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BRCA2, but not *BRCA1*, mutations account for familial ovarian cancer in Iceland: a population-based study☆

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Abstract

A single founder mutation in each of the BRCA genes has been identified in Iceland. The frequency of the *BRCA1 G5193A* and *BRCA2 999del5* mutations in all ovarian cancer patients diagnosed over the period 1991–2000 was determined. Mutation status was correlated with family history, tumour morphology and age at diagnosis. Samples from 86% of cases (179 carcinomas and 74 borderline tumours) were available. In the carcinomas, *BRCA1* and *BRCA2* mutations were present in 1.2% and 6% of cases, respectively. No BRCA mutations were found in the borderline tumours. Odds Ratio (OR) of developing ovarian cancer was 20.65 for *BRCA2* carriers. Family history of breast/ovarian cancer was present for 70% of *BRCA2* carriers and approximately 14% for non-carriers with carcinoma. In conclusion, *BRCA2 999del5* is present in 6% of ovarian cancer cases in Iceland and is associated with a 20-fold increase in the risk of the disease. The *BRCA1 G5193A* mutation is too rare to contribute significantly to ovarian cancer in Iceland.

Keywords: Ovarian cancer; Iceland; *BRCA1 G5193A*; *BRCA2 999del5*; Population-based; Family history

1. Introduction

Mutations in the *BRCA1* and *BRCA2* genes predispose carriers to cancers of the breast, ovaries and other organs [1]. The prevalence of *BRCA1* and *BRCA2* mutations in ovarian cancer cases unselected for family history varies between populations, but ranges from 3% to 8% for *BRCA1* and 2% to 4% for *BRCA2* (reviewed in [2]). The average cumulative risk of ovarian cancer by the age of 70 years in cases unselected for family history is estimated to be approximately 40% and 11% for *BRCA1* and *BRCA2* mutation carriers, respectively [3]. Not surprisingly, the estimated risk of breast and ovarian cancer in BRCA mutation carriers is generally higher in studies that are based on families with multiple cases of cancer than in studies that are based on an unselected series of cases. In addition, it has been reported that a significant proportion of BRCA-associated ovarian cancers has no family history [4,5]. Thus, it is clear that the overall risk of familial ovarian cancer depends to a large extent on the type of mutation and the genetic and environmental context.

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Studies of the genomic regions around *BRCA1* and *BRCA2* in patients with family history of breast cancer strongly suggest that only a single founder mutation in each of the genes exist in Iceland [6,7]. The *BRCA1* mutation, *BRCA1 G5193A*, is a single base pair substitution that disrupts a splice site in the mRNA [8]. This mutation is extremely rare and is present in less than 1% of breast cancer cases in Iceland [8]. Its occurrence in ovarian cancer has so far not been examined. The *BRCA2* mutation, *BRCA2 999del5*, is found in 7–8% of female and 40% of male breast cancers in the country [6]. The mutation explains a substantial proportion of the familial risk of breast cancer in Iceland and accounts for most of the familiality of prostate and ovarian cancer observed in families of breast cancer patients [9]. The cumulative risk of breast cancer by the age of 70 years associated with this particular mutation was originally estimated to be 37.2% [10], but re-analysis of the data suggests that it may be higher, approximately 45% [3]. A pilot study that tested the frequency of *BRCA2 999del5* in various tumours in Iceland included approximately 30 archived ovarian cancer samples and reported the mutation to be present in approximately 8% of these cases [11].

In this study, we determined the contribution of both *BRCA1* and *BRCA2* founder mutations to ovarian cancer in Iceland in consecutive ovarian cancer patients who were unselected for age or family history. By cross-referencing genealogy information with the National Cancer Registry, it was possible to accurately determine the family history of cancer for all ovarian cancer patients.

2. Patients and methods

2.1. Patient samples

Information on all women who were diagnosed with ovarian cancer in the 10-year period from 1991 to 2000, a total of 293 women, was obtained from the Icelandic Cancer Registry. Samples from 133 live patients were collected through the Icelandic Cancer Project [12], while paraffin-embedded tissue specimens from 120 deceased patients were obtained from the Department of Pathology, Landspitali University Hospital. In all, samples from 86% of patients diagnosed in this period were available for study. The genealogy of patients was constructed by the Genetical Committee of the University of Iceland. Data on age at diagnosis, type and stage of tumour and cancer in relatives were obtained from the Icelandic Cancer Registry and the Department of Pathology, Landspitali University Hospital. The study protocols were approved by the National Bioethics Committee and the Privacy and Data Protection Authority.

2.2. Isolation of DNA and genotyping

DNA was isolated from blood using standard methods employing digestion by proteinase K and phenol–chloroform extractions. For paraffin-embedded tissue, DNA was extracted from 15-μm thick sections, as described by Wright and Manos [13].

