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A founder mutation of the *BRCA1* gene in Western Sweden associated with a high incidence of breast and ovarian cancer

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Abstract

The aim of this study was to describe and characterise a founder mutation of the *BRCA1* gene in western Sweden. Of 62 families screened for BRCA mutations, 24 had *BRCA1* mutations and two had *BRCA2* mutations. Tumours that occurred in family members were histologically reviewed and mutational status was analysed using archival paraffin-embedded tissues. The same *BRCA1* mutation, 3171ins5, was found in 16 families who were clustered along the western coast of Sweden. Mutation analysis revealed a maternal linkage in 13 families and a paternal linkage in 3. There was complete agreement between mutation analysis results obtained from blood and archival tissues. The penetrance of breast or ovarian cancer by age 70 years was estimated to be between 59 and 93%. There were no differences in survivals between breast or ovarian cancer patients with the mutation and age-matched controls. Thus, a predominant *BRCA1* gene founder mutation associated with a high risk of breast and ovarian cancer has been identified and found to occur in a restricted geographical area, thereby allowing timely and cost-effective mutation screening using blood samples or archival histological material.

Keywords: *BRCA1* gene; Founder effect; Breast neoplasms; Ovarian neoplasms

1. Introduction

Well-documented cases of familial breast cancer have been reported since the 19th century. Most cases with identified mutations are associated with the recently discovered tumour suppressor genes *BRCA1* and *BRCA2* [1,2]. Families harbouring mutations of one of these two genes have a high incidence of breast cancer that occurs at a considerably younger age than in the general population [3]. *BRCA1* gene mutations, and to a lesser extent *BRCA2* mutations, are also associated with an increased risk of developing ovarian cancer [4]. *BRCA1* and *BRCA2* gene mutations have been reported to be highly penetrant [5], although the estimated risk of developing cancer is believed to be somewhat less than previously reported based on more recent population-based studies of Ashkenazi Jews in the United States [6] and studies from Iceland [7].

Identification of *BRCA1* and *BRCA2* gene mutations has made it possible to trace individuals who are particularly at risk for developing breast and ovarian cancer. However, *BRCA1* and *BRCA2* gene mutations are rare and unevenly distributed in the general population. Moreover, several different mutations have been described for both BRCA genes. While screening for mutations in the two *BRCA* genes is laborious and costly, identification of a single unique mutation is fairly simple. The frequent occurrence of a unique mutation in a defined population indicates a common ancestor in all families that harbour the mutation. Such a mutation is called a founder mutation because it supposedly originates from a single founder. The identification of founder mutations and their geographical distribution is important for designing mutational screening. A few Swedish founder mutations have been previously

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described [8,9]. This study is a detailed report of a Swedish founder mutation that occurs primarily along the western coast of Sweden and is associated with a high risk of developing breast and ovarian cancer.

2. Patients and methods

The Cancer Genetic Counselling Unit in Göteborg, Sweden was established in 1995 as a joint venture between the Departments of Oncology and Clinical Genetics at Sahlgrenska University Hospital. Patients seen at the clinic come from the western Swedish Health Care Region, which has a population of 1.6 million.

As of October 1999, 195 families had been concerned about breast and/or ovarian cancer in their families. Screening for *BRCA1* and *BRCA2* mutations was offered if their pedigree indicated a dominant inheritance pattern. Mutation screening was completed in 62 families. In 26 of these families (42%), *BRCA1* or *BRCA2* gene mutations were identified ($n=24$ and 2, respectively). In 16 families, an insertion of five bases (TGAGA) at nucleotide 3171 of exon 11 of the *BRCA1* gene was found (*BRCA1* 3171ins5; also known as nt3166ins5). Eight different mutations were found in the remaining 10 families (data not shown). Three of the 16 families with the 3171ins5 mutation had family members whose mutation was originally identified in Stockholm ($n=2$) or Lund ($n=1$). However, the earliest documented case of breast and/or ovarian cancer in the 16 families came from western Sweden. An additional six families with this founder mutation (3171ins5) were identified in our genetic counselling clinic after October 1999. These families have been included in the estimation of penetrance only.

