A Screening for BRCA1 Mutations in Breast and Breast-Ovarian Cancer Families from the Stockholm Region

Moraima Zelada-Hedman, Brita Wasteson Arver, Antonio Claro, Jindong Chen, Barbro Werelius, Helen Kok, Kerstin Sandelin, Sara Håkansson, Tone Ikdahl Andersen, Åke Borg, Anne-Lise Berresen Dale, and Anna Lindblom

Departments of Clinical Genetics [M. Z. H., B. W. A., A. C., J. C., B. W., H. K., A. L.], Surgery [K. S.], and Molecular Medicine [A. L.], Karolinska Hospital, S-171 76 Stockholm, and Institute of Oncology, University Hospital, S-221 83 Lund [S. H., Å. B.], Sweden; and Department of Genetics, Institute for Cancer Research, The Norwegian Radium Hospital, N-0310 Oslo, Norway [T. I. A., A.-L. B. D.]

ABSTRACT

To identify BRCA1 germ-line mutations in the breast and breast-ovarian cancer families in the Stockholm region, a total of 127 families were screened. DNA from 174 patients from these families were studied using various mutation screening techniques, followed by direct DNA sequencing. Mutations were identified in 7 of 20 families with breast and ovarian cancer and in one family with ovarian cancer only, whereas only 1 family of 106 with breast cancer showed a mutation. Thus, germ-line mutations in BRCA1 were found in one-third of the families with both breast and ovarian cancer, but in only 1% of the breast cancer families. The low frequency of germ-line mutations in the site-specific breast cancer families means that other genes are likely to segregate in these families.

INTRODUCTION

Breast and ovarian cancer often segregate in families (1). One criterion for defining hereditary disease is that the patient has at least three close relatives (first- and second-degree relatives) with breast and/or ovarian cancer. In 1990, a breast cancer susceptibility gene, BRCA1, was localized by linkage analysis in a number of families with early-onset breast cancer (2). Linkage to the same region was also obtained in families with the breast-ovarian cancer syndrome (3) and in families with site-specific ovarian cancer (4). The responsible gene from this region was identified in October 1994 (5). Tumor studies showed that loss of heterozygosity of the region involved the wild-type chromosome, suggesting that the breast-ovarian cancer gene is a tumor suppressor gene (6). Although sporadic breast and ovarian cancer frequently show allelic loss on the long arm of chromosome 17, one study suggested that somatic BRCA1 mutations might not be involved in the development of most cases of sporadic breast cancer (7). However, somatic alteration in the gene seemed to be involved in at least some sporadic ovarian cancers (8). Since the identification of the gene, a number of germ-line mutations segregating in families with hereditary breast and/or ovarian cancer have been published (9—13). Recently, mutations in a second gene, BRCA2, have been shown to be responsible for the disease in some families with breast and breast-ovarian cancer. In contrast to BRCA1, germ-line mutations in BRCA2 (14, 15) also seem to confer an increased risk for male breast cancer.

Genetic epidemiological studies have estimated that BRCA1 is involved in 1—5% of all cases of breast or ovarian cancer (16). Germ-line mutations in BRCA1 were suggested to be present in almost all families with breast and ovarian cancer and in 45% of families with breast cancer only (17, 18). In Ashkenazi Jewish women, a common founder mutation segregates and is responsible for 16% of the breast cancer and 39% of the ovarian cancer diagnosed before the age of 50 (19). This mutation is also strongly associated with early onset (patient <40 years old) of breast cancer in Jewish women without any family history of cancer (20). In a study of breast cancer patients diagnosed before 35 years of age and not selected for family history, alterations in BRCA1 were identified in 10% of the cases (21).

To date, many families screened for BRCA1 have belonged to kindreds used in linkage studies leading to the identification of the gene. These families are often very large and cannot be used to give reliable estimates of the number of families in which a BRCA1 mutation is responsible for the disease. Heterogeneity between populations may also give rise to different estimates of families segregating BRCA1 mutations. In the present study, a cohort of women from breast and breast-ovarian cancer families in the Stockholm region were screened for BRCA1 mutations. Various mutation screening techniques followed by direct sequencing were used to search for germ-line mutations in 127 families with hereditary breast and/or ovarian cancer. These families are likely to represent a large part of existing breast and breast-ovarian cancer families in the region.