Genotyping of the founder mutation, *BRCA1 G5193A* was performed by polymerase chain reaction (PCR) amplification of a 258-bp fragment centred over the site of the *BRCA1* mutation, followed by denaturing high-performance liquid chromatography (DHPLC) analysis to detect mismatched fragments. The sequences of the *BRCA1* PCR primers were: forward primer 5′-TGCTCGTGTACAAGTTTGCC, reverse primer 5′-GTAGAGACGGGGTTTCACCA. The reaction mixture contained 1 μl tissue extract from paraffin samples or 30 ng DNA from blood, 5 μl Thermostart PCR Master Mix (ABgene) and 6 pmol of each primer in a 10-μl reaction. The PCR reaction proceeded as follows: 94 °C for 15 min; 40 cycles of 94 °C for 30 s, 62 °C for 45 s, 72 °C for 45 s; followed by heating to 72 °C for 5 min and cooling to 4 °C. Five μl water were added to each reaction and heteroduplex formation induced by heating the samples to 94 °C for 5 min before cooling slowly to room temperature. The samples were analysed by DHPLC (WAVE System, Transgenomic) using the following profile: Loading of samples in 54% Acetonitrile for 0.1 min followed by a linear gradient from 57% to 62% acetonitrile during a period of 3.1 min. Heterozygotes were detected as two double peaks. Fragments from heterozygous individuals were sequenced to confirm the mutation, using the same primers as for PCR. Genotyping for *BRCA1 G5193A* was successful in 244 samples out of 253, the 9 samples that failed to amplify were all from paraffin-embedded tissue.

Samples containing the *BRCA2 999del5* mutation were identified by PCR amplification of a 273-bp fragment spanning the site of the 5-bp deletion, followed by size fractionation of the resulting product. The sequences of the *BRCA2* PCR primers were: forward primer 5′-CTAGGTTGATTGCAGATAACTGAA, reverse primer 5′-AAAACCTGTAGTTCAACTAAACAG. The PCR reaction mixture contained 1 μl tissue extract from paraffin samples or 15 ng DNA from blood, 5 μl Thermostart PCR Master Mix and 3 pmol of each primer in a 10 μl reaction. The PCR reaction proceeded as follows: 94 °C for 15 min; 40 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 80 s; followed by heating to 72 °C for 5 min and cooling to 4 °C. The PCR products were diluted 40-fold, denatured at 98 °C for 1 min and analysed on MegaBace1000 Genotyping Instrument (Amersham Biosciences) and analysed with Genetic Profiler Software from the same manufacturer. Genotyping for *BRCA2 999del5* was successful in

241 samples, the 12 samples that failed were all from paraffin-embedded tissue.

2.3. Statistical analysis

Confidence Intervals (95% CI) for the binomial proportions were calculated using the continuity corrected version of the score interval, since it has better coverage probability than the standard method [14]. Odds Ratios (OR) were calculated using modified empirical logits, since they behave better than the usual OR for small numbers [15]. Difference in distribution was assessed using the Wilcoxon two-sample rank-sum test [16]. The non-parametric Wilcoxon-test was used instead of the parametric *t*-test, since the distribution of the age at diagnosis could not be reliably estimated for the small number of *BRCA2* carriers. All statistical analyses were done using the statistical system R [17].

2.4. Analysis of family history

The occurrence of ovarian, breast and other cancers was documented in first- and second-degree relatives of patients. A patient was determined to have a family history of breast/ovarian cancer if she had (i) at least one first-degree relative diagnosed with ovarian cancer at any age, or breast cancer when younger than 40 years, or bilateral breast cancer when younger than 60 years, or at least one first-degree male relative with breast cancer or (ii) two first- or second-degree relatives with breast cancer diagnosed when younger than 60 years or ovarian cancer at any age on the same side of the family or (iii) three first- or second-degree relatives with breast or ovarian cancer at any age on the same side of the family. These criteria are similar to the familial ovarian cancer criteria used by the United Kingdom Coordinating Committee on Cancer Research (UKCCCR) [2].

3. Results

A total of 293 women were diagnosed with ovarian cancer in Iceland from 1991 to 2000. Of those, 98 had tumours of borderline malignancy and 195 had invasive carcinomas. Samples and information from 253 of these patients (86%) were available for study (Table 1).

3.1. BRCA1 G5193A and BRCA2 999del5 and their association with ovarian cancer in Iceland

Only 2 out of 244 samples contained the *BRCA1 G5193A* founder mutation or 0.82% (Table 2). These cases were a mother/daughter pair and both had tumours of the serous type. Both cases had invasive carcinomas so if cases with borderline tumours are excluded, the frequency of this mutation is 1.2%. Therefore, although the risk of ovarian cancer in *BRCA1 G5193A* carriers may be high, this mutation is too rare to contribute significantly to ovarian cancer in Iceland.

