BRCA1 and *BRCA2* Mutation Prevalence and Clinical Characteristics of a Population-Based Series of Ovarian Cancer Cases from Denmark

Marie Soegaard,[1] Susanne Kruger Kjaer,[1,2] Mark Cox,[3] Eva Wozniak,[3] Estrid Høgdall,[1] Claus Høgdall,[2] Jan Blaakaer,[4] Ian J. Jacobs,[3] Simon A. Gayther,[3] and Susan J. Ramus[3]

Abstract

Purpose: To evaluate the prevalence of *BRCA1* and *BRCA2* mutations and associations with clinical correlates of disease in a population-based series of ovarian cancer cases from Denmark.

Methods: DNA sequencing and multiplex ligation-dependent probe amplification analysis were used to analyze the *BRCA1* and *BRCA2* genes for coding sequence mutations and large genomic rearrangements in 445 confirmed cases of ovarian cancer. We evaluated associations between mutation status and clinical characteristics, including cancer risks for first-degree relatives and clinicopathologic features of tumors.

Results: Deleterious *BRCA1* or *BRCA2* mutations were identified in 26 cases; thus, mutations in these genes are responsible for at least 5.8% of ovarian cancer cases in this population. Five different mutations were identified in more than one individual, suggesting that they may be founder mutations in Denmark. We identified several differences between mutation carriers and noncarriers: mutation carriers were diagnosed at a significantly early age (median, 49 and 61 years, respectively; $P = 0.0001$); the frequency of BRCA1 mutation carriers was 23% for women diagnosed <40 years, 15% for 40 to 49 years, 4% for 50 to 59 years, and 2% for ≥60 years ($P = 0.00002$); ovarian cancer in carriers was diagnosed at a later stage ($P = 0.002$) and tumors were of poorer grade ($P = 0.0001$); and first-degree relatives of mutation carriers had greater relative risks of both ovarian cancer [10.6 (95% confidence interval, 4.2-26.6); $P < 0.0001$] and breast cancer <60 years [8.7 (95% confidence interval, 3.0-25.0); $P < 0.0001$].

Conclusion: These data may have a significant effect on risk assessment and clinical management of individuals from Denmark who are predisposed to ovarian cancer because they carry a *BRCA1* or *BRCA2* mutation.

Epithelial ovarian cancer is the leading cause of death from gynecologic malignancy in the western world. The strongest known risk factor is a family history of the disease; an individual with a first-degree relative affected with ovarian cancer has a 3-fold increased risk of developing the disease (1). Two genes, *BRCA1* and *BRCA2* (2, 3), are responsible for the majority of families containing multiple cases of breast and ovarian cancer (4–6). The cumulative lifetime risks of ovarian cancer associated with these genes have been estimated as 40% to 50% for a *BRCA1* mutation carrier and 20% to 30% in *BRCA2* carriers (4, 7).

There have been several studies reporting the prevalence of *BRCA1* and *BRCA2* mutations in ovarian cancer cases unselected for family history (8–13). Together, these studies suggest that mutations in these genes may cause 3% to 15% of all ovarian cancers. The first published study of 374 ovarian cancer cases from southern England, which analyzed just *BRCA1*, identified truncating mutations in 3% of cases (8). Another larger study reported a higher prevalence (8%) in 977 patients from Canada (13). Less data are available for *BRCA2*, but the Canadian study reported deleterious mutations for 5.9% of cases (13).

There are several reports of *BRCA1* and *BRCA2* analysis in ovarian and breast cancer cases from some Scandinavian countries (Finland, Norway, Sweden, and Iceland; refs. 13–19), but the data from the Danish population are much more limited. The largest study of *BRCA1* and *BRCA2* mutations to date from Denmark is the analysis of 103 multifocal or bilateral early-onset breast cancers, which identified mutations in 20% of cases (20). There are no reports in the literature describing

Authors' Affiliations: [1]Institute of Cancer Epidemiology, Danish Cancer Society; [2]The Gynaecologic Clinic, The Juliane Marie Centre, Copenhagen, Denmark; [3]Translational Research Laboratory, University College London Elizabeth Garrett Anderson Institute for Women's Health, University College London, London, United Kingdom; and [4]Department of Gynaecology and Obstetrics, Aarhus University Hospital, Aarhus, Denmark

Received 11/2/07; revised 3/6/08; accepted 3/6/08.

Grant support: Mermaid/Eve Appeal. This work was partly undertaken at University College London Hospital/University College London, which received a proportion of funding from the Department of Health's National Institute of Health Research Biomedical Research Centres funding scheme.

