Mutations in two major cancer susceptibility genes, BRCA1 and BRCA2, predispose to early onset breast and ovarian cancer. Since 1994, genetic testing for germline mutations in these genes has been carried out in many countries in both diagnostic and research settings. Mutation analysis is usually done on the basis of a (family) history of breast or ovarian cancer—for example, (very) early age of onset, multiple affected close relatives, multiple tumours in one patient, and breast cancer in men. Ethnic background may also play a role in decisions about DNA testing, as in some populations founder BRCA1 or BRCA2 mutations are known to occur at relatively high prevalence (for example, 185delAG and 5382insC in BRCA1 and 6174delT in BRCA2 in Ashkenazi Jews).

In recent years several families have been described in which more than one BRCA mutation segregated, predominantly involving Ashkenazi Jewish founder mutations. These reports describe families that harbour two pathogenic BRCA1 mutations, one BRCA1 and one BRCA2 mutation, or even three pathogenic mutations in BRCA genes. Some of these families were uncovered because the index case appeared to carry two (founder) mutations, and only rarely was cosegregation of two different mutations suspected beforehand on the basis of the family history. This has led to the recommendation that one should always test for all three founder mutations in individuals of known Jewish ancestry. However, DH has also been reported without prior knowledge of Jewish ancestry. In this paper we present four new cases with mutations in both BRCA1 and BRCA2 and review and update the 30 cases reported in the literature, in order to investigate the phenotypic consequences of double heterozygosity (DH)—that is, the presence of pathogenic mutations in both BRCA1 and BRCA2 in one individual.

**CASE REPORTS**

The first proband had papillary serous ovarian cystadenocarcinoma stage IIB at age 40 and unilateral infiltrative ductal breast carcinoma at age 45. She was referred to the clinical genetics department because she was considering prophylactic contralateral mastectomy if she harboured a genetic predisposition. She has 10 siblings and a positive maternal history and family history, BRCA1 and BRCA2 testing was carried out.

**Key points**

- Detection of double heterozygosity (DH) for BRCA1 and BRCA2 mutations has implications for genetic counselling and possibly for clinical management. We review 34 women with DH to assess phenotypic expression.

- In the diagnostic setting DH occurs in 0.09–0.36% of index cases—that is, in 0.22–0.87% of proven BRCA mutation carriers, rising to 1.8% in Ashkenazi Jews. At least one of the detected mutations is usually a founder mutation, mainly 185delAG and 5382insC in BRCA1 and 6174delT in BRCA2.

- The phenotypic expression in DH varied from unilateral breast cancer at age 26 to cancer-free survival at age 70. Twenty-four cases had a total of 32 primary cancers; the mean age at first breast cancer was 40.8 years (range 26 to 70), and for ovarian cancer, 45.7 years (range 36 to 57). The age related incidence for first cancer was 84% (38% to 99%) at age 70, and median cancer-free survival was 45 years (range 33 to 57). The genotype 5382insC/6174delT seemed to confer the highest cancer risk.

- In non-Ashkenazi populations DH is rare. Compared with carriers of a single BRCA1 or BRCA2 mutation, DH does not seem to lead to a more severe phenotype. The presence of a second mutation has important consequences for genetic counselling. We suggest that index cases should always be tested for all known founder mutations, that co-segregation in BRCA positive families must if possible be confirmed, and that mutation analysis should be extended where there is a tentative phenocopy with a positive history on both sides, in order to avoid missing a second BRCA mutation.

**Protein truncation testing of exon 11 of BRCA1 and exon 10 and 11 of BRCA2 indicated a truncating mutation in BRCA1. Sequencing demonstrated a two base pair deletion (2804delAA) in BRCA1, a known Dutch founder mutation.** Because the sample was used to validate the denaturing gradient gel electrophoresis (DGGE) analysis we were developing for BRCA1 and BRCA2, additional testing was carried out even though the causative mutation had already been found. Unexpectedly, this revealed a second mutation in

**Abbreviations:** DH, double heterozygosity; LOH, loss of heterozygosity
BRCA2: 3715delG, a novel mutation that leads to a stop codon at position 1167.

Subsequently, these mutations were detected in brothers of our proband, confirming that both are inherited and neither is a de novo mutation. Maternal and paternal family members declined DNA testing, and ovarian tumour specimens of the patient’s mother were no longer available. We were therefore unable to determine whether one mutation came from either parent or whether both were inherited from the mother.

