Increased Rate of Phenocopies in All Age Groups in BRCA1/BRCA2 Mutation Kindred, but Increased Prospective Breast Cancer Risk Is Confined to BRCA2 Mutation Carriers

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Abstract

Background: To establish, if among unaffected noncarrier relatives in a family with an established BRCA1/2 mutation, there is an increased risk of breast cancer.

Methods: We identified 49 women with breast cancer who were first-degree relatives of a pathogenic mutation carrier among 807 BRCA1/2 families but who tested negative for the specific mutation. A prospective analysis of breast cancer from date of family ascertainment was performed for first-degree relatives of proven BRCA1/2 mutation carriers and compared with population-expected incidence rates.

Results: Women who prospectively test negative for BRCA1/2 mutations showed excess risk of breast cancer to be confined to BRCA2 noncarriers with an observed:expected (O/E) ratio of 4.57 [95% confidence interval (CI) 2.50–7.67; \(P < 0.0001\); O/E in BRCA1 noncarriers, 1.77]; this dropped to 2.01 for BRCA2 [relative risk (RR), 1.99; 95% CI, 0.54–5.10] from date of predictive test. Genotyping of 18 breast cancer susceptibility single-nucleotide polymorphisms (SNP) defined an RR of 1.31 for BRCA2 breast cancer phenocopies with a breast cancer diagnosis at age less than 60 years.

Conclusion: Noncarriers remain at risk in the prospective follow-up of women who tested negative for BRCA1/2. Women testing negative in BRCA2 families may have increased risk of breast cancer compared with population levels, particularly with strong breast cancer history in close relatives. Any increased risk in BRCA1 families is likely to be insufficient to recommend additional interventions.

Impact: Our work can help with counseling women from BRCA1/2 families who have tested negative, and could impact on how individual breast cancer risk is related back to these women. Cancer Epidemiol Biomarkers Prev; 1–8. ©2013 AACR.
(4, 10–15). Indeed, a recent article suggested that all women testing negative for a family mutation were at no greater risk than the average population (14). Without clear information on what risks to provide women testing negative for the family mutation, it is likely that there will be differences in how women are counseled. Therefore, we have reexamined our combined database of families with BRCA1 and BRCA2 mutations, to assess the risk of breast cancer in women testing negative for the family-specific BRCA1 or BRCA2 mutation after the date of ascertainment of the family and individual (when the first family member joined the Family History Clinic) and after the date of mutation testing.

Materials and Methods

Families with individuals with breast and/or ovarian cancer have been screened for mutations in BRCA1/2 since 1996 in the overlapping regions of Manchester and Birmingham in Mid-/Northwest England, encompassing approximately 10 million people. Women with a family history of breast/ovarian cancer who attend specialist genetic clinics in these two regions have a detailed three-generation family tree constructed. The date the first family member was referred to the genetic service was considered as the family ascertainment date. If a BRCA1/2 mutation is identified, further attempts are made to ensure that all individuals relevant to discussions on risk are represented on the family tree. All cases of breast/abdominal cancers are confirmed by means of: hospital/pathology records, Regional Cancer Registries (from 1960) or death certification. When a family-specific pathogenic BRCA1/2 mutation is identified, predictive testing is offered to all blood relatives.

Details of all tested relatives and first-degree untested female relatives were entered onto a Filemaker Pro-7 database. The initial individual in which a mutation was identified was designated the "index" case, with all other individuals being classified as to their position in the pedigree compared with a proven mutation carrier. All women reaching 18 years were entered on the database even if untested for a mutation carrier. The exception was for mothers of a mutation carrier when it was clear that the mutation was paternally inherited (i.e., there was no maternal family history but a very convincing paternal history of breast/ovarian cancer). A total of 807 index cases were studied. Date of birth and date of last follow-up, breast cancer status, ovarian cancer status, dates of diagnoses, and date of death (if applicable), gene mutation identified in the family, the individual’s relationship to a known mutation carrier, and their mutation status were entered. The resultant combined series is referred to as the M6-ICE (Inherited Cancer in England) Study (4).

Women with breast or ovarian cancer who tested negative for the family mutation were defined as phenocopies. In 90% of cases, at least two independent blood draws from every phenocopy have been genotyped to firmly establish negative mutation status. Only first-degree relatives (FDR) of proven pathogenic mutation carriers were included in the study.