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Requests for reprints: Simon A. Gayther, Translational Research Laboratory, Elizabeth Garrett Anderson Institute for Women's Health, University College London, Windeyer Building, 46 Cleveland Street, London W1T 4JF, United Kingdom. Phone: 44-20-7679-9204; Fax: 44-20-7679-9687; E-mail: s.gayther@ucl.ac.uk.

doi:10.1158/1078-0432.CCR-07-4806

the analysis of *BRCA1* and *BRCA2* in population-based ovarian cancer cases from Denmark.

For all but one of the population-based studies described above, screening for mutations in *BRCA1* and *BRCA2* was limited to the coding sequence and splice sites of both genes, which will only identify single-base changes or small deletions and insertions of a few nucleotides. However, several studies have now reported the common occurrence of large, genomic alterations (rearrangements and deletions) in *BRCA1* and *BRCA2* (21, 22). For *BRCA1*, this type of mutation represents 8% to 40% of all *BRCA1* mutations identified in families from the United Kingdom, United States, France, Germany, The Netherlands, and Italy, but may be rare in the Danish population (6, 23, 24). This suggests that most studies will have underestimated the prevalence of *BRCA1* and *BRCA2* mutations in ovarian and breast cancer populations.

Some studies have also reported on the associations between *BRCA1* and *BRCA2* mutation status and clinical features of disease. These studies suggest that tumors from *BRCA1* carriers are more likely to be of serous histology and higher grade (25); *BRCA2* tumors appear to be similar by pathology to *BRCA1* tumors (25). Patients with *BRCA1* mutations are also diagnosed at a younger age (12, 13). Reports describing associations between survival and *BRCA1* and *BRCA2* mutation status are conflicting (26–29).

The purpose of the current study was to establish the prevalence of *BRCA1* and *BRCA2* mutations, including coding sequence alterations and large genomic deletions/rearrangements in a population-based series of 445 invasive ovarian cancer cases unselected for family history of cancer from Denmark. A secondary aim was to correlate *BRCA1* and *BRCA2* mutation status with both a family history of ovarian and breast cancer and clinical features of disease including age at diagnosis and histopathologic characteristics of ovarian tumors.

Materials and Methods

Patient samples. The MALOVA study is an epidemiologic, population-based study of ovarian cancer cases from Denmark. Details of this population have been published previously (30). Briefly, eligible cases were women ages 35 to 79 years with suspected ovarian cancer, who were diagnosed with an ovarian tumor from December 1994 to May 1999. Study participants were recruited from 16 different hospitals throughout Denmark. To ensure that all eligible cases in the study area were included, we also identified cases by searching the Danish Cancer Registry, which is a nationwide register, every 2 months. Participants provided blood and tissue samples and completed an epidemiologic, questionnaire. Ethics committee approval was obtained for the collection and genetic analysis of all samples, and an informed written consent was obtained from all participants. Likewise, approval from the Danish Data Protection Agency was obtained. In total, 681 patients with invasive ovarian cancer, 235 cases of borderline ovarian cancer, and 450 cases with a benign ovarian tumor were enrolled in the study. Lymphocyte DNA samples of sufficient quantity and suitable quality were available from 445 invasive epithelial ovarian cancer cases.

We attempted to establish the history of cancer in first-degree relatives of all ovarian cancer cases in this study using the Danish Civil Registration System, which assigns a unique 10-digit personal identification number to every Danish person alive on April 1, 1968 and born thereafter. Index cases were asked about the name and date of birth of their mother and biological sister(s) and daughter(s). The Civil Registration System was then used to confirm this information. When information on the relatives could not be found, we contacted the National Register (Folkeregisteret) in the municipality where the woman was born. The National Register includes information on every inhabitant in 271 different municipalities from 1924 up to the time the Civil Registration System was introduced. If information was unavailable from these systems, we manually went through parish registers. In total, we obtained information on 427 mothers (96% complete), 389 fathers (87% complete), 494 sisters, and 310 brothers. We used the Danish Cancer Registry, established in 1942 to obtain information about cancer incidence in this cohort. Since 1987, it has become compulsory to notify the registry of all cases of cancer. Even before this date, the register was 96% to 98% complete. Cancers diagnosed up to December 31, 2003 were included. The Civil Registration System contains information on dates of death and emigration and was used to perform active clinical follow-up of cases up until 2004.

Genetic analysis. All cases were screened for mutations in the coding region and splice site (intron/exon) boundaries by sequencing. DNA samples were amplified using Variant SeqR primer sets for *BRCA1* (v1) and *BRCA2* (v2; Applied Biosystems). Custom primers were designed for regions not covered by these kits (primer sequences available on request). Sequencing reactions were done using the Big Dye terminator v1.1 kit (Applied Biosystems). M13 forward and reverse primers were used to sequence each fragment for both sense and antisense strands. Sequencing reactions were size fractionated on a 3730xl, 96 capillary DNA analyzer (Applied Biosystems). SeqScape software version 2.5 (Applied Biosystems) was used to analyze sequence traces. Pass rates for the number of samples aligned by SeqScape were determined for each PCR fragment and those with <70% pass were repeated. All detectable mutations were confirmed in an independent PCR amplified product.

