Risk factors for detecting germline BRCA1 and BRCA2 founder mutations in Ashkenazi Jewish women with breast or ovarian cancer

Shirley V Hodgson, Elizabeth Heap, June Cameron, David Ellis, Christopher G Mathew, Rosalind A Eeles, Ellen Solomon, Cathryn M Lewis

Abstract

We ascertained 184 Ashkenazi Jewish women with breast/ovarian cancer (171 breast and 13 ovarian cancers, two of the former also had ovarian cancer) in a self-referral study. They were tested for germ-line founder mutations in BRCA1 (185delAG, 5382insC, 188del11) and BRCA2 (6174delT). Personal/family histories were correlated with mutation status. Logistic regression was used to develop a model to predict those breast cancer cases likely to be germ-line BRCA1/BRCA2 mutation carriers in this population. The most important factors were age at diagnosis, personal/family history of ovarian cancer, or breast cancer diagnosed before 60 years in a first degree relative.

A total of 15.8% of breast cancer cases, one of 13 ovarian cancer cases (7.7%), and both cases with ovarian and breast cancer carried one of the founder mutations. Age at diagnosis in carriers (44.6 years) was significantly lower than in non-carriers (52.1 years) (p=0.001), and was slightly lower in BRCA1 than BRCA2 carriers. Thirty three percent of carriers had no family history of breast or ovarian cancer in first or second degree relatives. Conversely, 12% of non-mutation carriers had strong family histories, with both a first and a second degree relative diagnosed with breast or ovarian cancer.

The predicted values from the logistic model can be used to define criteria for identifying Ashkenazi Jewish women with breast cancer who are at high risk of carrying BRCA1 and BRCA2 mutations. The following criteria would identify those at approximately 10% risk: (1) breast cancer <50 years, (2) breast cancer <60 years with a first degree relative with breast cancer <60 years, or (3) breast cancer <70 years and a first or second degree relative with ovarian cancer.

Keywords: breast cancer; Ashkenazi Jewish; BRCA1; BRCA2

Epidemiological evidence suggests that in the general population, approximately 5% of breast cancers are the result of inherited mutations in genes resulting in a strong susceptibility to breast cancer. Inherited mutations in BRCA1 may confer up to an 80% lifetime risk of breast cancer and a lifetime risk of <40% of ovarian cancer in females. Mutations in BRCA2 also confer a high risk of breast cancer and are associated with a broader spectrum of cancer susceptibility (including pancreatic and fallopian tube cancers and breast cancer in males), but with a lesser risk of ovarian cancer. A family history of breast and ovarian cancer is strongly predictive of a BRCA1 mutation. However, approximately 30% of high risk families have no detectable mutation in BRCA1 or BRCA2.

Studies from the USA and Israel, in particular, have shown that in the Ashkenazi Jewish population there is an increased prevalence of three specific founder mutations in these genes (185delAG and 5382insC in BRCA1 and 6174delT in BRCA2), together occurring in 2-3% of this ethnic group. In Ashkenazi Jewish women with breast cancer, the prevalence of these mutations is as high as 20% in women with breast cancer diagnosed at 40 years of age or less (even greater (30%) in women with a family history of the disease), and in 39-45% of women with ovarian cancer. These founder mutations may also account for 39% of ovarian cancer diagnosed before 50 years of age.

Epidemiological studies indicate that a family history of breast cancer in Ashkenazi women confers a stronger relative risk of breast cancer than in other ethnic groups and that the absence of clinical features, such as early age at diagnosis, family history of breast or ovarian cancer, or a personal history of both breast and ovarian cancer, significantly reduces the chance of detecting a germline BRCA1 or BRCA2 founder mutation. There are obvious implications for genetic counselling.

Over 50% of Ashkenazim with breast cancer and a strong family history of breast/ovarian cancer do not carry a detectable germline founder mutation, so other mutations may cause the breast cancer susceptibility in these families. Additionally, although germline BRCA1 and BRCA2 founder mutations are very common in this ethnic group, overall breast cancer incidence may be only slightly increased (89.8 per 100 000) and may vary in different Jewish groups (for example, incidence is 70.7 per 100 000 in Asian-born Jews). This suggests that the penetrance of these mutations may vary. It has been found to be reduced in the Ashkenazim when probands were not selected for a strong family history, the cumulative risk for breast cancer
being estimated at 55% and for ovarian cancer 30-65% in BRCA1 and 10-20% in BRCA2 mutation carriers.15-16

Systematic studies have not previously been performed to determine whether the prevalence of these founder mutations is as high in an unselected series of cases of breast and ovarian cancer in Ashkenazi Jewish women in the UK as has been reported in Israel and the USA. This, and the determination of family history variables predictive of the presence of a founder mutation, derived from these data, will improve the accuracy of genetic counselling.