An analysis was undertaken assessing prospective breast cancer risk in individuals testing negative for the family mutation using date of ascertainment of the family by the genetic service as the start date. If the reason for family ascertainment was due to an index case in another region or country being identified as a mutation carrier, then the date of mutation report was used as ascertainment date. Standard incidence ratios were derived using age- and year-specific data from the population-based North West Cancer Intelligence Service (NWCIS) as previously described (4). Follow-up was censored at July 1, 2011, or date of breast cancer, date of death, or date of bilateral risk-reducing breast surgery, whichever was the earlier. Person-years at risk analyses were performed to assess expected cancers in the general female population using data from the NWCIS. Observed/expected ratios were assessed for statistical significance using the common method from Clayton and Hills based on the Poisson assumption (16). A subset of women testing negative for the family mutation were part of family history (FH) risk, an assessment program looking at women who have a high familial risk of breast cancer, and is part of the PROCAS program grant, for which we had ethical approval to check details against the NWCIS for cancer incidence. This was carried out in September 2011. A final analysis was carried out using date of testing of unaffected FDRs as start date.

In addition, an assessment of the strength of family history of breast cancer was included by summing the BRCA2 element of the Manchester scoring system for each affected family member (17). This system scores breast cancers in the direct lineage based on age at diagnosis, giving higher scores for earlier age at diagnoses. In addition, an assessment was made of close breast cancer family history (FDR and second-degree) using diagnosis at less than 40 years in an FDR; less than 50 years in at least two relatives (including an FDR); or at least 3 (including an FDR) diagnosed less than 60 years as a surrogate for increased degree of breast cancer family history.

DNA testing for SNPs

DNA was extracted from blood samples provided by women attending the genetic clinics. In addition to the BRCA1/2 mutation analysis, women were genotyped for 18 SNPs that have been shown to be associated with breast cancer risk in the general population (7) and a subset of SNPs in familial breast cancer (18). Using the published per SNP ORs and risk-allele frequencies (RAF) from Turnbull and colleagues (e.g. FGFR2 per allele OR is 1.43 with RAF of 0.42; ref. 7), we calculated the ORs for each of the three SNP genotypes (no risk alleles, one risk allele, and two risk alleles), assuming independence (Table 1). To obtain an overall breast cancer risk score for each woman, we multiplied the ORs for each of her 18 genotypes together.
Results

Among 809 families (two index cases had a mutation in both BRCA1 and BRCA2 providing two families each; Fig. 1) with a proven pathogenic mutation (428 BRCA1 or 381 BRCA2), 290 FDR female relatives with breast cancer have undergone genetic testing following identification of the family mutation; there were also 110 deceased obligate FDR mutation carriers with breast cancer (inferred by other family testing). An additional 383 deceased FDRs with breast cancer were of unknown mutation status as well as 77 living breast cancer FDRs. Fortynine (17%) FDR relatives with breast cancer tested

Table 1. SNP scores taken from the article by Turnbull and colleagues (7)

