding T3, complex hormone-receptors modulate the transcription of mRNA of genes encoding enzymes and structural proteins associated with thyroid hormone action e.g. synthesis of Na^+/K^+ ATPase in order to increase oxidative metabolism. However, the mechanism by which thyroid hormones might trigger ovarian carcinogenesis remains unclear. Recently, a new hypothesis was introduced: that inflammation plays a role in ovarian cancer risk [35]. Hyperthyroidism may be associated with the inflammatory responses of the ovarian epithelium [36]. Therefore patients with a genetic predisposition to ovarian cancer (*BRCA1* pathogenic or unclassified variant mutation) may have a higher risk of developing tumour when exposed to hyperthyroidism. The pathogenic role of *BRCA* unclassified variants remains unclear. Clinically, unclassified variant-linked ovarian tumours do not differ significantly from sporadic cases. The higher frequency of hyperthyroidism and infertility in patients with unclassified variant mutations than in sporadic cases might suggest a hormonal background for ovarian carcinogenesis observed in this subgroup. This finding warrants further confirmation.

Conflict of interest statement

None declared.

Acknowledgements

We cordially thank Jeannelle Kraan for her excellent technical assistance. The present study was supported by a grant from the Eurogendis project for Dr. Ewa Majdak, Dutch Cancer Institute and the State Committee for Scientific Research, Poland.

References

1. ACOG Committee Opinion. Genetic risk and screening techniques for epithelial ovarian cancer. *Int J Gynec Obstet* 1993, **41**, 321–323.
2. Xu CF, Chambers JA, Nicolai H, *et al*. Mutations and alternative splicing of the *BRCA1* gene in the UK breast/ovarian cancer families. *Gene Chromosom Cancer* 1997, **18**, 102–110.
3. Mazoyer S, Puget N, Perrin-Vidonz L, *et al*. A *BRCA1* nonsense mutation causes exon skipping. *Am J Hum Genet* 1998, **62**, 713–715.
4. Ozcelik H, Nedelcu R, Chan VWY, *et al*. Mutations in the coding region of the *BRCA1* gene leads to aberrant splicing of the transcript. *Hum Mutat* 1999, **14**, 540–541.
5. Liu HX, Cartegni L, Zhang MQ, *et al*. A mechanism of exon skipping caused by nonsense or missense mutations in *BRCA1* and other genes. *Nat Genet* 2001, **27**, 55–58.
6. Vega A, Campos B, Bressac de Pailleets B, *et al*. The R71G *BRCA1* is a founder Spanish mutation and leads to abberant splicing of the trancript. *Hum Mutat* 2001, **17**, 520–521.
7. Feckenthal JD, Cartegni L, Krainer AR, *et al*. *BRCA2* T2722R is a deleterious allele that causes exon skipping. *Am J Hum Genet* 2002, **71**, 625–631.
8. Williams RS, Glover JN. Structural consequences of a cancer-causing *BRCA1*-BRCT missense mutation. *J Biol Chem* 2003, **278**, 2630–2635.
9. Fleming MA, Potter JD, Ramirez CJ, *et al*. Understanding missense mutations in the *BRCA1* gene: an evolutionary approach. *Proc Natl Acad Sci USA* 2003, **100**, 1151–1156.
10. Easton DF, Ford D, Bishop DT, *et al*. Breast and ovarian cancer incidence in *BRCA1* mutation carriers. *Am J Hum Genet* 1995, **56**, 265–271.