Since our group of affected women was unselected for age at diagnosis, it is more representative of cancer in the Ashkenazi population than previous studies.

**Methods**

Information about the study was provided to relevant clinicians and Jewish agencies, and it was advertised in lectures, broadcasts, and newspaper articles. Women coming forward to participate were contacted personally and given written information. A family history was taken and arrangements were made to obtain a blood sample, with informed consent for the study. Diagnosis in probands was confirmed by their physicians in the majority of cases. Confirmation of cancer in relatives was not attempted in most families.

**MUTATION DETECTION METHODS**

A heteroduplex assay was used to screen for the common BRCA1 and BRCA2 mutations. The PCR was performed in a volume of 25 ml using the following primer sets. BRCA1 exon 2: forward: 5' GAA GTT GTC TTT TAT TAA ACC TTT 3', reverse: 5' GAA GTT GTC TTT TAT AAA ACT TT 3'; BRCA1 exon 20: forward: 5' ATA TGA CGT GTC TGC TCC AC 3', reverse: 5' TGC AAA GGG GAG TGG AAT ACA GAG 3'; BRCA2 exon 11: forward: 5' CAC CTT GTG ATG TTA GTT TG 3', reverse: 5' TGG GGA TAT TAA TTC TGG AGT A 3'.

Following initial denaturation at 94°C for three minutes, the DNA was amplified separately for each exon through 30 cycles of 94°C for 30 seconds, 55°C for one minute, and 72°C for one minute. Following the PCR, heteroduplex formation was carried out by heating the PCR product to 94°C, followed by incubation at 65°C for one hour. Heteroduplex products were electrophoresed on a 10% polyacrylamide gel (9.9% acrylamide/0.1% piperazine diacrylamide) (Biorad) 20 × 20 cm vertical gel, and visualised by ethidium bromide staining.

**STATISTICAL METHODS**

Differences between mutation carriers and non-carriers were determined for variables of family history, age at diagnosis, and cancer site. Similarly, differences between BRCA1 and BRCA2 mutation carriers were tested. Fisher’s exact test and t tests were used, as appropriate. A cut off for age at diagnosis of 50 years in probands and 60 years in relatives was used to define a young onset group. The significant variables from the univariate analysis above were used in a logistic regression model, to predict the mutation carrying status of the proband, given age at diagnosis and family history. All statistical analyses were carried out using Splus (Cary, NC).

Only probands with breast cancer were included in the statistical analysis, since too few ovarian cancer cases were available to provide meaningful conclusions. Women diagnosed with both breast and ovarian cancer were classified by their breast cancer diagnosis. For the breast cancer probands, a family history of ovarian cancer was included as a risk variable, defined as having either a second primary ovarian cancer, or a first or second degree relative diagnosed with ovarian cancer (at any age).

**Results**

We ascertained 184 unrelated affected Ashkenazi Jewish women and obtained pedigree information and blood samples from them. Of these probands, 169 had been diagnosed with breast cancer, 13 with ovarian cancer, and two with both breast and ovarian cancer.

All subjects were screened for the three mutations 185delAG, 5382insC, and 188del11 (BRCA1), and 6174delTT (BRCA2). Thirteen 185delAG and three 5382insC mutations in BRCA1 and 11 6174delTT mutations in BRCA2 have been detected in 171 breast cancer cases (giving a mutation frequency of 15.8%). One of the 13 cases of ovarian cancer (7.7%) carried a 5382insC mutation. Both cases with breast and ovarian cancer were mutation carriers (185delAG, 6174delTT). Age at diagnosis of ovarian cancer was not significantly different from age at diagnosis of breast cancer. The mean age at diagnosis of breast cancer in carriers was 44.6 years and in non-carriers was 52.1 years (p<0.001), indicating a significantly earlier age at diagnosis in mutation carriers compared to non-carriers.

Fifteen of 17 BRCA1 mutations and 7 of 11 BRCA2 mutations were detected in women with breast cancer diagnosed below the age of 50 years (table 1). The remaining BRCA1 positive cases were diagnosed with breast cancer at 58 years and ovarian cancer at 49 years. The four remaining BRCA2 cases were diagnosed between 52 and 75 years. The age at diagnosis of breast cancer was slightly lower in BRCA1 than in BRCA2 mutation carriers (41.8 years for BRCA1 versus 48.5 years for BRCA2 carriers; difference not significant).