Successful sequencing was achieved for 91% of all PCR fragments for both genes (93% for *BRCA1* and 89% for *BRCA2*). It was not possible to sequence across all of the coding region for each gene. We achieved 98% coverage of *BRCA1* and 95% coverage of *BRCA2*. Thus, by combining successful sequencing rates and gene coverage, we estimate that we were able to achieve complete gene sequencing of at least 91% of *BRCA1* and 85% of *BRCA2* for the study population. These figures are based on pass rates per fragment and are likely to be an underestimate because many of the fragments are overlapping.

Cases were screened for large genomic alterations of *BRCA1* and *BRCA2* by multiplex ligation-dependent probe amplification (MLPA) using the SALSA P002-*BRCA1* or P045-*BRCA2* Exon Copy Number Test Kits (MRC-Holland). Details of MLPA analysis have been described previously (6). MLPA was successfully done in 93% of cases for *BRCA1* and 85% of cases for *BRCA2*. We attempted to characterize the breakpoints of the one genomic rearrangement we found by long-range PCR using TaKaRa LA enzyme (TaKaRa). However, it was not possible to amplify the genomic fragment containing the MLPA alteration. The naming of mutations was based on the nucleotide number of the cDNA sequences in GenBank U14680.1 (*BRCA1*) and GenBank U43746.1 (*BRCA2*). For deletions and insertions in short tandem repeats, the most 3′ nucleotide was arbitrarily assigned as recommended by Antonarakis and the Nomenclature Working Group (31).

Statistical analysis. *t* tests were used to analyze differences in the mean ages of diagnosis. χ^2 tests were used to analyze differences in family history and the proportions for age at diagnosis. Fisher's exact tests were used to analyze differences in histology, stage, and grade, by mutation status. Unconditional logistic regression analysis was used to calculate the relative risks and 95% confidence intervals of ovarian cancer, breast cancer, prostate cancer, or any cancer in first-degree relatives of mutation carriers.

Results

BRCA1 ***and*** **BRCA2** ***mutations in ovarian cancer cases from Denmark.*** We evaluated the prevalence of germ-line *BRCA1* and *BRCA2* mutations in a population-based series of 445 invasive epithelial ovarian cancer cases from Denmark. Both

genes were screened using a combination of DNA sequencing to identify coding sequence and splice-site alterations and MLPA to identify large genomic rearrangement mutations.

Deleterious *BRCA1* mutations were found in 22 cases (4.9%) and *BRCA2* mutations in 4 cases (0.9% Table 1). Thus, *BRCA1* and *BRCA2* mutations are responsible for at least 5.8% of ovarian cancer cases in this population. Twenty-five mutations were in the coding region and were either frame-shift deletions or insertions of 1 to 5 bp or nonsense substitutions. We identified one genomic alteration by MLPA, which resulted in an in-frame deletion of exons 17 to 19. We have included this as a deleterious mutation, although it is not predicted to lead to protein truncation. The deletion would remove 207 bp of the *BRCA1* coding sequence and 69 amino acids (3.7% of the predicted protein). Five different mutations were found in more than one individual from the study, suggesting that they may be founder mutations in the Danish/Scandinavian population. Combined, these mutations accounted for nearly half of all the mutations identified. The most common mutations were 2594delC and 3829delT, both in *BRCA1*, which were found in four and three cases, respectively.

No alterations that are predicted to affect splicing were identified. Neither did we find any of a small number of previously reported missense mutations that are known to be deleterious. However, we did identify several rare nonsynonymous base changes, some of which have been reported before in the Breast Cancer Information Core (BIC) database (32); others are previously unreported variants (see Table 2). Thirteen variants in *BRCA1* and 27 variants in *BRCA2* had an allele frequency of <0.01 and were nonsynonymous sequence changes for which there may potentially be disease associated function (Table 2). We attempted to model the predicted functional effect for these alterations using the programs PMut, MuPro, and SIFT (33–35). Eight of these variants were predicted to be pathogenic using at least two of the measures. These were L1198W and R1347G in *BRCA1* and A75P, S1832P, D2665G, T2766I, N2781I, and K2860T in *BRCA2*. However, two of these variants (L1198W and T2766I) were present in two patients that also had different termination mutations in *BRCA1*, which suggests that they are unlikely to be pathogenic. The variants R1347G, A75P, and D2665G have been reported on the BIC database 154, 49, and 26 times, respectively, where they are described as of "unknown" or of "no" clinical significance. Finally, several known common single nucleotides polymorphisms in the coding region were also found (data not shown).