11. Claus EB, Schildkraut JM, Thomposn WD, *et al*. The genetic attributable risk of breast and ovarian cancer. *Cancer* 1996, **77**, 2318–2324.
12. Anton Culver H, Cohen PF, Gldeea ME, *et al*. Characteristics of *BRCA1* mutations in a population-based case series of breast and ovarian cancer. *Eur J Cancer* 2000, **36**, 1200–1208.
13. Dorum A, Hovig E, Trope C, *et al*. Three per cent of Norwegian ovarian cancers are caused by *BRCA1* 1675delA or 1135insA. *Eur J Cancer* 1999, **35**, 779–781.
14. Geisler JP, Hatterman–Zogg MA, Rather JA, *et al*. Frequency of *BRCA1* dysfunction in ovarian cancer. *J Natl Cancer Inst* 2002, **94**, 61–67.
15. Gotlieb WH, Friedman E, Bar-Sade RB, *et al*. Rates of Jewish ancestral mutations in *BRCA1* and *BRCA2* in borderline ovarian tumours. *J Natl Cancer Inst* 1998, **90**, 995–1000.
16. Janezic SA, Ziogas A, Krumroy LM, *et al*. Germline *BRCA1* alterations in a population-based series of ovarian cancer cases. *Hum Mol Genet* 1999, **8**, 889–897.
17. Lu KH, Cramer DW, Muto MG, *et al*. A population-based study of *BRCA1* and *BRCA2* mutations in Jewish women with epithelial ovarian cancer. *Obstet Gynecol* 1999, **93**, 34–37.
18. Modan B, Gak E, Sadebruchim RB, *et al*. High frequency of *BRCA1* 185delAG mutation in ovarian cancer in Israel. *JAMA* 1996, **276**, 1823–1825.
19. Moslehi R, Chu W, Karlan B, *et al*. *BRCA1* and *BRCA2* mutation analysis of 208 Ashkenazi Jewish women with ovarian cancer. *Am J Hum Genet* 2000, **66**, 1259–1272.
20. Mullen P, Miller WR, Mackay J, *et al*. *BRCA1* 5382insC mutation in sporadic and familial breast and ovarian carcinoma in Scotland. *Br J Cancer* 1997, **75**, 1377–1380.
21. Sarantaus L, Vahteristo P, Bloom E, *et al*. *BRCA1* and *BRCA2* mutations among 233 unselected finnish ovarian carcinoma patients. *Eur J Hum Genet* 2001, **9**, 424–430.
22. Stratton JF, Gayther SA, Russell P, *et al*. Contribution of *BRCA1* mutations to ovarian cancer. *N Engl J Med* 1997, **336**, 1125–1130.
23. Shephard JH. Revised FIGO staging for gynaecological cancer. *Br J Obst Gynaecol* 1989, **96**(8), 889–892.
24. Boyd J, Sonoda Y, Federici MG, *et al*. Clinicopathologic features of *BRCA*-linked and sporadic ovarian tumours. *JAMA* 2000, **283**, 2260–2266.
25. Górski B, Byrski T, Lubinski J, *et al*. Founder mutations in the *BRCA1* gene in Polish families with breast–ovarian cancer. *Am J Hum Genet* 2000, **66**, 1963–1968.
26. Perkowska M, Brożek I, Wysocka B, *et al*. *BRCA1* and *BRCA2* mutation analysis in breast–ovarian families from Northeastern Poland. *Hum Mutat* 2003, **21**, 553–554.
27. Boyd J, Rubin S. Hereditary ovarian cancer: molecular genetics and clinical implications – review. *Gynecol Oncol* 1997, **64**, 196–206.
28. Johannsson OT, Tanstam J, Borg A, *et al*. Survival of *BRCA1* breast and ovarian cancer patients: a population-based study from southern Sweden. *J Clin Oncol* 1998, **16**, 397–404.
29. Lu KH, Broaddus RR. Gynecologic tumours in HNPCC: we know they are common – now what?. *Gynecol Oncol* 2001, **82**, 337–340.
30. Lynch HT, Watson P, Bewtra C, *et al*. Hereditary ovarian cancer. Heterogeneity at age of diagnosis. *Cancer* 1991, **67**, 1460–1466.
31. Marcus JN, Watson P, Page DL, *et al*. Hereditary breast cancer pathobiology, prognosis and *BRCA1* and *BRCA2* gene linkage. *Cancer* 1996, **77**, 687–709.
32. Narod SA, Sun P, Ghadirian P, *et al*. Tubal ligation and risk of ovarian cancer in carriers of *BRCA1* and *BRCA2* mutations – a case control study. *Lancet* 2001, **12**(357), 1467–1470.
33. Martinez MB, Ruan M, Fitzpatrick LA. Altered response to thyroid hormones by breast and ovarian cancer cells. *Anticancer Res* 2000, **20**, 4141–4146.
34. Skjoldebrand Sparre L, Kollind M, Carlstrom K. Ovarian ultrasound and ovarian and adrenal hormones before and after treatment for hyperthyroidism. *Gynecol Obstet Invest* 2002, **54**, 50–55.
35. Ness RB, Cottreau C. Possible role of ovarian epithelial inflammation in ovarian cancer. *J Natl Cancer Inst* 1999, **91**(17), 1459–1467.
36. Ness RB, Cottreau C, Kapplar J, *et al*. Factors related to inflammation of the ovarian epithelium and risk of ovarian cancer. *Epidemiology* 2000, **11**(2), 97–98.