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Original Paper

Three per cent of Norwegian Ovarian Cancers are Caused by *BRCA1* 1675delA or 1135insA

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Our aim was to determine the prevalence of two Norwegian *BRCA1* founder mutations in ovarian cancer patients, to identify carriers and their families for medical follow-up, and to study histopathological factors. Of a cohort of 727 ovarian cancer patients, 615 gave informed consent to testing. 2.9% (18/615) of the tested patients were found to be carriers of *BRCA1* 1675delA ($n = 13$) or 1135insA ($n = 5$). The total frequency of the mutations was 4.7% (8/171) in patients below 50 years of age, and zero (0/144) in patients above 70 years of age. In patients below 70 years of age, the frequency of 1675delA and 1135insA mutations was 2.8% and 1.0%, respectively. Out of 13 patients with 1675delA mutation, 4 had breast cancer. 14/16 (87.5%) families fulfilled clinical criteria for familial breast-ovarian cancer. Median age of onset of ovarian and breast cancer was 51 years and 37 years, respectively. Mutation carriers tended to have tumours with unfavourable prognostic factors. This is, to our knowledge, the highest reported frequency of founder mutations in a national ovarian cancer cohort (less than in the Ashkenazis). It seems justified to offer such testing to ovarian cancer patients below 70 years of age in Norway, identify their risk of breast cancer and offer medical follow-up to the families.

Key words: hereditary breast-ovarian cancer, *BRCA1*, founder mutations, prognostic factors
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INTRODUCTION

STUDIES HAVE shown that 3–10% of breast and ovarian cancer may be caused by *BRCA1* or *BRCA2* mutations [1]. The Nordic countries, except from Finland, exhibit some of the highest incidence rates of ovarian cancer in the world, 13.6 per 100 000 person years and the median age at diagnosis is 65 years. Early diagnosis is important for survival, as the 5-year survival rate is only 36% because 60% of the women are diagnosed in FIGO stage III–IV in advanced stage [2]. Screening for ovarian cancer is generally not recommended. However, in breast-ovarian cancer families medical follow up as screening for early diagnoses, treatment, and cancer prevention is urgently needed, together with evaluation of such programmes. Use of prophylactic surgery, chemoprevention in the form of contraceptive pills [3], and anti-oestrogens are all insufficient or may have unwanted side-effects. Studies have shown that regular examinations of breasts (mammography) and/or ovaries (vaginal ultrasound), and biomarkers (CA 125) in this high risk group may be useful [4, 5], but, results of long-term survival are not available.

Reports of founder mutations and their population frequencies have shown significant variation [6–12]. Owing to the unavailability of largescale mutation testing, laboratory costs and the plethora of different mutations in these genes, a family history of breast and ovarian cancer has been used for clinical diagnosis, and as criteria for identifying families to be tested. Knowledge of founder mutations and their geographic and ethnic origin may permit a more rational and less costly approach to genetic testing.

For the purpose of identifying *BRCA1* mutation carriers in a rational manner and offering them medical follow-up, we searched for founder mutations. A Norwegian *BRCA1* founder mutation (1675delA) was found by detecting a common haplotype in affected families in a high incidence region of familial breast-ovarian cancer in South West of Norway [9]. Another founder mutation 1135insA was detected by systematic search in DNA from 25 breast-ovarian cancer

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families [9]. We examined the prevalence of the two Norwegian *BRCA1* founder mutations in ovarian cancer patients, and studied histopathological findings.

PATIENTS AND METHODS

During May 1993 to January 1996 a total of 727 consecutive Caucasian, ovarian cancer patients were admitted to our hospital. All gynaecological cancer cases from the Southern region of Norway, making up 60% of the population, is referred to our hospital. Of the patients, 84.6% (615/727) agreed to participate, and blood samples were examined after informed consent. Of the 727 patients, 561 were below 70 years of age, and 84.0% ($n = 471$) agreed to participate.

Women found to be mutation carriers were offered genetic counselling, and classified according to our clinical criteria for familial breast-ovarian cancer on the basis of family history (Table 1) [4]. Complete family history was obtained including first and second degree relatives. All diagnoses of ovarian cancer were confirmed by histopathological reports whenever possible (always confirmed if classified familial breast-ovarian cancer); diagnosis of breast cancer has been shown to be reported with 99% accuracy when given by relatives. The families were approached through the index women, and offered appropriate genetic counselling and medical follow-up. As the activity was part of our health service, no separate research register was established.