Table 1. Mutation and family history information and histologic classification in patients with a *BRCA1* or *BRCA2* mutation

Case ID*	Age (y) at diagnosis†	Mutation details‡						Family history§ ovary	Family history§ breast	Histologic diagnosis		
		Gene	Mutation ID	Coding exon	Nucleotide number	Type	Termination codon			Type	Grade	Stage
1857	61	*BRCA1*	1675delA	11	1675	FS	531	+	+	S	—	III
2122	55	*BRCA1*	Q563X	11	1806	NS	563	-	-	S	2	III
3969	45	*BRCA1*	Q563X	11	1806	NS	563	+	-	S	3	III
1690	48	*BRCA1*	2594delC	11	2594	FS	845	-	-	C	3	IV
4184	66	*BRCA1*	2594delC	11	2594	FS	845	-	-	S	3	IV
922	49	*BRCA1*	2594delC	11	2594	FS	845	-	-	S	3	III
4961	38	*BRCA1*	2594delC	11	2594	FS	845	-	-	S	3	IV
4335	52	*BRCA1*	2644insG	11	2644	FS	851	-	+	S	3	III
1806	47	*BRCA1*	2644insG	11	2644	FS	851	+	-	S	3	III
4216	37	*BRCA1*	3172insTGAGA	11	3172	FS	1023	+	-	S	3	III
298	51	*BRCA1*	E1060X	11	3297	NS	1060	-	+	C	2	III
1940	67	*BRCA1*	E1107X	11	3438	NS	1107	+	+	P	3	II
717	52	*BRCA1*	3582insCTGTT	11	3582	FS	1156	-	-	E	3	III
2958	45	*BRCA1*	3819delGTAAA	11	3819	FS	1242	-	-	E	3	III
3054	45	*BRCA1*	3829delT	11	3829	FS	1263	-	-	S	3	III
4538	35	*BRCA1*	3829delT	11	3829	FS	1263	-	-	S	3	IV
4385	43	*BRCA1*	3829delT	11	3829	FS	1263	-	-	P	3	III
50	42	*BRCA1*	4780delC	15	4780	FS	1558	-	-	S	3	III
4084	52	*BRCA1*	del exons17-19	17	5106	IFD	1795	+	-	S	2	III
4368	70	*BRCA1*	5386insC	20	5386	FS	1829	-	-	P	3	III
1641	66	*BRCA1*	5386insC	20	5386	FS	1829	-	-	S	3	III
1182	49	*BRCA1*	5460delG	22	5460	FS	1792	+	-	U	3	III
737	57	*BRCA2*	2021delA	10	2021	FS	613	-	-	E	3	III
142	45	*BRCA2*	3036delACAA	11	3036	FS	959	+	-	P	3	III
3751	53	*BRCA2*	4075delGT	11	4075	FS	1284	+	+	S	2	II
691	48	*BRCA2*	7297delCT	14	7297	FS	2358	-	+	C	3	III

Abbreviations: S, serous; E, endometrioid; C, clear cell; P, papillary adenocarcinoma; U, undifferentiated.
*For purposes of patient confidentiality, all case IDs have been coded and do not correspond to any previously used nomenclature.
† Age at which the diagnosis of ovarian cancer was made.
‡ Mutation details include gene name (*BRCA1* or *BRCA2*), mutation identification nomenclature (based on nomenclature from the BIC database), coding exon, nucleotide number, mutation type [frame-shift (FS), nonsense (NS), or in-frame deletion (IFD)], and termination codon.
§ Family history of ovarian cancer or breast cancer <60 y among first-degree relatives.

Associations between **BRCA1** *and* **BRCA2** *mutation status, family history, and clinical characteristics of disease.* We examined the relationship between mutation status and a family history of breast, ovarian, colorectal, prostate, and "other" cancers in first-degree relatives of cases from this population (Table 3). Nine *BRCA1* and *BRCA2* mutation carriers (35%) had one or more first-degree relative(s) with confirmed epithelial ovarian cancer. This contrasts with cases in which no mutations were found; only 5% of these cases had a first-degree relative with ovarian cancer (P = 0.00001). Similarly, six mutation carriers (23%) had a family history of breast cancer <60 years in first-degree relatives compared with 3% of noncarriers (P = 0.0007). The relative risks (95% confidence intervals) of ovarian and breast cancer <60 years in first-degree relatives of mutation carriers were 10.6 (4.2-26.6) and 8.7 (3.0-25.0), respectively (P < 0.0001 for both). We found no statistically significant differences in the risks for prostate or any other cancers in carriers compared with noncarriers, although the numbers were small (data not shown).