The woman was counselled about the presence of the two distinct genetic predispositions and informed that her risk of a second breast cancer could be around 60% (the figure for women with only one mutation) or even higher. She subsequently underwent a prophylactic mastectomy (with no malignancy on pathological examination) and is free of disease at the age of 51 years.

The second Dutch case attended the hereditary cancer clinic at age 50 years. She was treated for unilateral ductal breast cancer with positive axillary lymph nodes at age 28. She was referred because of her daughter, who was 32 years old at that time. Family history was not informative. She is the only child of her parents’ relationship, and they both died young from unknown causes. There were maternal half brothers and sisters but she had no contact with them. DNA from unknown causes. There were maternal half brothers and sisters but she had no contact with them. DNA

The second Dutch case attended the hereditary cancer clinic at age 50 years. She was treated for unilateral ductal breast cancer with positive axillary lymph nodes at age 28. She was referred because of her daughter, who was 32 years old at that time. Family history was not informative. She is the only child of her parents’ relationship, and they both died young from unknown causes. There were maternal half brothers and sisters but she had no contact with them. DNA

Figure 1: Anonymised pedigree of case 1. The proband is indicated by an arrow. Squares, male; circles, female. BC, breast cancer; d, deceased; OC, ovarian cancer; PP, postpartum.

Case 4 was affected by left sided invasive lobular carcinoma at age 51. She had no prophylactic surgery and had no further cancer or recurrence to date at age 68. She has a BRCA1 5382insC mutation and a BRCA2 6174delT mutation. Her unaffected brother’s daughter had breast cancer at 34 years and harbours the BRCA1 5382insC mutation. Her unaffected daughter, who is 40 years old, has the 6174delT mutation. The proband’s mother had no cancer at age 71, but a maternal aunt had breast cancer aged 70 years. One maternal cousin had bowel cancer in his 50s and 60s. The proband’s father did not have cancer, but a paternal cousin had a cancer of unknown origin at 57 years. Unfortunately segregation analysis could not be carried out in this family.

REVIEW OF CASES WITH DOUBLE HETEROZYGOSITY

Data on various cases with DH described by Frank et al. were updated and extended with respect to mutations and phenotypic expression. We found an additional 22 cases with DH from a total of 13 reported families. The report by Caldes et al. is the only one involving a missense mutation, which initially raised doubts about its pathogenicity. We decided to include this family, based on the literature on this mutation which is at an evolutionarily highly conserved residue in a functional motif of the BRCA1 protein (BRCT repeat); the missense change has a high chemical difference score, the mutation is absent in appropriate control populations, and the mutant alleles are always found to be retained in the tumours. It is striking, however, that the phenotypic expression in all these cases (from one family) turned out to be less severe than in the other DH cases. One case report on a woman with breast cancer before the age of 35 is not included, because presumed DH was based on tumour sample analysis only—that is, without constitutional DNA analysis or family history. In the series of Frank et al., there were nine women who were diagnosed with DH. In one of these the clinical data were missing, so she was excluded from analysis. In the series of

