A population study of mutations and LOH at breast cancer gene loci in tumours from sister pairs: two recurrent mutations seem to account for all BRCA1/BRCA2 linked breast cancer in Iceland

Adalgeir Arason, Adalbjörg Jonasdottir, Rosa Björk Barkardottir, Jon Thor Bergh thorsson, M Dawn Teare, Douglas F Easton, Valgurard Egilsson

Abstract
The majority of breast cancer in high risk families is believed to result from a mutation in either of two genes named BRCA1 and BRCA2. A germline defect in either gene is usually followed by chromosomal deletion of the normal allele in the tumour. In Iceland two recurrent mutations have been identified, 999del5 BRCA2 and G5193A BRCA1. In this study, randomly selected pairs of sisters diagnosed with breast cancer at the age of 60 years or younger were analysed to evaluate the proportion of breast cancer resulting from BRCA1 and BRCA2. Genotypes and allele loss in tumour tissue from 42 sister pairs were compared using markers within and around the BRCA1 and BRCA2 genes. Eleven sister pairs were highly suggestive of BRCA2 linkage, and no obvious BRCA1 linkage was seen. Screening for the G5193A BRCA1 and 999del5 BRCA2 mutations showed the 999del5 mutation in the 11 BRCA2 suggestive pairs plus three pairs less indicative of linkage, and the G5193A BRCA1 mutation in one pair. When known mutation carriers are removed from the group, no indication of further linkage to BRCA1 or BRCA2 is seen. The results of our studies suggest that a large proportion of familial breast cancer in Iceland is the result of the 999del5 BRCA2 mutation, and it is unlikely that BRCA1 and BRCA2 germline mutations other than 999del5 and G5193A play a significant role in hereditary breast cancer in Iceland. Furthermore it can be concluded that most families with BRCA1 or BRCA2 linkage are easily identified by studying LOH around the defective gene in as few as two affected relatives.

(J Med Genet 1998;35:446–449)

Keywords: LOH; BRCA2; BRCA1

Breast cancer is the most common malignancy among women in western countries, with a cumulative life time risk of more than 10%. It has been estimated that 5-10% of all breast cancers are caused by genetic predisposition. Two major breast cancer susceptibility genes, BRCA1 and BRCA2, have recently been identified by linkage analysis and positional cloning. Mutations in BRCA1 and BRCA2 may confer up to 90% life long risk of female breast and ovarian cancer, while mutations in BRCA2 have in addition been associated with an increased risk of male breast cancer.

BRCA1 and BRCA2 proteins have been shown to associate with Rad51 and may therefore function in the maintenance of genome stability. A germline defect in either BRCA1 or BRCA2 in breast tumours is most often followed by chromosomal deletion of the wild type allele. The deletion usually also affects the surrounding chromosomal region and is therefore detected by nearby markers as loss of heterozygosity (LOH). In mutation carriers, this occurs in ~85% of informative tumours.

In sporadic breast tumours, a 30-40% frequency of copy loss is observed for each of these two genes, and then usually without any sign of a somatic mutation in the retained allele. In Iceland, one recurrent mutation has been described for each gene. In BRCA1, a splice site mutation in exon 17 (G5193A) has been found in three families, but BRCA1 linkage is relatively uncommon in Iceland. A much more common mutation, a 5 bp deletion in exon 9 in BRCA2 (999del5), accounts for most of the hereditary breast cancer families studied and is found in 8.5% of randomly selected breast cancer patients, as compared to 0.5% of controls from the Icelandic population. A 999del5 BRCA2 mutation has also been detected in Finland and in an American family of Puerto Rican Hispanic ancestry. The prevalence of BRCA2 linkage in Iceland contrasts with the experience in other countries where BRCA1 linkage predominates.

BRCA1 and BRCA2 are quite large genes, with transcripts of 7.8 and 11.4 kb respectively, and a wide spectrum of disease associated mutations have been described, as well as rare variants and common polymorphisms. It is therefore a laborious task to screen the coding...
region of both genes for mutations in order to estimate their proportional contribution to breast cancer in the general population. Here, an indirect approach was used to evaluate proportions of BRCA1 and BRCA2 linked breast cancer in the Icelandic population by studying families that were not preferentially selected on the basis of dense clustering of affected relatives. Tumours from a group of randomly selected sisters diagnosed with breast cancer at or below the age of 60 years were analysed for LOH. From the different frequencies of LOH in ‘linked’ and sporadic breast tumours, it can be inferred that most (>70%) sister pairs from families with linkage to either gene will display LOH around that gene in both sisters’ tumours and with a common haplotype retained, whereas only ≈5% of pairs without linkage would yield such results by chance. The patients were also screened for the presence of either of the two known BRCA1/BRCA2 mutations in Iceland and LOH and the mutation results were compared.