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Age at diagnosis of breast cancer</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;40</td>
<td>41–50</td>
</tr>
<tr>
<td>185delAG</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>5382insC</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>6174delTT</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>All carriers</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Non-carriers</td>
<td>21</td>
<td>41</td>
</tr>
<tr>
<td>Relative risk</td>
<td>2.79</td>
<td>1.28</td>
</tr>
</tbody>
</table>

Table 1  Age at diagnosis of breast cancer by mutation
Details of cancer diagnosis and family history for the probands are given in table 2, which shows the numbers of mutation carriers and non-carriers positive for each risk factor and the associated p values. Significantly more mutation carriers than non-carriers were diagnosed with breast cancer before 50 years (81.5% compared to 46.5%, p=0.001). The presence of breast and ovarian cancer in a proband was strongly predictive of being a mutation carrier.

The frequency of family history risk factors in mutation carriers and non-carriers is given in table 2. The total number of affected first or second degree relatives did not differ significantly between carriers and non-carriers, although there was a strong trend towards a greater prevalence of mutation carriers among all groups with a family history of cancer, especially early onset breast cancer. However, the presence of a first or second degree relative diagnosed before 60 years was highly predictive of a mutation. For example, 37% of mutation carriers had a first degree relative diagnosed under the age of 60 years, compared to 14.6% of non-carriers (p=0.012). A similar difference was seen when second degree relatives diagnosed before 60 years were included. There was no significant difference in the proportion of bilateral breast cancer cases among carriers and non-carriers (18.5% versus 7.6%; p=0.14). The most significant difference between carriers and non-carriers concerned a family history of ovarian cancer, with 33.3% of carriers reporting ovarian cancer in a first or second degree relative compared to 2.8% of non-carriers (p<0.001). Among the non-carriers, 12% had a strong family history with both first and second degree relatives diagnosed with breast cancer, indicating that other susceptibility genes (or mutations) may be important in the UK Ashkenazi Jewish population. A total of 33% of carriers and 50% of non-carriers, respectively, had no family history of breast/ovarian cancer in first or second degree relatives.

Ten of the 27 BRCA1/BRCA2 mutation carriers (breast cancer cases only) had a mother diagnosed with breast or ovarian cancer. Assuming that half the mutation carriers would have inherited their cancer paternally and half maternally, this figure would imply a high penetrance for the BRCA1/BRCA2 mutations (10/27 × 2=74%).

Similar penetrance estimates are obtained from affected and unaffected sisters. This value is higher than the penetrance previously reported in Jewish BRCA1 and BRCA2 carriers, but our small sample size relative to the published series limits the significance of this observation. In addition, it is probable that women with a close relative affected with breast cancer were more likely to participate in the study, and that probands with affected mothers or sisters are therefore over-represented, thus inflating the estimates of penetrance.

Cancers other than breast or ovarian cancer were reported, and those found in first degree relatives of BRCA1/BRCA2 mutation carriers are noted in table 3, with corresponding numbers from non-carriers. The prevalence was not significantly different in BRCA1 mutation carriers, BRCA2 mutation carriers, or non-carriers. Although colorectal and prostate cancer have previously been associated with BRCA1 mutations, these families contain no supporting evidence: of the 20 cases of CRC, four cases occurred in the first degree relatives of BRCA2 carriers and none in the relatives of BRCA1 carriers. Only one case of prostate cancer was seen, in a BRCA1 family. There was no significant difference in the total number of non-breast/ovarian cancers diagnosed in the first degree relatives of mutation carriers and non-carriers (19 in the relatives of 28 mutation carriers, compared to 88 in the relatives of 156 non-carriers).

Logistic modelling was used to predict breast cancer cases who may be BRCA1 or BRCA2 mutation carriers, using family history variables and age at diagnosis in the model. The best fitting model included age (as a continuous variable), family or personal history of ovarian cancer (first or second degree relative at any age), presence of a first degree relative diagnosed with breast or ovarian cancer before 60 years, and an interaction between age and a history of ovarian cancer.