www.jmedgenet.com
<table>
<thead>
<tr>
<th>No.</th>
<th>DH (index) cases</th>
<th>BRCA1 Mutations</th>
<th>BRCA2 Mutations</th>
<th>FH: paternal/paternal origin confirmed?</th>
<th>Inheritance confirmed?</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>OC 40 and BC 45</td>
<td>BRCA1 2804delAA</td>
<td>BRCA2 371delIG</td>
<td>Pos/Neg</td>
<td>Both mutations familial, maternal/paternal origin undetermined</td>
<td>This report (AZG)</td>
</tr>
<tr>
<td>2</td>
<td>BC 28 (49)</td>
<td>BRCA1 2804delAA</td>
<td>BRCA2 4677delA</td>
<td>Neg/Neg</td>
<td>No information available</td>
<td>This report (UMCN)</td>
</tr>
<tr>
<td>3</td>
<td>Healthy 40</td>
<td>BRCA1 185delAG</td>
<td>BRCA2 6174delT</td>
<td>Pos/??</td>
<td>Two maternal cousins 185delAG, 6174delT, probably paternal</td>
<td>This report (UK)</td>
</tr>
<tr>
<td>4</td>
<td>BC 51 (68)</td>
<td>BRCA1 5382insC</td>
<td>BRCA2 6174delT</td>
<td>Pos/??</td>
<td>Maternal niece 5382insC, origin 6174delT unknown</td>
<td>This report (UK)</td>
</tr>
<tr>
<td>5</td>
<td>BC &lt;39</td>
<td>BRCA1 187delAG</td>
<td>BRCA2 6174delT</td>
<td>Neg/Neg</td>
<td>No information available</td>
<td>This report/Frank et al</td>
</tr>
<tr>
<td>6</td>
<td>BC 40</td>
<td>BRCA1 5385insC</td>
<td>BRCA2 6174delT</td>
<td>??/??</td>
<td>No information available</td>
<td>This report/Frank et al</td>
</tr>
<tr>
<td>7</td>
<td>BC 41</td>
<td>BRCA1 187delAG</td>
<td>BRCA2 6174delT</td>
<td>Pos</td>
<td>No information available</td>
<td>This report/Frank et al</td>
</tr>
<tr>
<td>8</td>
<td>Bilat BC 34</td>
<td>BRCA1 187delAG</td>
<td>BRCA2 6174delT</td>
<td>Pos</td>
<td>No information available</td>
<td>This report/Frank et al</td>
</tr>
<tr>
<td>9</td>
<td>Bilat BC 33</td>
<td>BRCA1 5385delAG</td>
<td>BRCA2 6174delT</td>
<td>??/??</td>
<td>No information available</td>
<td>This report/Frank et al</td>
</tr>
<tr>
<td>10</td>
<td>Bilat BC 55</td>
<td>BRCA1 187delAG</td>
<td>BRCA2 6174delT</td>
<td>Pos</td>
<td>No information available</td>
<td>This report/Frank et al</td>
</tr>
<tr>
<td>11</td>
<td>Healthy 61</td>
<td>BRCA1 5385insC</td>
<td>BRCA2 6174delT</td>
<td>Pos/??</td>
<td>No information available</td>
<td>This report/Frank et al</td>
</tr>
<tr>
<td>12</td>
<td>Healthy 66</td>
<td>BRCA1 187delAG</td>
<td>BRCA2 6174delT</td>
<td>Neg/??</td>
<td>No information available</td>
<td>This report/Frank et al</td>
</tr>
<tr>
<td>13</td>
<td>BC 48 and OC 50</td>
<td>BRCA1 185delAG</td>
<td>BRCA2 6174delT</td>
<td>??/??</td>
<td>No information available</td>
<td>This report/Frank et al</td>
</tr>
<tr>
<td>14</td>
<td>Index BC 38</td>
<td>BRCA1 185delAG</td>
<td>BRCA2 6174delT</td>
<td>Pos</td>
<td>Father had neither mutation, mother assumed carrier of both mutations</td>
<td>Frieden et al</td>
</tr>
<tr>
<td>15</td>
<td>Mother OC 50†</td>
<td>BRCA1 185delAG</td>
<td>BRCA2 6174delT</td>
<td>Pos</td>
<td>Father had neither mutation, mother assumed carrier of both mutations</td>
<td>Frieden et al</td>
</tr>
<tr>
<td>16</td>
<td>OC 57 (62)</td>
<td>BRCA1 185delAG</td>
<td>BRCA2 6174delT</td>
<td>Pos</td>
<td>No information available</td>
<td>Frieden et al</td>
</tr>
<tr>
<td>17</td>
<td>Healthy 50</td>
<td>BRCA1 185delAG</td>
<td>BRCA2 6174delT</td>
<td>??/??</td>
<td>No information available</td>
<td>Frieden et al</td>
</tr>
<tr>
<td>18</td>
<td>BC 45 (46)</td>
<td>BRCA1 5382insC</td>
<td>BRCA2 6174delT</td>
<td>??/??</td>
<td>5382insC assumed to be maternal</td>
<td>Frieden et al</td>
</tr>
<tr>
<td>19</td>
<td>BC 35</td>
<td>BRCA1 2508G→T</td>
<td>BRCA2 3295insA</td>
<td>Pos</td>
<td>Mother neither mutation, no other family members available</td>
<td>Liede et al</td>
</tr>
<tr>
<td>20</td>
<td>BC 30 and OC 41</td>
<td>BRCA1 3888delGA</td>
<td>BRCA2 6174delT</td>
<td>Pos</td>
<td>Mother and sister 6174delT, father 3888delGA</td>
<td>Randall et al</td>
</tr>
<tr>
<td>21</td>
<td>BC &lt;40</td>
<td>BRCA1 3888delGA</td>
<td>BRCA2 6174delT</td>
<td>??/??</td>
<td>Father 6174delT; neither parent 3888delGA; 3888delGA assumed paternal de novo</td>
<td>Tesoriero et al</td>
</tr>
<tr>
<td>22</td>
<td>Index healthy 36</td>
<td>BRCA1 185delAG</td>
<td>BRCA2 6174delT</td>
<td>Pos</td>
<td>Brother and father neither mutation; sister only 185delAG; mother assumed carrier of both mutations</td>
<td>Mosleh et al</td>
</tr>
<tr>
<td>23</td>
<td>Mother OC 36†</td>
<td>BRCA1 185delAG</td>
<td>BRCA2 6174delT</td>
<td>Pos</td>
<td>Brother and father neither mutation; sister only 185delAG; mother assumed carrier of both mutations</td>
<td>Mosleh et al</td>
</tr>
<tr>
<td>24</td>
<td>BC 33 and 44 and 47</td>
<td>BRCA1 5382insC</td>
<td>BRCA2 6174delT</td>
<td>??/??</td>
<td>No information available</td>
<td>Bell et al</td>
</tr>
<tr>
<td>25</td>
<td>Index BC 28</td>
<td>BRCA1 5382insC</td>
<td>BRCA2 6174delT</td>
<td>Neg</td>
<td>Mother both mutations</td>
<td>Caldes et al</td>
</tr>
</tbody>
</table>