Table 2. Rare, nonsynonymous sequence variants identified in patients from the MALOVA study

Gene	Sequence variant			BIC report	BIC function	MuPro stability	SIFT conservation	PMut effect	PMutconf. value
	Nucleotide change	Amino acid	Allele frequency						
BRCA1	684 G>A	D189N	0.001	0	—	-1.2	A low conf	Neutral	6
BRCA1	1219 C>G	P371A	0.001	0	—	-0.9	T 0.09	Neutral	4
BRCA1	1606 G>A	R496H	0.001	85	Unknown	-1.1	T 0.98	Path	2
BRCA1	1841 C>T	P568S	0.001	0	—	-1.2	T 0.05	Neutral	8
BRCA1	1998 G>A	V627I	0.001	2	Unknown	-0.04	T 0.17	Neutral	9
BRCA1	2110 G>A	R664K	0.001	0	—	-0.9	T 0.68	Neutral	9
BRCA1	3238 G>A	S1040N	0.009	45	Unknown	-0.8	T 0.09	Neutral	4
BRCA1	3491 C>G	F1124L	0.001	0	—	-0.6	T 0.14	Path	4
BRCA1	3537 A>G	S1140G	0.001	28	Unknown	-1.5	T 0.23	Neutral	4
BRCA1	3712 T>G	L1198W*	0.002	0	—	-1.3	A 0	Path	8
BRCA1	4158 A>G	R1347G	0.008	154	Unknown	-1.3	A low conf	Path	7
BRCA1	4654 G>T	S1512I	0.003	53	NO	0.2	A low conf	Path	3
BRCA1	5075 G>A	M1652I	0.005	39	Unknown	-0.9	T 0.17	Path	0
BRCA2	451 G>C	A75P	0.002	49	Unknown	-1.2	A 0.02	Path	8
BRCA2	1379 C>T	S384F	0.002	141	NO	-0.5	T 0.32	Path	7
BRCA2	1688 C>A	A487E	0.001	12	Unknown	-0.3	T 0.06	Path	7
BRCA2	1694 C>G	S489C	0.001	0	—	-0.8	T 0.22	Path	0
BRCA2	1742 T>C	I505T	0.002	128	NO	-2.4	T 0.76	Path	0
BRCA2	2014 C>G	D596H	0.001	49	NO	-0.6	A 0.02	Path	1
BRCA2	2020 A>G	T598A †	0.004	62	NO	-1.1	T 0.51	Neutral	5
BRCA2	2032 G>A	G602R	0.001	10	Unknown	-1.3	T 0.07	Path	5
BRCA2	3031 G>A	D935N	0.001	105	NO	-0.8	T 0.09	Neutral	3
BRCA2	3079 C>T	L951F	0.001	0	—	-0.8	T 0.83	Neutral	5
BRCA2	5299 A>C	K1691Q	0.001	0	—	-0.3	T 0.23	Path	2
BRCA2	5642 A>G	N1805S	0.001	3	Unknown	-0.8	T 0.24	Path	4
BRCA2	5722 T>C	S1832P	0.001	0	—	-1.5	T 0.30	Path	7
BRCA2	7049 G>T	G2274N	0.001	15	Unknown	-0.4	A 0.02	Path	3
BRCA2	7844 A>G	Q2539R	0.001	0	—	-0.8	A 0.03	Path	5
BRCA2	8222 A>G	D2665G	0.001	26	NO	-1.3	T 0.08	Path	8
BRCA2	8377 G>T	A2717S	0.005	110	NO	-1	T 0.75	Neutral	8
BRCA2	8410 G>A	V2728I ‡	0.006	87	NO	-0.9	T 2.74	Neutral	7
BRCA2	8525 C>T	T2766I *	0.001	0	—	-0.006	A 0.01	Path	7
BRCA2	8570 A>T	N2781I	0.001	0	—	-0.1	A 0	Path	9
BRCA2	8795 A>C	E2856A	0.004	185	NO	-0.7	T 0.09	Path	6
BRCA2	8807 A>C	K2860T	0.001	0	—	-0.7	A 0.03	Path	8
BRCA2	9078 G>T	K2950N	0.001	111	Unknown	-0.6	T 0.07	Path	7
BRCA2	9133 G>A	V2969M	0.001	24	Unknown	-0.6	A 0.01	Path	1
BRCA2	9415 C>T	P3063S	0.001	2	Unknown	-1	T 0.07	Path	3
BRCA2	9812 C>T	T3195I	0.001	0	—	-0.5	T 0.50	Path	1

*One patient also had a termination mutation.
† Two patients also had a termination mutation.
‡ One patient was homozygous for this change. BIC function, predictions from BIC if change is clinically important. No structural information for full BRCA1 and BRCA2 proteins. MuPro, protein stability from sequence using support vector machines. Value is energy change (ΔΔG). Negative, decreased stability; positive, increased stability. Known functional missense mutation C61G MuPro value -1.5. SIFT prediction of tolerated amino acid changes based on protein sequence conservation across species <0.05 = A (affects protein function) and ≥0.05 = T (tolerated). Low conf, there is low confidence in this result. Note that G at 2274 in normal sequence of BRCA2 is not tolerated by prediction. PMut prediction and also confidence value 1 to 10 from neural networks trained on large numbers of neutral and pathogenic mutations. C61G PMut Path 3. Q2539R changes a base in the donor 5′ splice site sequence. Consensus sequence is AG/gtaagt, splice boundary exon 18 AG/gtatgt, and missense mutation GG/gtatgt. Changes splice site score from 90 to 81, max 100 (http://ast.bioinfo.tau.ac.il/SpliceSiteFrame.htm). PMut, MuPro, and SIFT (33–35).