Materials and methods

PATIENT MATERIAL

Two sources of information were used to identify pairs of sisters with breast cancer from the Icelandic population. Firstly, the Icelandic Cancer Registry provided a list of all sister pairs (100 in total) in which at least one sister was a proband in a population based study of 947 families, representing nearly half of the breast cancer patients diagnosed in Iceland since 1910. Secondly, we have collected blood samples and clinical family history information from consecutive cases with breast cancer diagnosed in Icelandic hospitals since 1987. All probands with a confirmed history of breast cancer in a sister were considered. In the current study, we restricted the selection to pairs of sisters with breast cancer at or below 60 years of age, and from whom DNA comparison was possible between both malignant and normal tissue in two or more affected sisters. A total of 42 sister pairs were obtained; 34 pairs were derived from the Cancer Registry list and eight from the consecutive patients. Altogether 87 patients were included in the study (in three families, a third affected sister was included); the number of tumours analysed was 91 (in four of 10 cases with bilateral breast cancer both tumours were available for analysis).

Table 1. The frequency of LOH involving BRCA1 or BRCA2, according to carrier status of the 87 patients with breast cancer.

<table>
<thead>
<tr>
<th>Carrier status</th>
<th>No of patients</th>
<th>No of tumours with LOH at BRCA1/BRCA2</th>
<th>No of tumours with LOH at BRCA1/BRCA2</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA1-G5193A</td>
<td>2</td>
<td>2/2 (100%)</td>
<td>2/2 (100%)</td>
<td>2/2 (100%)</td>
</tr>
<tr>
<td>BRCA2-999del5</td>
<td>27</td>
<td>8/12 (28%)</td>
<td>29/31 (94%)</td>
<td>29/31 (94%)</td>
</tr>
<tr>
<td>No known mutation</td>
<td>58</td>
<td>26/59 (44%)</td>
<td>19/59 (32%)</td>
<td>45/78 (59%)</td>
</tr>
<tr>
<td>Total</td>
<td>87</td>
<td>36/90 (40%)</td>
<td>48/92 (52%)</td>
<td>48/92 (52%)</td>
</tr>
</tbody>
</table>

*In two sisters, microsatellite instability was seen at chromosome 17q markers but not at markers on chromosome 13q. These sisters belong to a family with segregation of an unknown mutation causing HNPPC in addition to the BRCA2-999del5 mutation (present study). The two mutations have apparently cosegregated in both sisters. All informative markers at chromosome 13q in the sisters show LOH with the common associated alleles retained. On the other hand, with microsatellite instability present at chromosome 17q markers, these sisters provide inconclusive results for LOH involving BRCA1. Therefore, the number of patients and samples informative at 17q is correspondingly reduced.

TISSUE SAMPLES

Blood was used if available, otherwise normal and tumour tissue was obtained from paraffin blocks. Areas rich in tumour cells (>90%) were selected for analysis by microscopy. All samples were taken from invasive primary tumours. Histology of the tumours was verified for the study.

DNA ANALYSIS

Peripheral blood and samples taken from paraffin blocks were processed as previously described. All samples were typed for the following microsatellite markers: D17S588, D17S579, D17S1323, D17S855, and THRA1 on chromosome 17q21 (from distal to proximal location) and D13S220, D13S267, D13S171, D13S260, and D13S1246 on chromosome 13q12-q13. The markers D17S1323 and D17S855 are intragenic to BRCA1; the BRCA2 gene is centromeric and very close to D13S171. In addition, all samples were screened for the BRCA1-G5193A mutation (using SSCP) and the BRCA2-999del5 mutation. PCR amplification and genotype scoring was as previously described. Briefly, PCR was carried out in 96 well plates using a hot start procedure followed by amplification for 35 cycles. Genotypes were visualised using a non-radioactive procedure.

Results

Altogether 91 primary breast cancer tissues from 87 patients in 42 sister pairs were screened for the 999del5 BRCA2 and the G5193A BRCA1 mutations, and analysed for LOH involving either BRCA1 on chromosome 17q or BRCA2 on chromosome 13q. Five markers were used for each chromosomal region. Table 1 shows the frequency of LOH according to the carrier status of patients.