Thus, 16 families harbouring the *BRCA1* 3171ins5 mutation form the basis of this study. Data regarding family history was provided by the proband in each family. A case was defined in this study as a woman in the line of inheritance with breast and/or ovarian cancer. The occurrence of cancer was verified through clinical records, death certificates and/or histological review. Cancers reported by hearsay (two breast and one ovarian) were not included in the study. The geographical distribution was studied by ascertaining the birthplace of the patient with the earliest case of breast/ovarian cancer in each family.

Histological material was retrieved from 15 of the 16 families with *BRCA1* 3171ins5 mutations and included 30 primary breast carcinomas (27 women), 22 ovarian carcinomas (22 women) and 10 other carcinomas (9 patients). Paraffin blocks were recut and haematoxylin and eosin-stained sections were re-evaluated. For the mutation analysis of paraffin-embedded tissues, non-neoplastic tissues removed at the time of tumour surgery or other occasions were chosen.

The aims of the mutation analysis of archival histological material were to: (1) compare the results obtained using archival tissues and blood samples from the same individuals ($n=35$), thereby evaluating the reliability of results based on archival material; (2) determine the line of inheritance by establishing the mutation status in as many individuals as possible; and (3) identify phenocopies.

Estimation of penetrance was essentially performed according to Dørum and colleagues [10] based on 22 families ($16+6$) with the founder mutation. Daughters and sisters of known mutation carriers, obligate carriers and women who had breast or ovarian cancer were traced with regard to survival and the occurrence of breast and ovarian cancer. The index case of each family and her sisters, as well as females who tested negatively for the mutation were all excluded. If information was lacking regarding a woman, she and her sisters were also excluded. Thus, the analysed series was comprised of 101 individuals who were either mutation carriers ($n=49$) or untested first degree relatives of mutation carriers ($n=52$). In the former group there were 28 women and in the latter group 10 women with breast or ovarian cancer.

Kaplan–Meier analyses were used to illustrate the cumulative incidence of the first occurrence of either of the two cancers. Two separate analyses were performed: one assuming that only individuals with a known mutation, obligate carriers or individuals with breast or ovarian cancer were mutation carriers; and the other assuming that all individuals with unknown mutation status were also carriers. The true penetrance lies somewhere between these two estimates. Confidence intervals were calculated according to the Greenwood formula.

The survival rates for patients with breast ($n=30$) and ovarian cancer ($n=20$) were calculated using the Kaplan–Meier methods (the two phenocopies and patients diagnosed prior to the establishment of the Swedish Cancer Registry in 1958 were excluded). Survival rates were compared with age-matched controls obtained from the Swedish Cancer Registry and diagnosed during the same time period. Four breast cancer controls and four ovarian cancer controls were used for each case (120 and 80 cases, respectively). There was no information from the Cancer Registry regarding tumour stage or grade. For the analysis of survival in the breast cancer patients with *BRCA1* mutations, two separate tests were run; one with and one without censoring for the occurrence of subsequent ovarian cancer.

2.1. DNA isolation

Genomic DNA was prepared from 78 samples of archival histological material and from 64 samples of venous blood, anticoagulated in EDTA. 30 micron sec-

tions of archival paraffin-embedded normal tissue specimens were prepared. The paraffin was removed by incubation in xylene and extraction of DNA was performed using Nucleon HT (Amersham Life Science) according to protocol. DNA preparation from blood was performed using the Puregene® DNA Isolation Kit (Gentra Systems) according to the manufacturer's recommendations.