PATIENTS AND METHODS

Patients and Their Families. One hundred twenty-seven families with hereditary breast and/or ovarian cancer were ascertained. The inclusion criterion for this study was having three or more first- or second-degree relatives with breast and/or ovarian cancer. All families fulfilling this criterion were included if at least one affected relative was alive and willing to participate in the study. Included were 106 families with breast cancer only, 20 families with breast and ovarian cancer, and 1 family with ovarian cancer only.

The family histories from 1975 breast cancer patients were available from a registry of all existing breast cancer patients in Stockholm in 1988—1989. In this cohort, 133 families with hereditary breast cancer were identified, and 52 were available for study (22). The remaining families were recruited through the Cancer Family Clinic at the Karolinska Hospital from 1990 to 1995. During this period, a total of 263 patients from the Stockholm area were counseled at the clinic because of a possible increased risk of breast or ovarian cancer, and 75 of these fulfilled the above criterion. Blood samples from 174 patients from these 127 families were obtained, and DNA was extracted according to routine procedures.

Characteristics of the families in terms of total number of cancer cases and age of onset are given in Table 1.

The majority of the families (106) were of the breast-cancer-only type. Half of the families (67) were small, having only three individuals with cancer. Mutation Screening. Twenty-two primer pairs were used to amplify all exons and exon-intron boundaries (except exons 1 and 11) from genomic DNA for all 174 samples.

SSCP Analysis. All 127 families were screened by SSCP for exons 2—10, 12, 15, and 17—23. For exons 13—16 and 24, 20 families were screened by SSCP, and the rest of the families (107) were screened by CDGE (see below). Most of the primers and SSCP conditions used to screen for mutations were

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To whom requests for reprints should be addressed, at Department of Molecular Medicine, Karolinska Hospital, S-171 76 Stockholm, Sweden. Phone: 46-8-729 52 48; Fax: 46-8-32 77 34.

3 The abbreviations used are: SSCP, single-strand conformation polymorphism; CDGE, constant denaturant gradient gel electrophoresis; PTT, protein truncation test.
Table 1 Characteristics of breast cancer families

<table>
<thead>
<tr>
<th>No. of families</th>
<th>No. of breast cancer cases per family</th>
<th>Mean age of earliest onset of breast cancer</th>
<th>Mean age of mean age of onset of ovarian cancer</th>
<th>Mean age of earliest onset of ovarian cancer</th>
<th>No. of BRCA1 mutations</th>
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<td>44</td>
<td>53</td>
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<td>Total, 127</td>
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Table 2 BRCA1 mutations found in Swedish breast and ovarian cancer families

<table>
<thead>
<tr>
<th>Family no.</th>
<th>Exon</th>
<th>Sequence change</th>
<th>Mutation type</th>
<th>No. of members with breast cancer</th>
<th>No. of members with ovarian cancer</th>
<th>Other types of cancer</th>
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<td>11</td>
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<td>1</td>
<td>P, G</td>
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<td>11</td>
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<td>Frameshift</td>
<td>5</td>
<td>1</td>
<td>M, G, CML</td>
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<td>2594 delC</td>
<td>Frameshift</td>
<td>5</td>
<td>1</td>
<td>P, L, Lip</td>
</tr>
<tr>
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<td>2594 delC</td>
<td>Frameshift</td>
<td>0</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>4028</td>
<td>11</td>
<td>3166 instTGAGA</td>
<td>Frameshift</td>
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<td>1</td>
<td></td>
</tr>
<tr>
<td>4047</td>
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<td>3166 insTGAGA</td>
<td>Frameshift</td>
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<td>1</td>
<td></td>
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<td>11</td>
<td>Not identified</td>
<td>Aberrant PTT band</td>
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<td>1</td>
<td></td>
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<tr>
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<td>23</td>
<td>5563 G → A</td>
<td>Stop</td>
<td>3</td>
<td>0</td>
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</tbody>
</table>