Loss of a BRCA1 allele occurred in 40% of all patients, with the frequency apparently differing according to carrier status. Two tumours were from G5193A carriers and both had lost the wild type copy in accordance with the high expected frequency of 85%. BRCA1 allele loss was seen in 28% of tumours from BRCA2 carriers and 44% of tumours from patients with unknown mutation status. Both numbers are close to the level of 30-40% that is observed in sporadic tumours.

BRCA2 allele loss was inferred in 52% of all patients, and this also varied according to carrier status; this is three fold higher in BRCA2 carriers (94%) than in presumed non-carriers (32%) (table 1).

In table 2 the sister pairs are classified according to whether BRCA2-LOH occurred in none, one, or both sisters, and if LOH occurred in both sisters, whether or not the retained alleles (haplotypes) were alike. For this analysis, in the case of a third affected sister
Table 2  Summary of LOH results involving BRCA2 in the 42 sister pairs with breast cancer

<table>
<thead>
<tr>
<th>Tumours with LOH involving BRCA2</th>
<th>Retained alleles: comparison between sisters</th>
<th>Total No of pairs</th>
<th>No of pairs segregating BRCA2-999del5</th>
<th>No of pairs with no known BRCA1 or BRCA2 mutation</th>
<th>Observed</th>
<th>Expected*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both</td>
<td>Common</td>
<td>17 (12+5)</td>
<td>3 (2+1)</td>
<td>14 (10+4)</td>
<td>11</td>
<td>12.1</td>
</tr>
<tr>
<td></td>
<td>Unlike</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>2.1</td>
</tr>
<tr>
<td>One of two†</td>
<td></td>
<td>17 (12+5)</td>
<td>3 (2+1)</td>
<td>14 (10+4)</td>
<td>11</td>
<td>12.1</td>
</tr>
<tr>
<td>Neither‡</td>
<td></td>
<td>12 (2+8+2)</td>
<td>0</td>
<td>11 (2+8+1)</td>
<td>27</td>
<td>12.5</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>42</td>
<td>14</td>
<td>27</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*If LOH events occur at random, the probability of two, one, or no LOH within a pair of tumours would be p^2, 2pq, and q^2, respectively, where p and q are the frequencies of tumours with and without LOH respectively. The value of p is taken to be 0.32, of the LOH frequency involving BRCA2 in BRCA1/BRCA2 non-carriers in table 1. In case of LOH in both tumours of a pair (ie derived from two sisters), the ratio of pairs with the retained haplotypes being identical v unlike would be expected to be on average 1:3 (first two lines of the last column), depending on the similarity of the sisters’ genotypes.12

†The first number in the parentheses is the number of sister pairs in which the retained allele in a patient’s tumour is present in both sisters. In general, this subset is more likely to include pairs with possible BRCA2 linkage; however, an exception is seen in column 4 (italicised), in which a 999del5 carrier with LOH has a sister who is a phenocopy.

‡The number of sister pairs compatible with the number of shared haplotypes between sisters being 0, 1, or 2, respectively, is shown in parentheses.

Table 3  Summary of LOH results involving BRCA1 in 41 sister pairs with breast cancer

<table>
<thead>
<tr>
<th>Tumours with LOH involving BRCA1</th>
<th>Retained alleles: comparison between sisters</th>
<th>Total No of pairs</th>
<th>No of pairs segregating BRCA1-G5193A</th>
<th>No of pairs with no known BRCA1 or BRCA2 mutation</th>
<th>Observed</th>
<th>Expected*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both</td>
<td>Common</td>
<td>17 (12+5)</td>
<td>3 (2+1)</td>
<td>14 (10+4)</td>
<td>11</td>
<td>12.1</td>
</tr>
<tr>
<td></td>
<td>Unlike</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>2.1</td>
</tr>
<tr>
<td>One of two†</td>
<td></td>
<td>17 (12+5)</td>
<td>3 (2+1)</td>
<td>14 (10+4)</td>
<td>11</td>
<td>12.1</td>
</tr>
<tr>
<td>Neither‡</td>
<td></td>
<td>16 (4+8+4)</td>
<td>0</td>
<td>9 (2+5+2)</td>
<td>27</td>
<td>8.5</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>41</td>
<td>14</td>
<td>27</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*See explanation to table 2. The value of p here is 0.44, cf the LOH frequency involving BRCA1 in BRCA1/BRCA2 non-carriers in table 1.