2.2. PCR

The quality and approximate concentration of genomic DNA extracted from paraffin-embedded tissue were examined on agarose gel. Amplification of DNA extracted from paraffin-embedded tissue was carried out using nested polymerase chain reaction (PCR). Both reactions were performed in a reaction volume of 50 μl containing 200 μM each of deoxyadenosine triphosphate (dATP), deoxycytosine triphosphate (dCTp), deoxyguanine triphosphate (dGTP) and deoxythymidine triphosphate (dTTP) and 25 pmol of both forward primer (fp) and reverse primer (rp). The first reaction also contained approximately 20–30 ng of genomic DNA, 1 U of the Expand™ High Fidelity PCR enzyme mix and the Expand™ High Fidelity PCR buffer (Boehringer Mannheim). In the nested PCR step, 3 μl of the first PCR reaction mixture was used as a template. The reaction mix included 2.5 U of AmpliTaq Gold DNA polymerase and AmpliTaq Gold buffer (PE Biosystems). Primers used in PCR 1 were located between nucleotide (nt) 2997 and nt 3250: #38 (fp) 5′-GGC AAC GAA ACT GGA CTC ATT ACT-3′ and 95 (rp) 5′-ATA TTG CTT GAG CTG GCT TC-3′. Nested primer sequences in the second PCR-round were located between nt 3060 and nt 3201: #50 (fp) 5′-CCA CCA CTT TTT CCC ATC AAG TC-3′ and #51 (rp) 5′-GGC TAA TTG TGC TCA CTG TAC TTG G-3′. The first reaction was after an initial denaturation step at 95 °C for 2 min amplified through 30 cycles consisting of 1 min denaturing at 94 °C, 1 min annealing at 56 °C, 1 min extension at 72 °C and one final extension cycle at 72 °C for 7 min. The second reaction had an initial denaturation step at 95 °C for 12 min followed by 35 cycles of 1 min denaturing at 94 °C, 1 min annealing at 60 °C, 1 min extension at 72 °C and one final cycle of extension at 72 °C for 7 min.

Genomic DNA extracted from blood samples was amplified using 25 pmol of primers #38 (see above) and #39 (rp) 5′-ATT TTG GCC CTC TGT TTC TAC CTA-3′, located between nt 2997 and nt 3357, in a reaction volume of 25 μl. The reaction mixture also contained 50–100 ng DNA, 200 μM deoxynucleoside triphosphate (dNTPs), 1 U of the Expand™ High Fidelity PCR enzyme mix and the Expand™ High Fidelity PCR buffer (Boehringer Mannheim). The PCR conditions used for amplification were: 94 °C/1 min, 66 °C/1 min, 72 °C/1 min for 30 cycles. An initial denaturation, 95 °C for 2 min, was performed.

2.3. SSCP/HD (single strand conformation polymorphism/heteroduplex) analysis

Denaturation: 2 μl of the PCR reaction mixture was mixed with 6 μl of formamide–dye solution (98% formamide, 20 mM ethylene diamine tetra-acetic acid (EDTA), 0.05% xylene cyanole and 0.05% bromophenol blue). The samples were heated at 98 °C for 5 min and then immediately put on ice. Electrophoretic separation: SSCP and HD-band pattern were detected on the same gel using the Phast system (Pharmacia Biotech) with a subsequent automated silver-staining step to visualise DNA fragments. Electrophoretic separation was performed using DNA buffer strips equilibrated overnight in a 0.2 M Tris–Tricine buffer, pH 8.3, and homogenous 20% polyacrylamide Phast gels. 1.3 μl of each sample was applied to the gel. Running conditions were: (a) Prerun: 400 V, 5.0 mA, 1.0 W for 10 AVh; (b) Run: 400 V, 5.0 mA, 1.0 W for 200 AVh. The running temperature was 15 °C.