Mutation analyses

DNA was extracted from frozen blood using the Nucleon kit (Amersham Life Sciences, Oslo, Norway).

Fragments analysis. 50 ng of sample DNA was used for a multiplex PCR-based fragment analysis. The reaction was performed in 50 µl at pH 8.3, containing 0.1 M Tris, 0.5 M KCl, and 1.5 mM MgCl, 1 mM dNTP, 50 pM of the four primers *BRCA1A*, *BRCA1B* (for analysis of a fragment containing the 1135insA), 1675U and 1675L (for analysis of a fragment containing the 1675delA (MedProbe, Oslo, Norway) and 1 unit of Taq polymerase produced in-house. The 30 PCR cycles were performed in a Gene Amp System 7600 cycles (PE-AB, Oslo, Norway) at: 94, 60 and 72°C with 30 sec at each step. The resulting two fragments were subjected to gel electrophoresis after denaturation using the Alf Express system (Pharmacia Biotech, Uppsala, Sweden) for 160 minutes and both fragments were scored for single insertions or deletions (fragments with primers *BRCA1A*/*B* and 1675U/L, respectively).

Sequencing. Following the identification of mutants, the verification procedure consisted of sequencing a PCR product. The PCR amplification was performed using the same conditions with some modifications. The two fragments were amplified separately, using 50 pM non-Cy5-labelled primers *BRCA1A* and *BRCA1B* for insA1135, or a different primer set, 11624 and 11716, for delA1675. The 25 PCR cycles were performed at: 94, 55 and 72°C with 30 sec at each step. Cycle sequencing was performed with 1 µl PCR product using a Thermo Sequenase 7-deaza-dGTP kit (Amersham Life Sciences, Oslo, Norway). The 30 PCR cycles were performed at: 45 s at 94°C, 45 s at 58°C and 30 sec at 72°C with Cy-5-labelled primers. Electrophoresis of the sequencing products was performed using the Alf Express with the same conditions as for fragment analysis.

Primers. The primer sequences were as follows (*BRCA1* cDNA base number from start codon (A = + 1)):

BRCA1A (950): 5′-Cy5-AACATAACAGATGGGCTG-GAAGT-3′

BRCA1B (1090): 5′-GATTCTCTGAGCATGGCAGT-TTC-3′

1675U (1524): 5′-TACATCAGGCCTTCATCCT-3′

1675L (1587): 5′-Cy5-AGGAGTCTTTTGAACTGC-3′

11624 (1505): 5′-TAAAGCGTAAAAGGAGAC-3′

RESULTS

We found that 2.9% (18/615) of the tested patients were carriers of *BRCA1* 1675delA ($n = 13$) or 1135insA ($n = 5$), 2.5% of the total cohort of 727 patients in the given period, as seen in Table 2. The total frequency of the mutations was 4.7% (8/171) in patients below 50 years of age, 3.3% (10/300) in patients between 50 and 70 years, and zero (0/144) in patients above 70 years of age. In patients below 70 years of age, the frequency of 1675delA and 1135insA mutations was 2.8% and 1.0%, respectively. The 18 mutation carriers represented 16 unrelated families, 11 families with the mutation 1675delA and 5 with 1135insA.

Our clinical criteria for familial breast-ovarian cancer on the basis of family history (Table 1) were fulfilled in 14/16 families (87.5%). Two families did not; one index woman had a grandmother (father's mother) with an undiagnosed cancer of the abdomen at the age of 78 years and a grandmother (mother's mother) with breast cancer at 65 years of age; the other index woman had a mother with cancer of the cervix at the age of 52 years.

In 7/14 families the mutation was inherited from the father by family history. However, the age of onset of ovarian cancer did not differ whether the mutation was inherited from the father or the mother, mean age was 51.6 years (range 39–68) versus 52.7 years (range 39–65), respectively. Inheritance of the mutation from one of the parents was verified by mutation testing in 6/14 families, 4/14 from the father and 2/14 from the mother. The rest of the families have not yet agreed to testing.

Mean and median age of onset of ovarian cancer among the mutation carrying women were 52.1 and 51.0 years (range 39–69), respectively. 4 of the 1675delA carriers had breast cancer as well, 2 had bilateral cancer. Mean age of onset of first breast cancer was 38.8 years (range 35.5–47.5).