* P, pancreas cancer; G, cancer of the gallbladder; M, mesothelioma; CML, chronic myelocytic leukemia; L, liver cancer; Lip, lip cancer; B, malignant tumor of the brain.
DISCUSSION

Individuals in families with germ-line mutations in BRCA1 have a high risk of cancer of the breast or ovary (27). We wanted to determine the frequency of Swedish breast and breast ovarian cancer families in the Stockholm region having a germ-line mutation in the BRCA1 gene. Epidemiological studies in women with a family history of cancer have shown the BRCA1 gene to be involved in almost all families with breast and ovarian cancer and in approximately 45% of the breast cancer-only families (16—18). In this study, 127 families with an autosomal dominant mode of inherited cancer susceptibility were screened. Germ-line mutations in BRCA1 were identified in only 7% (9 of 127) of all of the families studied: 7 of 20 (35%) families with breast-ovarian cancer, 1 of 106 (around 1%) breast cancer-only families, and 1 in the only site-specific ovarian cancer family. Three of the mutations have been reported before (12). Two of the mutations were founder mutations in Sweden (Table 2) and have already previously been shown to segregate in apparently nonrelated breast-ovarian cancer families in the south of Sweden (13).

It is difficult to identify all mutations in the large BRCA1 gene. In a previous study of early-onset breast cancer families in which linkage analysis gave clear evidence for BRCA1 involvement, SSCP analysis and direct sequencing of DNA and RNA revealed BRCA1 mutations in less than half of the families (9). The DNA-screening methods used in our study would be expected to identify most mutations, including missense mutations for all exons except exon 11.

Because our mutations and most of the published mutations in the BRCA1 gene are of the nonsense or frameshift types giving rise to a truncated protein, it would be convenient for clinical purpose to use PTT only and base the analysis on RNA. This would be a faster method and would probably identify most mutations of functional importance. On the other hand, SSCP and CDGE screening could detect missense mutations, but their biological relevance have to be established.

Most (107) families were screened using all three methods in a similar way. Twenty families were screened for exons 13—16 and 24 using SSCP instead of CDGE. No mutations were found in any of these exons using SSCP or CDGE. Thus, there is no bias in selecting any particular group of families for specific methods that could explain the difference in mutation frequency between breast-ovarian and breast cancer families.

The only germ-line mutation among the breast cancer-only families was found in exon 23. This is interesting because previous studies have suggested the risk of ovarian cancer to be lower when the mutation is located in the 3' part of the gene (28—30).

Although we may have underestimated the involvement of BRCA1, our findings indicate that BRCA1 germ-line mutations are not very frequent in families with breast cancer only in the Stockholm region. In a previous study in southern Sweden, a higher incidence of BRCA1 mutations was found (13). However, the inclusion criteria in that study were different. Only first-degree relatives were considered, and at least one case of cancer had to be diagnosed at an age of <50. In this study, we considered first- and second-degree relatives, and we did not exclude any family because of the age at onset. In addition, the majority of families included from the Stockholm region had only three affected relatives (Table 1). Moreover, it is possible that the frequency of BRCA1 carriers differs between geographic regions.

Another highly penetrant gene, BRCA2, has been identified. It seems to account for a lower percentage of hereditary early-onset breast and ovarian cancer than the BRCA1 gene (14, 15). We are presently screening the same families for mutations in the BRCA2 gene, and preliminary results indicate that the involvement of BRCA2 is similar to that of BRCA1. Still, we believe that these families segregate inherited mutations predisposing to breast cancer. Thus, it is likely from previous studies and from the present study that mutations in other, probably low-penetration, genes predispose for breast cancer (18, 30).

The present study indicates that, when BRCA1 mutation analysis is performed in women from families with breast-ovarian cancer, there is a high likelihood of finding a BRCA1 mutation. The majority of women counseled at our clinic are from breast cancer-only families. The low frequency of BRCA1 mutation carriers in this group suggests further studies to determine whether other genes are involved in these families.

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REFERENCES


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