3. Results

3.1. Patients

In the 16 families with *BRCA1* 3171ins5 mutations, there were 47 breast cancers in 39 women (eight bilateral cancers). The first breast cancer was diagnosed at a median age of 44 years (mean 45 years, range 30–70 years). Eight women had bilateral breast cancer; the median interval to the development of the second breast cancer was 5 years (mean 7 years, range 1–25 years). Twenty-five women developed ovarian cancer at a median age of 52 years (mean 53 years, range 40–70 years). Nine women had both breast and ovarian cancer. Eight of them developed breast cancer prior to ovarian cancer at a median interval of 9 years (range 2–23 years). In one case, the ovarian cancer preceded the breast cancer by 4 years. One woman with breast cancer also developed both cervical and colon cancer. Two women with ovarian cancer also had other malignancies, including a lung cancer in one and a malignant melanoma in the other.

The number of family members varied considerably. Genetic counselling was sought due to suspicion of inherited breast and/or ovarian cancer on the maternal side in 14 families and on the paternal side in two families. A maternal linkage was confirmed in 13 families and a paternal linkage in three families. Only two families were found to have a common ancestor within the last century (1900s). The earliest verified case in each family occurred in individuals from a limited geographical area along the western coast of Sweden.

3.2. Mutation analysis

Among the 16 families, 98 individuals were tested for the *BRCA1* 3171ins5 mutation. In 29 cases, only DNA extracted from blood was tested. In 72 cases, archival material was tested; the mutation status could be determined in 67 (93%) of these. In 35 cases, both archival material and blood were tested with completely congruous results; in 23 cases, both blood and archival material were positive for the mutation and in 12 cases both were negative. In one woman with ovarian cancer, only archival material was available for testing and no mutation was found. This woman is believed to be an obligate carrier since two daughters and a sister's daughter are known carriers of the mutation, raising the possibility of a false-negative test result. In addition, six randomly selected archival specimens from patients without known hereditary breast/ovarian cancer were tested for the mutation in order to validate the method. All tested negatively.

Based on blood and archival material, mutational status was determined in 96 individuals. Sixty-five of these were positive for the mutation and 31 were negative. Among 28 women with breast carcinoma whose mutational status was analysed, 26 were positive for the mutation (including all nine women with breast and ovarian cancer), and two were negative (phenocopies). The mother of one of the latter two women developed breast cancer and was found to be a mutation carrier. An aunt of the other woman who tested negatively was a carrier; the mutation status of the woman's mother was, however, unknown. 22 patients with ovarian carcinoma were tested; 21 tested positively and 1 tested negatively, although the pedigree suggested she might be a carrier.

In addition, 9 patients (from 8 of the 16 families) with 10 histologically verified malignant tumours of other types were also tested for the mutation. Seven individuals tested positively; their malignancies included lung carcinoma ($n=2$), pancreatic carcinoma ($n=2$), colon carcinoma ($n=1$), urethral carcinoma ($n=1$), renal cell carcinoma ($n=1$) and prostatic carcinoma ($n=1$). Two individuals with medullary thyroid carcinoma and bile duct carcinoma tested negatively.

Eight other individuals with cancer (from six of the 16 families) were not tested for the mutation. Malignancies in this latter group included cancers with an unknown primary site (3 cases), gastric carcinoma (2), colon carcinoma (1), lung carcinoma (1), and prostatic carcinoma (1).

3.3. Penetrance and survival

The Kaplan–Meier estimate of the cumulative incidence of breast or ovarian cancer (penetrance) was 59% at 70 years of age (95% confidence interval (CI): 46–73%) when all women with unknown mutation status were considered as positive. When only known mutation carriers or women with breast or ovarian cancer were included, the estimated penetrance was 93% (95% CI: 80–99%) (Fig. 1).

The crude 5- and 10-year survivals for women after the development of breast cancer were 72% (95% CI: 51–85%) and 56% (95% CI: 35–72%), respectively. The crude 5- and 10-year-survivals for patients with ovarian cancer were 38% (95% CI: 17–59%) and 17% (95% CI: 3–39%). There were no obvious differences in survival rates compared with the rates from controls selected from the Swedish Cancer Registry (Figs. 2 and 3). Censoring for the occurrence of subsequent ovarian cancer did not significantly alter the survival rate of breast cancer patients in this series ($P=0.50$).