Table 1. *Clinical criteria for families with hereditary breast/ovarian cancer*

1.	One with ovarian cancer who has a 1° relative *or* a 2° relative through male, with ovarian cancer or breast cancer (breast carcinoma ≤ 60 years of age)
2.	One with both ovarian and breast cancer (breast carcinoma ≤ 60 years of age)

Table 2. *Prevalences of* BRCA1 1675*delA and* 1135*insA according to age in consecutive ovarian cancer patients*

		Patients (%) with founder mutations		
Age (years)	Patients tested	1675delA	1135insA	Total
≤ 49	171	7 (4.1)	1 (0.6)	8 (4.7)
50–70	300	6 (2.0)	4 (1.3)	10 (3.3)
70 +	144	0	0	0
Total	615	13 (2.1)	5 (0.8)	18 (2.9)

Table 3. Type of mutation according to histology of the ovarian cancers, grade of differentiation, FIGO stage, and previous breast cancer (n = 18)

	Mutation		
	1675delA	1135insA	Total
Histology			
Serous	10	5	15
Mixed	2	0	2
Undifferentiated	1	0	1
Grade of differentiation			
High	0	0	0
Medium	1	1	2
Low	11	4	15
Unknown	1	0	1
FIGO stage			
I	1	1	2
II	1	0	1
III	9	2	11
IV	2	2	4
Breast cancer	4 (2 bilateral)		4

The histology, FIGO stage, and grade of the ovarian cancers in the mutation carriers are described in Table 3. All women had serous (one had undifferentiated) epithelial ovarian cancer, and 15/18 had stage III–IV disease.

DISCUSSION

In Norway the high proportion of inherited cancer is due to founder effects assumed to be caused by the rapid population expansion following the Bubonic plague (Black Death) in the 13th century, killing more than 50% of the population [10]. During the last 600 years (~23 generations) the population increased from approximately 200 000 to 4.5 million [10]. Our result, to our knowledge, is the highest reported frequency of founder mutations in a national ovarian cancer cohort, but less than in the Ashkenazis [7]. It is surprising that these mutations were not found in a study by Stratton and colleagues [6], since we assumed the populations of south-east England and the south of Norway, with historical trade and relations, could be genetically related. The findings of a variety of population prevalences of frequent mutations in the Norwegian, Dutch [11], Swedish [8] and U.K. [6] populations demonstrate that validity of population data are limited to the population examined.

In 50% of the mutation carriers the mutation was inherited from the father by family history. We found no sign of imprinting as the age of onset of ovarian cancer in the index women did not differ in the families whether the mutation was inherited from the father or mother (mean age of onset of ovarian cancer was 51.6 versus 52.7 years, respectively). The sensitivity of our clinical criteria for familial breast-ovarian cancer seems acceptable. The results, however, demonstrate the importance of making a thorough pedigree of the father's family, as this information is often not present at the first consultation.

The age of onset of cancer in the mutation carriers was as observed previously in hereditary breast-ovarian cancer [4, 12]. There is a high risk of breast cancer at premenopausal age as in other *BRCA1* families, and among the 1675delA mutation carriers, 4/13 had breast cancer, 2 had bilateral cancer. The penetrance of breast and ovarian cancer in these families will be demonstrated in a future study designed for this.

In our hospital, the 5-year survival of epithelial ovarian cancer patients of all stages is 40%, and 60% are diagnosed with stage III–IV disease (1990–1994) [13]. The distribution of the histology of these cancers in the same period was 54.7% serous; 8.5% mucinous; 11.7% endometroid; 8.5% clear call; 2.1% undifferentiated; 7.4% mixed; and 7.0% unclassified tumours. In comparison, the mutation carriers had 83.3% serous tumours and 83.3% had stage III–IV disease (Table 3), all considered to be unfavourable prognostic factors. In a previous prospective study with annual screening of women in breast-ovarian cancer families, we detected five out of nine ovarian cancers with stage I ($n = 4$) and stage II ($n = 1$) disease [4]. Compared with the present study with only 3 of the 18 mutation carriers with stage I–II disease, we cautiously conclude that screening in high risk families may help to identify ovarian cancer at an earlier stage and thereby improve survival, as patients with stage I disease, have 90% 5-year survival. Optimal cytoreductive surgery may also improve survival, and this implies that earlier detection of stage III disease may improve prognosis.

In view of the data presented, it seems appropriate to offer specific mutation testing of the two founder mutations to all ovarian cancer patients below the age of 70 years in the Norwegian population, and by this identify the risk of breast cancer in the patients themselves, and to offer appropriate genetic counselling and medical follow-up in the families.

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