Table 3. Clinical and family history information for all cases from the MALOVA study screened for *BRCA1* and *BRCA2* mutations and correlation with mutation status

	All cases, *n* (%)	Mutation status, *n* (%)			
		BRCA1	*BRCA2*	Non-*BRCA1/BRCA2*	
				Family history*	No family history*
Total no. subjects	445	22	4	33	386
Median (95% confidence interval) age (y) at diagnosis	61 (32-80)	49 (35-70)	51 (45-57)	61 (37-74)	61 (32-80)
Mean ± SE age (y) at diagnosis	60 ± 0.5	51 ± 2.11	51 ± 2.66	61 ± 1.59	60 ± 0.54
Age (y) at diagnosis (by mutation status)					
<40	13 (3)	3 (14)	0	1 (3)	9 (2)
40-49	71 (16)	9 (41)	2 (50)	2 (6)	58 (15)
50-59	130 (29)	5 (23)	2 (50)	12 (36)	111 (29)
≥60	231 (52)	5 (23)	0	18 (55)	208 (54)
Age (y) at diagnosis (by age group)					
<40	13	3 (23)	0	1 (8)	9 (69)
40-49	71	9 (13)	2 (3)	2 (3)	58 (82)
50-59	130	5 (4)	2 (2)	12 (9)	111 (85)
≥60	231	5 (2)	0	18 (8)	208 (90)
Family history					
≥1 ovarian/breast cancer	47 (10.1)	9 (41)	3 (75)	33 (8)	—
≥1 ovarian cancer	29 (6.5)	7 (32)	2 (50)	20 (5)	—
≥1 breast cancer	21 (4.7)	4 (18)	2 (50)	15 (4)	—
Prostate cancer	25 (5.6)	0	1 (25)	0	24 (6)
Colorectal cancer	47 (10.6)	3 (13.6)	0	4 (12)	40 (10)
Other cancer	272 (61)	13 (59)	4 (100)	33 (100)	222 (58)
Histopathologic type					
Serous	275 (62)	14 (64)	1 (25)	23 (70)	237 (61)
Endometrioid	56 (13)	2 (9)	1 (25)	4 (12)	49 (13)
Mucinous	43 (10)	0	0	3 (9)	40 (10)
Clear cell	33 (7)	2 (9)	1 (25)	2 (6)	28 (7)
Undifferentiated	8 (2)	1 (5)	0	0	7 (2)
Other	30 (7)	3 (14)	1 (25)	1 (3)	25 (7)
Clinical stage (FIGO)					
I	106 (24)	0	0	8 (24)	98 (25)
II	41 (9)	1 (5)	1 (25)	1 (3)	38 (10)
III	252 (57)	17 (77)	3 (75)	22 (67)	210 (54)
IV	46 (10)	4 (18)	0	2 (6)	40 (10)
Grade					
Well differentiated (1)	104 (23)	0	0	10 (30)	94 (24)
Moderately differentiated (2)	145 (33)	2 (9)	1 (25)	10 (30)	132 (34)
Poorly/undifferentiated (3)	168 (38)	19 (86)	3 (75)	11 (33)	135 (35)
Unknown	28 (6)	1 (5)	0	2 (6)	25 (6)

*Family history is defined as breast cancer <60 y and/or ovarian cancer at any age in a first-degree relative.

Age at diagnosis and family history of cancer were strong predictors of *BRCA1/BRCA2* mutation status (Table 3). Approximately half of all cases in this population were diagnosed with ovarian cancer over 60 years. The median age of ovarian cancer diagnosis in mutation carriers was substantially less than for individuals without a mutation (49 versus 61 years; $P < 0.0001$). Fifty-four percent of *BRCA1* and *BRCA2* carriers were diagnosed before age 50 years compared with 19% of noncarriers. Mutations were identified in 23% of cases aged <40 years, 13% of cases aged 40 to 49 years, 4% of cases aged 50 to 59 years, and 2% of cases aged ≥60 years; this trend was significantly different compared with noncarriers ($P = 0.00002$). Of 445 cases in this study, 45 (10%) had a family history of ovarian cancer or breast cancer <60 years in first-degree relatives; 12 of these cases harbored a *BRCA1* or *BRCA2* mutation (46% of all mutation carriers). In total, 20 of 26 mutation carriers (77%) were either diagnosed <50 years and/or had a family history of ovarian/breast cancer, although only 27% of cases from this cohort fulfilled one or both of these criteria.