3.4. Histopathology

Thirty breast carcinomas were available for histological review. These were classified as invasive ductal carcinoma in 23 cases and as atypical medullary carcinoma in 7. The Bloom–Richardson–Elston (BRE) score was 8 or 9 (high grade) in 23 cases. All 22 ovarian carcinomas

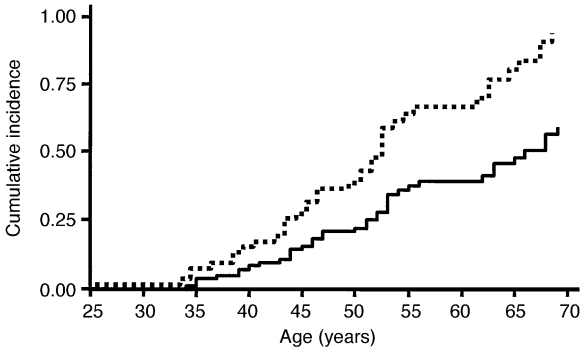

Fig. 1. The cumulative risk of developing breast or ovarian cancer in women with verified *BRCA1* 3171ins5 mutations. The maximum risk curve (dotted line) was obtained by assuming that all women with unknown mutation status were non-carriers, and the lower risk curve (solid line) that all were carriers.

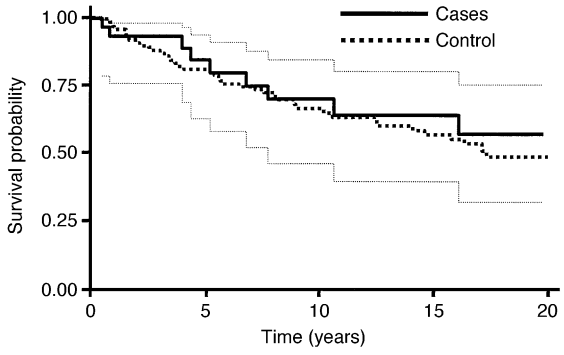

Fig. 2. Comparison of survivals for women with breast cancer from families with *BRCA1* 3171ins5 mutations ($n=30$, solid line with 95% Confidence Interval) and age-matched controls during the same time period ($n=120$, dotted line).

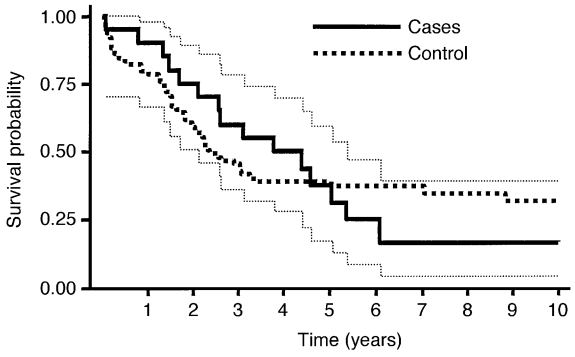

Fig. 3. Comparison of survivals for women with ovarian cancer from families with *BRCA1* 3171ins5 mutations ($n = 20$, solid line with 95% Confidence Interval) and age-matched controls during the same time period ($n = 80$, dotted line).

that were available for histological review were classified as serous carcinomas. The majority of these were predominantly poorly differentiated, although better differentiated areas were seen as well.

4. Discussion

The *BRCA1* 3171ins5 mutation described in this study is restricted to a relatively small, well-defined geographical area where it constitutes by far the most common type of BRCA gene mutation. We have found that carriers of the mutation from all families share the same haplotype indicating that this mutation represents a founder mutation [11]. Three individuals in our study with this particular mutation had initially been investigated in Stockholm or in Lund. However, all of the patients' ancestors could be traced back to the western coast of Sweden. Two individuals with the same mutation have also been described from Seattle, WA, USA [12], but the ethnic origin of this mutation carrier is not known. Considering the large percentage of Scandinavian immigrants in the Seattle area, a common ancestor from the Swedish western coast is plausible. Other Scandinavian *BRCA1* founder mutations occurring in restricted geographical areas have been reported [8,10]. The relative restriction of founder mutations to confined geographical areas such as the one we report here reflect the age of the mutation as well as the stability (or immobility) of the population.