The prediction curves from the model are illustrated in fig 1, showing the probability of being a mutation carrier by age at diagnosis and family history. The upper curves represent probands with a family or personal history of

---

**Table 2** Risk factors for age at diagnosis, site, and family history (breast cancer probands only)

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Mutation carriers (n=27)</th>
<th>Non-carriers (n=144)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosis &lt;50 y</td>
<td>22 (81.5%)</td>
<td>67 (46.5%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Bilateral breast cancer</td>
<td>5 (18.5%)</td>
<td>11 (7.6%)</td>
<td>NS</td>
</tr>
<tr>
<td>Breast + ovarian cancer</td>
<td>2 (7.4%)</td>
<td>0 (—)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*Family history: relatives diagnosed with breast or ovarian cancer

| 1° (any age)                          | 11 (40.7%)              | 40 (27.8%)           | NS       |
| 1° (dx <60 y)                         | 10 (37.0%)              | 21 (14.6%)           | 0.012    |
| 2° (any age)                          | 12 (44.4%)              | 49 (34.0%)           | NS       |
| 2° (dx <60 y)                         | 8 (29.6%)               | 26 (18.1%)           | NS       |

*Family history of ovarian cancer

| 1° or 2° (any age)                    | 18 (66.7%)              | 72 (50.0%)           | NS       |
| 1° or 2° (dx <60 y)                  | 15 (55.6%)              | 44 (30.6%)           | 0.016    |

*p values from Fisher’s exact test.

---

**Table 3** Other cancers in first degree relatives of mutation carriers, with corresponding numbers reported in non-carriers

<table>
<thead>
<tr>
<th>Site</th>
<th>BRCA1 (n=27)</th>
<th>BRCA2 (n=17)</th>
<th>Non-carriers (n=156)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>2</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>Colorectal</td>
<td>—</td>
<td>—</td>
<td>4</td>
</tr>
<tr>
<td>Stomach</td>
<td>1</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Prostate</td>
<td>1</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Throat</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Bladder</td>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Leukaemia</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>—</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Spinal tumour</td>
<td>1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Other sites</td>
<td>—</td>
<td>—</td>
<td>34</td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>10</td>
<td>88</td>
</tr>
</tbody>
</table>
ovarian cancer, and these are all at high risk of being a BRCA1 or BRCA2 mutation carrier, although the risk decreases rapidly with the proband’s age at diagnosis. The lower curves represent subjects with no family history of ovarian cancer, with or without a first degree relative diagnosed with breast cancer under the age of 60 years. These curves illustrate the parameters from the model, superimposed with points representing women from the current study. Three BRCA2 carriers diagnosed after 55 years are at a low predicted risk of being mutation carriers; they have no family history of close relatives with breast or ovarian cancer. From the total of 27 mutation carriers, 23 have a predicted risk of at least 10%. Using this risk as a baseline for identifying women at high risk of being mutation carriers gives the test a sensitivity of 85%. However, at this level, 41% of non-carriers would have been defined as having a high risk of carrying a mutation.

Discussion

Overall we found that 15.8% of our breast cancer cases had germline BRCA1/BRCA2 mutations (including both probands with breast and ovarian cancer). The prevalence of mutations was 7.7% in probands with ovarian cancer. These proportions are similar to those of previously reported series when unselected for positive family history. The strongest of previously reported series when unselected cancer. These proportions are similar to those mutations was 7.7% in probands with ovarian cancer, and these are all at high risk of BRCA1 or BRCA2 mutation carrier, although the risk decreases rapidly with the proband’s age at diagnosis. The lower curves represent subjects with no family history of ovarian cancer, with or without a first degree relative diagnosed with breast cancer under the age of 60 years. These curves illustrate the parameters from the model, superimposed with points representing women from the current study. Three BRCA2 carriers diagnosed after 55 years are at a low predicted risk of being mutation carriers; they have no family history of close relatives with breast or ovarian cancer. From the total of 27 mutation carriers, 23 have a predicted risk of at least 10%. Using this risk as a baseline for identifying women at high risk of being mutation carriers gives the test a sensitivity of 85%. However, at this level, 41% of non-carriers would have been defined as having a high risk of carrying a mutation.

Figure 1 Predicted risk that a woman diagnosed with breast cancer is a mutation carrier, by age at diagnosis and family history. BR = breast cancer. OV = ovarian cancer.

We gratefully acknowledge the support of the Dunhill Medical Trust.


Risk factors for detecting germline BRCA1 and BRCA2 founder mutations in Ashkenazi Jewish women with breast or ovarian cancer

Shirley V Hodgson, Elizabeth Heap, June Cameron, David Ellis, Christopher G Mathew, Rosalind A Eeles, Ellen Solomon and Cathryn M Lewis

J Med Genet 1999 36: 369-373
doi: 10.1136/jmg.36.5.369

Updated information and services can be found at:
http://jmg.bmj.com/content/36/5/369

These include:

References
This article cites 17 articles, 4 of which you can access for free at:
http://jmg.bmj.com/content/36/5/369#BIBL

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections
Breast cancer (239)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/