Out of 241 individuals that were genotyped for *BRCA2 999del5*, 10 carried the mutation, or 4.1% (95% CI 2.1–7.7; Table 2). No mutation carriers had borderline tumours, translating to a mutation frequency of 6.0% (95% CI 3.1–11.1) in the group with carcinomas.

Table 1
Frequency, age distribution, histology and family history of ovarian cancer patients diagnosed from 1991 to 2000

	All cases	Borderline tumours	Carcinomas	*BRCA1/2*-negative carcinomas[a]	*BRCA2 999del5*-positive carcinomas	*BRCA1 G5193A*-positive carcinomas[b]
Study population						
In Cancer Registry 1991–2000	293	98	195			
In study (% of total)	253 (86%)	74 (76%)	179 (92%)	153	10	2
Age (years)						
<51	75 (30%)	41 (55%)	34 (19%)	29 (19%)	3 (30%)	
51–70	114 (45%)	26 (35%)	88 (49%)	74 (48%)	6 (60%)	
>70	64 (25%)	7 (9%)	57 (32%)	50 (33%)	1 (10%)	
Age range	15–92	15–83	21–92	21–92	45–84	
Average age (95% CI)	59 (57–61)	50 (47–54)	63 (61–65)	63 (60–65)	56 (48–65)	
Histology						
Serous	134 (53%)	37 (50%)	97 (54%)	81 (53%)	8 (80%)	2 (100%)
Endometrioid	18 (7%)	1 (1%)	17 (9%)	16 (10%)		
Mucinous	48 (19%)	29 (39%)	19 (11%)	16 (10%)		
Clear cell	10 (4%)	0	10 (6%)	10 (7%)		
Undifferentiated	24 (9%)	0	24 (13%)	21 (14%)		
Other	19 (8%)	7 (9%)	12 (7%)	9 (6%)	2 (20%)	
Family history	37 (15%)	7 (9%)	30 (17%)	21 (14%)	7 (70%)	2 (100%)

95% CI, 95% Confidence Interval.

[a] Cases with invasive carcinomas where typing for both *BRCA1* and *BRCA2* was successful.

[b] Age information not shown due to patient privacy issues.

Table 2
BRCA1 and *BRCA2* genotypes of ovarian cancer patients diagnosed from 1991 to 2000

Cancer type	# In study	*BRCA1 G5193A*		*BRCA2 999del5*	
		# (Of genotyped samples)	% (95% CI)	# (Of genotyped samples)	% (95% CI)
Borderline tumours	74	0 (74)	0	0 (74)	0
Invasive carcinomas	179	2 (170)	1.2 (0.1–3.3)	10 (167)	6.0 (3.1–11.1)
Total cases	253	2 (244)	0.8 (0.2–3.4)	10 (241)	4.1 (2.1–7.7)
Controls	1708	–	–	5 (1708)	0.3 (0.1–0.7)

To obtain a reliable estimate of the *BRCA2 999del5* mutation in the population, 1708 randomly selected, adult controls were genotyped. Of those, five (0.29%) tested positive for the mutation. The OR of developing ovarian carcinoma was 20.65 (95% CI 7.75–57.02) for *BRCA2 999del5* mutation carriers.

The range of age at diagnosis for *BRCA2*-positive cases was 45–84 years, but 21–92 years for *BRCA*-negative cases with invasive carcinoma. The average age at diagnosis was 56 years (95% CI 48.0–64.6) for *BRCA2 999del5* mutation carriers as opposed to 63 years (95% CI 60.2–65.0) for BRCA-negative cases. However, the two distributions were not significantly different ($P = 0.09$, two-sided Wilcoxon rank-sum test). The average age at diagnosis for the borderline tumours (50 years, 95% CI 46.7–53.5) was significantly lower than for the carcinomas.

3.2. Histological characteristics of BRCA2 999del5 tumours

Most ovarian tumours from *BRCA2* mutation carriers have been shown to have a serous morphology [18]. In our study, eight out of ten *BRCA2 999del5*-positive tumours and 53% of the *BRCA2*-negative cases had the serous morphology. However, this difference, did not reach statistical significance ($P = 0.11$, Fisher's exact test). The remaining two *BRCA2 999del5*-positive tumours had mesodermal-mixed morphology.