* DH: double heterozygosity, FH: familial history of breast or ovarian cancer.

**Online mutation report for 30 of 84 mutations reported by www.jmedgenet.com**
Satagopan et al.,30 Warner et al.,31 and Robson et al.,32 there was mention of double heterozygotes, but they were not included because of lack of clinical information. We are aware of three males with DH: one man described by Caldes et al.16 who had prostate cancer at age 66; one healthy man of over 50 in the series of Frank et al.23; and one brother of case 1, who is healthy at age 63. These were also not included in the analysis.

RESULTS

The data on all 34 informative women are given in table 1. The women originated from 25 families, and most cases of DH were the only ones known in the family, the clearest exception being the large family from Spain.16 Excluding the two Korean families for which no population data were available,20 we found that in all but two families (index 19 and index 25) at least one of the detected mutations was a known founder in the population. In several families Ashkenazi Jewish descent was known before testing, and DNA analysis was often restricted to founder mutations. In 17 of 25 families (19 women) two Ashkenazi founder mutations were detected, whereas in two families (two women) such a founder mutation was detected only in BRCA2 (6174delT). In many of the 25 families, the information on family history of cancer was inadequate, most often on the paternal side; this is likely to reflect the method of ascertainment. From 18 informative families, the maternal (family) history was positive in 13 cases, the paternal family history was positive in only one case, and there was a history of breast/ovarian cancer on both sides of the family in four.

Of the 34 women with DH, 10 were without breast/ovarian cancer (mean age at last follow up 51.3 years (range 36 to 70)) and 24 (71%) had developed breast or ovarian cancer or both. Fourteen women had one primary breast cancer (mean age at diagnosis 41.3 years (range 26 to 70)), three had bilateral breast cancer (mean age at diagnosis 40.7 years (range 33 to 55)), and one had three primary breast cancers (at age 33, 44, and 47). Three women were diagnosed with both breast and ovarian cancer (mean age at first cancer 41.3 years (range 33 to 55)), and one had three primary breast cancers (at age 33, 44, and 47). Three women were diagnosed with both breast and ovarian cancer (mean age at first cancer 39.3 years (range 30 to 48)); mean age for breast cancer 41.0 years (range 30 to 48); mean age for ovarian cancer 43.7 years (range 40 to 50)). Three women had ovarian cancer only (mean age 47.7 years (range 36 to 57)). In 13 of 24 women (54%) the first cancer was diagnosed before the age of 40.

![Figure 2](image2.png)  
**Figure 2** Age related penetrance in 34 cases with double heterozygosity.

![Figure 3](image3.png)  
**Figure 3** Kaplan–Meier curve for tumour-free survival in cases with double heterozygosity. Cases are left censored at age of first cancer or at age at last follow up.