Breast and ovarian cancers occur at a considerably younger age in *BRCA1* and *BRCA2* mutation carriers than in the general population, with most occurring during the later reproductive years or afterwards. There is thus little selection against mutation carriers. This, as well as the relatively stable population contribute to the high frequency of families with the founder mutation in this geographical area.

Knowledge of penetrance is of special importance for genetic counselling. Estimates based on series of cancer genetic counselling clinics usually indicate a higher penetrance than population-based studies [6,7,10]. We reduced the ascertainment bias by excluding the index cases and their sisters from our estimate. Thus, we found a minimum penetrance of 59% (95% CI: 46–73) at 70 years of age, assuming that all women with unknown mutation status had the mutation. The maximum estimate of penetrance, assuming that none of the women with unknown mutation status were carriers, was 93% (95% CI: 80–99%) at 70 years of age. Since both assumptions are unrealistic, the true cumulative incidence of breast and ovarian cancer in this group lies between these two estimates and is similar to the estimated penetrance in Norwegian women with founder mutations [10].

The crude survivals of patients in our series after the development of breast or ovarian cancer were similar to those of patients from the regional cancer registry matched for cancer type, age, and time of diagnosis (Figs. 2 and 3). Eight of 30 women with breast cancer in our series developed subsequent ovarian cancer. Since ovarian cancer generally carries a worse prognosis than breast cancer, we also calculated the survival of breast cancer patients censoring at the time of ovarian cancer. However, this did not improve the survival of the group. The ovarian cancers in our series developed after a median interval of 9 years. The increased risk of dying from ovarian cancer may be counteracted by a selection for breast cancer patients who have survived long enough to develop ovarian cancer. While most studies have indicated similar survival rates for breast cancer patients with *BRCA1* mutations and controls, some reports have described a better or worse prognosis [13]. Further detailed and larger studies that take the complexity of other prognostic factors into account, such as stage, morphology and the development of multiple malignancies, will be needed to more accurately analyse this issue.

Results of mutational analysis based on archival material appear to be reliable. In 93% of cases, sufficient DNA could be extracted for the mutation analysis. There was complete agreement between the results obtained using blood and archival material in 35 patients.

All of the histologically reviewed breast carcinomas in this series were either invasive ductal carcinoma (23 cases) or so-called atypical medullary carcinoma (7 cases). The morphological distribution in our series is distinctly different from that of breast carcinoma in the general population. Moreover, an unusually high percentage was high grade (BRE score 8 or 9). There was also a predominance of poorly differentiated serous carcinomas of the ovary. The histological findings in our study are similar to those reported in other studies of hereditary breast and ovarian cancers associated with *BRCA1* and *BRCA2* mutations [14].

The high frequency of this founder mutation among families with breast/ovarian cancer in western Sweden makes it the most suitable BRCA candidate for mutation screening. Thus, all new cases of breast and ovarian cancer in our geographical area that are potentially hereditary are first screened for this mutation. Further testing for other *BRCA1* and *BRCA2* mutations is carried out only in those patients who do not have the *BRCA1* 3171ins5 mutation. This strategy is cost-effective and minimises the time to report test results to patients.

We are currently planning a population-based retrospective analysis of women who have a high risk of hereditary breast and ovarian cancer, such as those with bilateral breast cancer before the age of 50 years as well as women with both breast and ovarian cancer based on archival material. Such a study could more clearly define the frequency of this mutation among women with breast and ovarian cancer and the mutation's geographical distribution.

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