3.3. Family history of BRCA2 999del5 patients

The families of all cases were traced to the third-degree and information on cancer in relatives was obtained from the Icelandic Cancer Registry. Family history of breast/ovarian cancer was observed in 37 of all cases or 15% (Table 1). Family history was present in 9% of cases with borderline tumours, but 17% of cases with invasive carcinomas, this difference did not reach statistical significance (chi-square test: $\chi^2 = 2.23$, $P = 0.13$).

The 14 samples that could not be genotyped for either *BRCA1* or *BRCA2* were from paraffin blocks and hence came from deceased patients. To assess the possibility that we were missing BRCA-positive samples, we analysed the family history of all these individuals and, where possible, genotyped close living relatives with cancer. Of the 14 cases where typing of either *BRCA1* or *BRCA2* failed, 4 had a family history of breast/ovarian cancer; however, in all cases an affected first-degree relative was negative for the respective mutation. In 6 of 10 cases that were not genotyped and did not meet the criteria for family history, a first- or second-degree affected family member tested negative for the mutation.

4. Discussion

Our results show that while *BRCA1* mutations are very rare in ovarian cancer in Iceland, the founder mutation *BRCA2 999del5* alone accounts for close to 6% of all cases of ovarian carcinoma. This is somewhat higher than what has been observed in other population-based studies where *BRCA2* mutations have been found in 2–4% of ovarian cancer cases [2]. Studies of European and North American populations estimate that mutations in the BRCA genes are present in close to 10% of invasive epithelial ovarian cancer cases, with mutations in *BRCA1* being the more common. In some Jewish populations, the frequency of BRCA mutations has been estimated to be approximately 20–30% [3,5], again with a predominance of *BRCA1* mutations. It is notable that the world age-standardised incidence of ovarian cancer in Iceland is similar to that observed in other Western European populations or $15.8/10^5$.

The OR of developing ovarian cancer was 20.65 for carriers of the *BRCA2 999del5* mutation. This is in agreement with the conclusions from a recent meta-analysis of 22 studies involving 8139 index case patients, unselected for family history, with breast or ovarian cancer [3]. According to this analysis, the OR for developing ovarian cancer in *BRCA2* mutation carriers reached a maximum of 19 in the 50–59 year age group and then decreased.

In addition to *BRCA1* and *BRCA2*, several genes have been reported to have modest effect on ovarian cancer risk. In particular, the androgen receptor and the progesterone receptors have been extensively studied, but recently their link to ovarian cancer has been questioned [19]. A study of 112 families, containing at

least two confirmed cases of epithelial ovarian cancer, identified mutations in *BRCA1* or *BRCA2* in less than half of the families. However, segregation analysis suggested that familial clustering not due to *BRCA1* or *BRCA2* may be explained by the combination of chance clustering of sporadic cases and insensitivity of BRCA mutation detection [20]. Thus, it is possible that no additional highly penetrant ovarian cancer susceptibility genes remain to be found.

Considerable effort has been put into determining if other functional mutations in addition to *BRCA1 G5193A* and *BRCA2 999del5* are present in the Icelandic population. A study on 42 randomly selected sister pairs, diagnosed with breast cancer at the age of 60 years or younger, did not support the presence of other mutations [7]. Furthermore, genetic analysis of 1400 breast cancer patients and 150 ovarian cancer patients with flanking microsatellite markers showed strong association to the *BRCA1* and *BRCA2* regions; however, when the known *BRCA1 G5193A* and *BRCA2 999del5* mutation carriers were removed this association was lost (data not shown from the Icelandic Cancer Project [12]).

The epidemiological and clinical impact of the *BRCA2 999del5* founder mutation on cancer in Iceland has been extensively studied, particularly with regard to breast and prostate cancer [6,9–11,21–23]. The mutation precedes the so-called ovarian cancer cluster region, a central portion of the *BRCA2* gene where mutations seem to be associated with significantly higher risk of ovarian cancer, but lower risk of breast cancer than mutations in other regions of *BRCA2* [24,25]. Furthermore, functional studies on the *BRCA2 999del5* gene product showed it to be extremely unstable, suggesting that the *BRCA2 999del5* associated cancer risk is most likely due to haploinsufficiency [26].

To summarise, we analysed the contribution of the two founder mutations, *BRCA1 G5193A* and *BRCA2 999del5* to ovarian cancer in Iceland. Contrary to other populations, the contribution of *BRCA1* is very small. However, the *BRCA2 999del5* mutation alone accounts for approximately 6% of invasive ovarian carcinomas and carries a 20-fold increase in risk of the disease. Although non-significant, the same trends towards a lower age at diagnosis and serous morphology of *BRCA2*-associated tumours was observed as in other population-based studies. Family history of breast/ovarian cancers was more prevalent in cases with invasive carcinomas than in cases with borderline tumours; however, this difference was non-significant.

Conflict of Interest Statement

None declared.

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