Table 1

<table>
<thead>
<tr>
<th>No</th>
<th>DH (index) cases*</th>
<th>Mutations†</th>
<th>FH: maternal/ paternal‡</th>
<th>Inheritance confirmed?</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
<td>Index BC 33</td>
<td>BRCA1 E1661X</td>
<td>Neg</td>
<td></td>
<td>Choi et al (pat 60071)</td>
</tr>
<tr>
<td>33</td>
<td>Mother stomach cancer 62</td>
<td>BRCA1 6174del4 6174del4</td>
<td>Neg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>Index BC 26</td>
<td>BRCA1 1653del5</td>
<td>Neg</td>
<td></td>
<td>Choi et al (pat 60351)</td>
</tr>
<tr>
<td></td>
<td>BRCA2 3026delCA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Diagnosis, age at diagnosis (years); (_ _), age at last follow up.
†Assumed to have both mutations.
‡Documented founder mutations in bold type; 185delAG and 187delAG are identical, 5382insC and 5385insC are identical.
§Family history of breast and/or ovarian cancer.
©AZG, Groningen University Hospital; BC, breast cancer; Bilat, bilateral; DH, double heterozygosity; FH, family history; Neg, negative; OC, ovarian cancer; Pos, positive; UMCN, University Medical Centre Nijmegen; ?, no information available.
which is 38% of the total group (13/34). These figures are likely to be skewed owing to differences in follow up time.

The total number of primary cancers in 24 affected women was 32 (26 breast, six ovarian) at a mean onset age of 42.3 years (range 26 to 70). The mean age at diagnosis for all breast cancers was 41.1 years (range 26 to 70), and for all ovarian cancers, 45.7 years (range 36 to 57). Cancer events in all 34 women with DH are depicted longitudinally in fig 2. Follow up data on reported cases often stopped at the age of cancer diagnosis or at the age of DNA testing. Only in four cases with DH was detailed information available on prophylactic surgery: case 1, unilateral mastectomy at age 50; case 2, bilateral salpingo-oophorectomy at age 49; case 3, bilateral mastectomy at age 35 and salpingo-oophorectomy at age 36; and case 4, no prophylactic surgery. These data influence their residual risk of developing cancer in the following years, and for this reason they were censored in the Kaplan–Meier analysis. Information on the extent of breast cancer treatment (that is, mastectomy or breast conserving therapy, radiotherapy, chemotherapy, or hormonal therapy), which may also affect the risk of subsequent cancers, was also unavailable on most cases, so this could not be taken into account. Of the 32 cancers that developed in 24 women with DH, 15 (47%) were diagnosed before the age of 40. Because the follow up time varied among the cases, the cancer-free survival curve (which only takes first cancers into account) was censored for missing follow up years (fig 3); it shows a median cancer-free survival of 45 years (95% confidence interval, 33 to 57). Figure 4 gives the cumulative incidence proportion of cancer in DH; by 70 years of age this was 84% (38% to 99%) for breast and ovarian cancer combined.

In our series there were three recurrent genotypic combinations of DH. The phenotypic expression is summarised in table 2. Thirteen women had 185delAG/6174delT, of whom four were healthy (mean age 48 years (range 36 to 66)) and nine (69%) had a total of 12 cancers (four ovarian, eight breast). Three women had multiple cancers. Six cases had 5382insC/6174delT, of whom one was without cancer at 61 years, and five (83%) had a total of eight cancers (eight breast cancers, no ovarian). Two women had multiple cancers. Seven cases had the 5242C→T/6503delTT genotype involving a missense mutation in BRCA1, of whom four were healthy (mean age 50 years (range 41 to 70)) and three (43%) had a total of three cancers (all breast).

To assess the contribution of both mutations in tumour development in case 1, loss of heterozygosity (LOH) analysis was carried out on DNA isolated from paraffin embedded breast and ovarian tumour tissue. DNA from peripheral blood and from paraffin embedded normal tissue from the proband was used as control. Sequence analysis around the mutation sites revealed loss of the wild type allele at the BRCA1 locus in the breast tumour tissue as well as in the ovarian tumour specimen. LOH data for the BRCA2 gene showed predominant loss of the wild type allele in breast cancer tissue. However, in ovarian cancer tissue predominant loss of the mutant allele was detected.

The data above were added to the LOH analyses that have been reported previously in seven breast cancer and two ovarian cancer specimens (table 3). In three of eight breast cancers, LOH for BRCA1 was detected, and in four (although incomplete in case 1) of eight for BRCA2. Two breast cancers did not show LOH for either gene. The data on ovarian tissue are very inconclusive: one tumour had LOH for both genes, one had no LOH for either gene, and one tumour (case 1) had loss of the wild type allele for BRCA1 but predominant loss of the mutant allele for BRCA2.