Previous studies have suggested that the presence of a germline *BRCA1* or *BRCA2* mutation may correlate with the histologic subtype of ovarian cancers. In this series, 62% of tumors from nonmutation carriers were serous histology compared with 64% of mutation carriers. The only notable difference between the two groups was in the proportion of mucinous cases, although the numbers were small; no tumors from carriers were mucinous compared with 10% of non-*BRCA1/BRCA2* tumors that were. We found significant differences between carriers and noncarriers for stage at diagnosis and tumor grade. Ninety-five percent of carriers were diagnosed at stages III/IV compared with 65% of noncarriers ($P = 0.002$); 86% of *BRCA1/BRCA2* tumors were poorly differentiated compared with only 35% of non-*BRCA1/BRCA2* tumors ($P = 0.0001$; Table 3). Ovarian cancer cases with no identifiable mutation, but with a family history of ovarian/breast cancer, more closely resembled the noncarriers without a family history rather than the mutation carriers (Table 3). We were unable to evaluate associations between mutation status and survival due to the small numbers of mutation carriers identified in the study.

Discussion

We have established the prevalence of *BRCA1* and *BRCA2* mutations in a population-based study of 445 ovarian cancer cases from Denmark. Twenty-six deleterious mutations were identified: 22 mutations in *BRCA1* (4.9%) and 4 mutations in *BRCA2* (0.9%). Thus, the combined prevalence of *BRCA1* and *BRCA2* mutations in this series was 5.8%. To our knowledge, this is the first such investigation in an ovarian cancer case series from Denmark, so we are unable to draw direct comparisons with other Danish studies. However, there have been a few studies that have reported the contribution of *BRCA1* and/or *BRCA2* to ovarian cancer in other populations. These studies provide different and wide-ranging estimates of mutation prevalence for these genes, suggesting a degree of genetic heterogeneity between different populations. Other possible explanations for the variation seen between studies include ascertainment bias in population collections, the presence of founder mutations and ethnic bias between different populations, and the completeness and accuracy of mutation screening.

The *BRCA1* and *BRCA2* prevalence estimates from this study are consistent with those reported in other populations, but they are substantially lower than for two other studies (11 – 13). Risch et al. initially reported on 515 invasive ovarian cancer cases and later on an additional 462 cases (977 cases in total) as part of the same cohort from Ontario, Canada (13). *BRCA1* and *BRCA2* mutations were found in 13.2% of cases. Pal et al. found *BRCA1* and *BRCA2* mutations in 15.2% of 208 ovarian cancer cases from west central Florida (12). A main reason for the differences between these studies and ours is the high frequency of *BRCA2* mutations identified (5.5% and 5.8%, respectively). The ratios of *BRCA1* to *BRCA2* mutations were 1.4:1 and 1.7:1, respectively, compared with 5.5:1 for our study. In this respect, the current study is more similar to a recent analysis of *BRCA1* and *BRCA2* in 283 ovarian cancer families, in which the ratio of *BRCA1* to *BRCA2* mutations was 5.9:1 in the UK population (6). Another contributing factor to the high frequency of mutations identified by Risch et al. and Pal et al. is the presence of Ashkenazi Jewish founder mutations, which represent 10.1% and 21.9%, respectively, of all mutations identified in each study. Finally, the studies of Risch et al. and Pal et al. both contain an unusually high proportion of cases with a family history of ovarian and/or breast cancer, which are much more likely to have a *BRCA1* and *BRCA2* mutation. Twenty-eight percent of cases in the study by Pal et al. and 25% of cases in the study by Risch et al. had at least one first-degree relative diagnosed with breast or ovarian cancer; this compares with only 10% of cases in our study.

Other *BRCA1* and *BRCA2* mutation screening studies from Scandinavia provide insights into mutation prevalence in northern Europe and of founder mutations that may also be common in Denmark. Most reports of mutation screening in Scandinavian ovarian cancer cases are based on the analysis of selected founder mutations in population studies. In a study from Finland, 20 different *BRCA1* and *BRCA2* mutations that had been identified previously in the Finish population were present in 6% of 233 unselected ovarian cancer cases (14). In another study, two common Norwegian *BRCA1* founder mutations were identified in 3% of 615 ovarian cancer cases (16). More recently, a Swedish study identified deleterious *BRCA1* and *BRCA2* mutations in 8% of 161 ovarian cancer cases (8).