†See explanation to table 2. In the BRCA1-G5193A carrying sister pair (cf the fourth column), one sister is a phenocopy so she did not segregate the haplotype which was retained in her sister’s tumour. A third affected sister (not included in the table) had both the mutation and BRCA1-LOH.

‡The number of sister pairs compatible with the number of shared haplotypes between sisters being 0, 1, or 2, respectively, is shown in parentheses.

Discussion

In this study we used LOH information to identify from a group of sister pairs with breast cancer the most likely pairs to belong to families with BRCA1 or BRCA2 linkage. All the most suggestive pairs turned out to have a mutation in the gene indicated. LOH is therefore a good predictor of linkage as regards BRCA1 and BRCA2, and presumably some other hereditary cancer genes as well, depending on how frequently wild type alleles are lost in carriers’ tumours. By comparing LOH results from as few as two affected members of a family, most mutation carriers seem to be easily identified. This appears to be a feasible approach to narrowing the search for mutation carriers in the population, who may then be subjected to gene mutation analysis. This could also help to solve problems in genetic counselling, when the number of affected cases in a family is insufficient for reliable linkage analysis by conventional methods.

BRCA2 is seen to contribute much more than BRCA1 to breast cancer in our study, and this accords with previous studies in Iceland.12 22 25 25 The observed ratio is 14:1. Altogether 15 (36%) of the 42 sister pairs are accounted for by the two mutations analysed, and it seems unlikely that other defects in BRCA1 or BRCA2 are yet to be found in these sister pairs. The remaining 27 sister pairs may include pairs with linkage to other loci (BRCA3, etc) and presumably also pairs with tumours of sporadic origin.

A slight excess in BRCA1-LOH was observed among non-carriers (26 of 59 tumours, or 44%) compared to BRCA2 mutation carriers (eight of 29 tumours, or 28%) (table 1). Although this difference is not statistically significant (Fisher exact probability=0.166), it could be taken to indicate additional BRCA1 linkage in presumed non-carriers. However, this possibility seems unlikely as the excess LOH is seen to raise the number of sister pairs with LOH in both sisters, but with different haplotypes retained (table 3). We have also collected information on breast cancer in relatives (first, second, and third degree) of these 27 sister pairs (data not shown). By subdividing the group according to whether or not more family members than two sisters were affected, BRCA1-LOH is observed more frequently in the group with no additional family history of the disease (12 tumours of 21 (57%) compared to 14 of 36 (39%)) (results not shown), indicating independence of heredity and LOH involving BRCA1 in these patients.

We have tried the method of LOH comparison in sib pairs to analyse the prevalence of BRCA1 and BRCA2 mutations in Iceland with...
promising results. It would be interesting to see this approach used in other populations, for these or other hereditary cancer genes, or as a strategy to identify unmapped tumour suppressor genes.

We thank Professor H Tulinius and G Olafsdottir at the Icelandic Cancer Society and A G Hafsteinsson and O Vilhjalmsdottir at the Genetic Committee of the University of Iceland for providing clinical and family information; Sir Walter Bodmer, Dr N Sparrow, D T Bishop, and M Stratton for discussions; and Professor J Hallgrimsson, S Kristiansdottir, H Helgadottir, G Eiriksdottir, and Dr G J Arason for their contribution. This work was funded by the Science Fund of Iceland and the University Hospital Research Fund. We are greatly indebted to the Imperial Cancer Research Fund for family information; in two breast cancer families: screening reveals low frequency of polymorphisms in Icelandic breast cancer patients. 

We thank Professor H Tulinius and G Olafsdottir at the Icelandic Cancer Society and A G Hafsteinsson and O Vilhjalmsdottir at the Genetic Committee of the University of Iceland for providing clinical and family information; Sir Walter Bodmer, Dr N Sparrow, D T Bishop, and M Stratton for discussions; and Professor J Hallgrimsson, S Kristiansdottir, H Helgadottir, G Eiriksdottir, and Dr G J Arason for their contribution. This work was funded by the Science Fund of Iceland and the University Hospital Research Fund. We are greatly indebted to the Imperial Cancer Research Fund for family information; in two breast cancer families: screening reveals low frequency of polymorphisms in Icelandic breast cancer patients.