### DISCUSSION

Serendipitous detection of DH for BRCA1 and BRCA2 prompted us to collect and review all available cases with DH. Thirty cases have been described since 1997, from different countries with highly variable ways of ascertainment ranging from research programmes targeted at Ashkenazi populations to case referral to clinical genetics.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Age*</th>
<th>FU years (25 and older)</th>
<th>AFFECTED/NON-AFFECTED BC†</th>
<th>OC†</th>
<th>Cancers per carrier</th>
<th>Cancers per FU-year &gt; 25</th>
</tr>
</thead>
<tbody>
<tr>
<td>5382insC/6174delT</td>
<td>6</td>
<td>52 (40 to 61)</td>
<td>151</td>
<td>5/1</td>
<td>8 (40; 33 to 51)</td>
<td>0</td>
<td>1.33 0.038</td>
</tr>
<tr>
<td>185delAG/6174delT</td>
<td>13</td>
<td>48 (36 to 66)</td>
<td>253</td>
<td>9/4</td>
<td>8 (43; 34 to 55)</td>
<td>0</td>
<td>0.92 0.047</td>
</tr>
<tr>
<td>5242C→T/6503delTT</td>
<td>7</td>
<td>52 (28 to 70)</td>
<td>177</td>
<td>3/4</td>
<td>3 (55; 28 to 70)</td>
<td>0</td>
<td>0.43 0.017</td>
</tr>
</tbody>
</table>

*Mean (range). †Mean (median age at diagnosis; range).

<table>
<thead>
<tr>
<th>Breast cancer tissue (case No)</th>
<th>Ovarian cancer tissue (case No)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13 20 21 24a 24b 24c 25 1 Total</td>
<td>13 20 1 Total</td>
</tr>
<tr>
<td>LOH BRCA1</td>
<td>– + – – – + – + 3/8 – + + 1/3</td>
</tr>
<tr>
<td>LOH BRCA2</td>
<td>– – + + + – – +/– 3/7 – + – 1/2</td>
</tr>
</tbody>
</table>

*Predominant loss of mutant allele.
LOH, loss of heterozygosity.
services. This has most probably led to a publication bias: some cases may be unreported, and an overrepresentation of Ashkenazi Jewish cases with founder mutations is likely.

**Prevalence**

The chance of detecting DH depends largely on the frequency of mutation carriers in the reference population, but also on the availability of clinical genetic knowledge and services, the extensiveness of family history taking, the inclusion criteria used for DNA testing, and the extent and procedures of molecular analysis. These are all very heterogeneous—for example, genetic testing may vary from only an analysis of the three well known Jewish founder mutations to comprehensive mutation analysis of the whole coding regions. The population carrier frequency of 185delAG, 5382insC (BRCA1), and 6174delT (BRCA2) is estimated to be 0.92, 0.26, and 1.20, respectively, in the Ashkenazi population, adding up to approximately 2.4%. Peto et al detected among at least 6724 non-Ashkenazi individuals in cases (1.8%); 11 of 3022 tested (0.36%). No cases of DH were of Ashkenazi descent (11 of 617 positive result (0.64%)—that is, 11 of 10 000 cases tested (0.1%). All DH were identified among 1720 patients with a positive test result (0.53%), which amounts to 0.14% (1/739) of all index cases tested to date.

**LOH analysis**

*BRCA1* and *BRCA2* both act as tumour suppressor genes, and their gene products play a role in different parts of the complex process of DNA repair. We tried to assess which mutation was the predominant one in oncogenesis in DH in the two different tumours in case 1. Analysis of LOH revealed that the mutation in *BRCA1* is most probably the causative mutation in both the breast tumour and the ovarian tumour. The role of the *BRCA2* mutation, however, is less clear. In the breast tumour there is loss of the wild type allele only in part of the cells. This is not a result of admixture with normal cells, because in the same DNA sample LOH is complete at the *BRCA1* locus. Perhaps loss of the second *BRCA2* allele plays a greater role in tumour progression than in the origin of this breast cancer. In the ovarian tumour there is loss of the mutant *BRCA2* allele. Some loss of alleles can be random owing to the known chromosomal instability that occurs with mutations in the BRCA genes. From table 3 one cannot deduce whether there is a preference for *BRCA1* or *BRCA2* to be the first gene to lose its function completely. This is especially clear from the data of Bell et al, who studied three breast tumours from one patient. In two of these they found LOH for *BRCA2* (previously detected in the family) is not found in a relative with breast or ovarian cancer, one could consider screening for other (founder) mutations before concluding that the index case is a phenocopy. However, genes other than *BRCA1* and *BRCA2* could also be involved. The likelihood that a (non-Ashkenazi) index case with a non-founding *BRCA* mutation has inherited a second mutation from the unaffected side is much less than 1%. It is probably a more logical use of resources to extend the availability of BRCA testing to moderate risk groups than to put more effort into full sequencing of *BRCA1* and *BRCA2* in these BRCA mutation carriers.