Four of the five *BRCA1* mutations found more than once in this study may be founder mutations in Denmark or Sweden. The 5386insC mutation (also known as 5382insC) is common throughout Europe and is also a founder mutation in the Ashkenazi Jewish population. The 2594delC mutation constituted 18% of *BRCA1* mutations in this study and was found at a similar frequency (17%) in the study of breast cancer cases from Denmark (20). The 3829delT and Q563X mutations have also been reported in studies from Sweden, suggesting that they may be Swedish/Danish founder mutations. However, we did not identify other north European founder mutations including the *BRCA2* 999del5 mutation common to Iceland, the *BRCA1* 1675delA and 1135insC mutations common to Norway, and the *BRCA1* 3172ins5 and 1201del11 mutation common to Sweden. Finally, the 2644insG mutation, which we found in two cases, has never been reported before in Scandinavia or elsewhere.

It is unlikely that any of the population-based studies described for ovarian cancer will have detected all of the mutations present in the populations studied, so the prevalence of *BRCA1* and *BRCA2* mutations will have been underestimated. Only one of the studies published thus far analyzed *BRCA1* and *BRCA2* for large genomic rearrangement mutations, although this type of alteration can be common, particularly in the *BRCA1* gene (23). In the current study, one of the 22 *BRCA1* mutations (~5%) was a large genomic deletion. Mutations may also have been missed due to the sensitivity of the mutation detection techniques. We used sequencing, which is widely considered the most accurate approach to mutation screening, but mutations may have been missed because we were unable to completely sequence the entire coding region in all samples. Finally, some missense alterations, which are often not recorded as deleterious alterations, may be pathogenic.

We identified several correlations between mutation status and either a family history of cancer or clinical features of disease. In the main, these analyses were restricted to *BRCA1* carriers due to the small number of *BRCA2* mutation carriers identified in this study. Not surprisingly, mutation carriers were more likely than noncarriers to have a history of ovarian and breast cancer. However, it is perhaps surprising that more than half of all mutation carriers had no affected first-degree relatives, suggesting that breast/ovarian cancer risk estimates are not as high as suggested from familial studies. Antoniou et al. (36) estimated the risks of breast and ovarian cancer at age 70 years to be 72% and 53%, respectively, for *BRCA1* carriers in a study of ovarian cancer families. In the same study, the risks associated with *BRCA2* were 71% for breast cancer and 31% for ovarian cancer. However, breast and ovarian cancer risks calculated from a meta-analysis of population-based breast/ovarian cancer studies suggests much lower risks; the average cumulative risks of breast and ovarian cancer by age 70 years in *BRCA1* carriers were 65% and 39% and in *BRCA2* mutation carriers 45% and 11%, respectively (37). In this study, the estimated relative risk (95% confidence interval) for ovarian cancer [10.6 (4.2-26.6)] is comparable with those of the study of 977 ovarian cancer cases from Canada [10.3 (6.01-17.6) for *BRCA1* mutation carriers and 3.46 (1.55-7.72) for *BRCA2* mutation carriers; ref. 13].

There are now several studies that have investigated links between germ-line genetic variation and clinicopathologic features of ovarian cancer. We found no evidence that *BRCA1* tumors are more likely to be serous compared with non-*BRCA1* tumors as suggested by a previous study (25). Lakhani et al. (25) examined 178 invasive ovarian cancers from *BRCA1* mutation carriers and found that 44% were serous compared with 31% of non-*BRCA1* tumors. As in the current study, Lakhani et al. also found a significant difference in grade between *BRCA1* and non-*BRCA1* tumors; in both studies, *BRCA1* tumors were more poorly differentiated. We additionally found that patients with *BRCA1* tumors were more likely to be diagnosed at a later stage.

Patients in which a mutation was identified were diagnosed at a younger age than noncarriers; this was a highly significant result (P = 0.00002). Given the strength of this finding, it is perhaps surprising that the Canadian and Florida studies described above did not report something similar (5.7% and 3.1% of *BRCA1* carriers, respectively, were <40 years compared with 23% in the current study; refs. 12, 13). However, this trend with decreasing age appears to be validated by data from the Canadian study, which shows a similar effect; 11% of cases were diagnosed ≤50 years, 5% were 51 to 60 years, and 3% were >60 years (13). If these results are true, then they could have implications for clinical genetic testing. In the current study, by stratifying the ovarian cancer case population by age at diagnosis and family history of ovarian/breast cancer, we would have identified 77% of *BRCA1*/*BRCA2* mutation carriers by screening only 27% of the population.

In conclusion, we have evaluated the contribution of *BRCA1* and *BRCA2* mutations to ovarian cancer in a population-based series of cases from Denmark. The data were used to identify correlates between mutation status and both a family history of ovarian and breast cancer and clinical features of disease. Because this study is the first of its kind for ovarian cancer cases from Denmark, the findings are likely to be of clinical benefit in the future for individuals undergoing genetic testing and counseling for *BRCA1* and *BRCA2* because they have a family history of ovarian and/or breast cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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