**Phenotypic expression of DH**

Assessment of the age related cancer incidence in DH is difficult for several reasons. First, it is a small and heterogeneous group from different populations and ascertained in different ways. Second, in most cases data on risk modifying factors (for example, the number of pregnancies, prophylactic surgery, oral contraceptives, hormone replacement therapy, and so on) are lacking. Third, in affected cases details of the mode of detection (screening or interval), multifocality, and treatment methods (mastectomy, breast

www.jmedgenet.com
conserving treatment, radiotherapy, chemotherapy, or adjuvant treatment) are missing.

The age related phenotypic expression in this small series of 34 female cases of DH appeared to be highly variable (fig 3). Ten women of the 34, with a mean age of 51.3 years (range 36 to 70), were without cancer. For most of these there were no data on risk modifying factors, so this group is not only very small but probably also heterogeneous. Kaplan–Meier analysis shows a median cancer-free survival of 45 years (95% confidence interval, 33 to 57), which is comparable with the average age for breast cancer diagnosis in \( BRCA1 \) and \( BRCA2 \) mutation carriers. The cumulative incidence proportion of breast cancer in DH at age 70 is 80% for breast cancer only and 84% for breast and ovarian cancer. This is comparable with the age related expression of breast cancer in large family based series of \( BRCA1 \) mutation carriers (85% and 84% risk of breast cancer at age 70 for \( BRCA1 \) and \( BRCA2 \), respectively).36 However, comparison with data from Ashkenazi populations may be more appropriate given the large number of Ashkenazi founder mutations in our series. The risks we found here in DH were higher than reported in population based data by Struwevg et al (40–73% at age 70 for \( BRCA1 \)),23 by Warner et al (60% at age 70 for \( BRCA1 \),13 or by Satagopan et al (31–80% at age 70 for \( BRCA1 \) and 14–50% for \( BRCA2 \)),37 but comparable to the family based data of Brose et al, with 37% Ashkenazi cases (74–82% by age 70 for \( BRCA1 \)).38 The population based data of King et al (69% for \( BRCA1 \) and 74% for \( BRCA2 \) at age 70).25

Brose et al, who had 37% Ashkenazi founder mutations in their study of 483 \( BRCA1 \) mutation carriers, estimated the risk of a second cancer in their population: 22% of women had a second breast cancer (age adjusted lifetime risk 34–47%), and 15% had ovarian cancer after a previous breast cancer (age adjusted lifetime risk 14–24%).39 In our series four breast cancer patients had a second breast cancer (21%) and two breast cancer patients subsequently had ovarian cancer (11%). If we include case 1, who had ovarian cancer before breast cancer, the percentage is 16% (3/19), and these percentages are remarkably similar to those of Brose et al. This means that on the basis of this small series there is no indication that double primary tumours occur more often in women with DH.

Genotype–phenotype correlation

Though the data on the three recurrent phenotypes are insufficient for statistical analysis, they indicate that 5382insC/185delAG gives the highest cancer risk—that is, the highest proportion of affected DH cases (5/6), the highest number of cancers per carrier (1.33) and per affected case (1.6), the highest ratio of multiple cancers, and the lowest mean age at diagnosis (40 years) (table 2). The 185delAG/6174delT is the only recurrent DH that caused ovarian cancer, leading to cancer in 69% of DH cases, with 0.92 cancers per carrier and 1.3 cancers per affected case. However, when the cancer incidence is correlated with the number of follow up years above the age of 25 (0.053 and 0.047, respectively), the difference becomes negligible. The 5242C→T/6503delT combination gives the lowest risk and the oldest average age of onset, which may be a reflection of the fact that we are dealing with a missense mutation in \( BRCA1 \) (which makes its pathogenicity less certain) and with members of only one family.

It is difficult to compare these results (that is, 185delAG/6174delT and 5382insC/6174delT) with the published data on founder mutation specific penetrance. Struwevg et al concluded that the apparent cancer risk was highest for the 5382insC mutation in \( BRCA1 \) and, surprisingly, that the ovarian cancer risk was highest in 5382insC and lowest in 185delAG.27 Brose et al found comparable results, with a lower ovarian cancer risk in 185delAG carriers.28 Others found that the penetrance of 185delAG and 5382insC was comparable, although the latter mutation was observed much more often than expected, suggesting a higher penetrance.29

Conclusions

Double heterozygosity for \( BRCA1 \) and \( BRCA2 \) mutations is a rare phenomenon, with an incidence of 0.22–0.87% among \( BRCA \) mutation carriers, though among Ashkenazi Jewish cases the percentage may be as high as 1.8%. Analysis of 34 cases with DH shows that the phenotypic expression is comparable to the severe end of the spectrum of \( BRCA1 \) mutation carriership. There is no indication of a more severe expression with respect to age of onset, cumulative lifetime risks, and the chance of multiple primary tumours. This implies that the usual cancer risk management and preventive options available to high risk women can be offered to women with DH as well. However, the numbers are small and it is therefore important to monitor the follow up of all women with DH. Data on LHI analysis in both breast and ovarian tumours of women with DH are very limited and do not suggest a predominant contribution of either \( BRCA1 \) or \( BRCA2 \) to oncogenesis in these women.

The main difference in counselling between carriers of a single mutation and people with DH lies in the risks for first degree relatives and other family members. These are confronted with a risk of carrying a mutation up to 75%, and could be falsely reassured if only one familial mutation is excluded while an unrecognised one could still be present. This underlines the need for thorough family investigation once DH is detected in an index case. Before DNA analysis, the presence of DH was suspected only in a small minority on the basis of family history. When a \( BRCA1 \) or \( BRCA2 \) mutation is detected in an index case, we suggest that co-segregation of the mutation with the diseases should always be assessed in order confirm that the detected mutation is sufficient to explain the family history. When there is a positive family history on both sides, when the first detected mutation is a founder mutation, or when there is Ashkenazi Jewish descent, further mutation analysis of \( BRCA1 \) and \( BRCA2 \) should be considered before concluding that an affected relative without the familial mutation is a phenocopy.

ACKNOWLEDGEMENTS

RE is funded by The Institute of Cancer Research. JB and EB are funded by Cancer Research UK. AS is funded by the Barclay Foundation. We thank M Schaapveld for methodological discussion and Professor D G Evans for critical comments on the manuscript.

Authors’ affiliations

B Leete, A H van der Hout, M K Bakker, I M Mulder, A ten Berge, E P Leenders, J C Oosterwijk, Department of Clinical Genetics, Groningen University Medical Centre, Groningen, Netherlands

A M Deffenbaugh, Myriad Genetic Laboratories Inc, Salt Lake City, Utah, USA

J Wesseling, Department of Pathology, Groningen University Medical Centre

J de Hullu, Department of Obstetrics and Gynaecology, University Medical Centre St Radboud, Nijmegen, Netherlands

N Hoogerbrugge, M J L Ligteneberg, Department of Clinical Genetics, University Medical Centre St Radboud

A Ardern-Jones, E Bancroft, Cancer Genetics Unit, Institute of Cancer Research, Royal Marsden Hospital, Sutton, Surrey, UK

A Salmon, Hadassah Oncology Centre, Hadassah, Jerusalem, Israel

J Barwell, Clinical Genetics Department, St George’s NHS Trust, London SW17, UK

R Eeles, Translational Cancer Genetics Team, Institute of Cancer Research, Sutton, Surrey, UK

Competing interests: none declared
REFERENCES


Randall TC, Bell KA, Rebane BA, Rubin SC, Boyd J. Germline mutations of the BRCA1 and BRCA2 genes in a breast and ovarian cancer patient. Gynecol Oncol 1998;70:432–5.


www.jmedgenet.com
Phenotypic expression of double heterozygosity for \textit{BRCA1} and \textit{BRCA2} germline mutations


\textit{J Med Genet} 2005 42: e20
doi: 10.1136/jmg.2004.027243

Updated information and services can be found at:
http://jmg.bmj.com/content/42/3/e20

These include:
References
This article cites 35 articles, 8 of which you can access for free at:
http://jmg.bmj.com/content/42/3/e20#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)
- Genetic screening / counselling (887)
- Screening (oncology) (234)
- CNS cancer (44)
- Neurooncology (78)
- JMG Online mutation reports (168)

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/