<table>
<thead>
<tr>
<th>SNP</th>
<th>Gene</th>
<th>Risk allele</th>
<th>RAF</th>
<th>Weight 0</th>
<th>Weight 1</th>
<th>Weight 2</th>
<th>0 Freq</th>
<th>1 Freq</th>
<th>2 Freq</th>
<th>RR</th>
<th>W</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2981579</td>
<td>FGFR2</td>
<td>T</td>
<td>42</td>
<td>0.72</td>
<td>1.03</td>
<td>1.47</td>
<td>34</td>
<td>49</td>
<td>17</td>
<td>1.43</td>
<td>100</td>
</tr>
<tr>
<td>rs10931936</td>
<td>CASP8</td>
<td>C</td>
<td>74</td>
<td>1.20</td>
<td>1.06</td>
<td>0.93</td>
<td>7</td>
<td>38</td>
<td>55</td>
<td>0.88</td>
<td>100</td>
</tr>
<tr>
<td>rs3803662</td>
<td>TOX3</td>
<td>T</td>
<td>26</td>
<td>0.86</td>
<td>1.12</td>
<td>1.45</td>
<td>55</td>
<td>38</td>
<td>7</td>
<td>1.3</td>
<td>100</td>
</tr>
<tr>
<td>rs889312</td>
<td>MAP3K</td>
<td>C</td>
<td>28</td>
<td>0.89</td>
<td>1.08</td>
<td>1.32</td>
<td>52</td>
<td>40</td>
<td>8</td>
<td>1.22</td>
<td>100</td>
</tr>
<tr>
<td>rs13387042</td>
<td>2q</td>
<td>A</td>
<td>49</td>
<td>0.82</td>
<td>0.99</td>
<td>1.20</td>
<td>26</td>
<td>50</td>
<td>24</td>
<td>1.21</td>
<td>100</td>
</tr>
<tr>
<td>rs1011970</td>
<td>cdkn2a</td>
<td>T</td>
<td>17</td>
<td>0.97</td>
<td>1.06</td>
<td>1.15</td>
<td>70</td>
<td>27</td>
<td>3</td>
<td>1.09</td>
<td>100</td>
</tr>
<tr>
<td>rs704010</td>
<td>10q22</td>
<td>A</td>
<td>39</td>
<td>0.95</td>
<td>1.01</td>
<td>1.08</td>
<td>37</td>
<td>48</td>
<td>15</td>
<td>1.07</td>
<td>100</td>
</tr>
<tr>
<td>rs1156287</td>
<td>COX11</td>
<td>A</td>
<td>71</td>
<td>0.87</td>
<td>0.96</td>
<td>1.05</td>
<td>8.5</td>
<td>41</td>
<td>50.5</td>
<td>1.1</td>
<td>100</td>
</tr>
<tr>
<td>rs11249433</td>
<td>notch</td>
<td>C</td>
<td>42</td>
<td>0.94</td>
<td>1.01</td>
<td>1.09</td>
<td>34</td>
<td>48.5</td>
<td>17.5</td>
<td>1.08</td>
<td>100</td>
</tr>
<tr>
<td>rs614367</td>
<td>11q13</td>
<td>T</td>
<td>15</td>
<td>0.96</td>
<td>1.10</td>
<td>1.27</td>
<td>72</td>
<td>26</td>
<td>2</td>
<td>1.15</td>
<td>100</td>
</tr>
<tr>
<td>rs10995190</td>
<td>10q21</td>
<td>G</td>
<td>85</td>
<td>0.77</td>
<td>0.90</td>
<td>1.04</td>
<td>2</td>
<td>24</td>
<td>74</td>
<td>1.16</td>
<td>100</td>
</tr>
<tr>
<td>rs4973768</td>
<td>3p24</td>
<td>SLC4A7</td>
<td>T</td>
<td>47</td>
<td>0.97</td>
<td>1.00</td>
<td>1.16</td>
<td>28</td>
<td>50</td>
<td>22</td>
<td>1.16</td>
</tr>
<tr>
<td>rs3757318</td>
<td>ESR1(6q25.1)</td>
<td>A</td>
<td>7</td>
<td>0.96</td>
<td>1.25</td>
<td>1.62</td>
<td>86.5</td>
<td>13</td>
<td>0.5</td>
<td>1.3</td>
<td>100</td>
</tr>
<tr>
<td>rs1562430</td>
<td>8q24</td>
<td>G</td>
<td>42</td>
<td>1.14</td>
<td>0.97</td>
<td>0.82</td>
<td>33.5</td>
<td>49</td>
<td>17.5</td>
<td>0.85</td>
<td>100</td>
</tr>
<tr>
<td>rs8009944</td>
<td>RAD51L1</td>
<td>A</td>
<td>75</td>
<td>1.21</td>
<td>1.06</td>
<td>0.94</td>
<td>6</td>
<td>38</td>
<td>56</td>
<td>0.88</td>
<td>100</td>
</tr>
<tr>
<td>rs809116</td>
<td>LSP1</td>
<td>T</td>
<td>53</td>
<td>0.84</td>
<td>0.98</td>
<td>1.15</td>
<td>22</td>
<td>50</td>
<td>28</td>
<td>1.17</td>
<td>100</td>
</tr>
<tr>
<td>rs9790879</td>
<td>5p12</td>
<td>C</td>
<td>40</td>
<td>0.92</td>
<td>1.02</td>
<td>1.12</td>
<td>36</td>
<td>48</td>
<td>16</td>
<td>1.1</td>
<td>100</td>
</tr>
<tr>
<td>rs713588</td>
<td>10q</td>
<td>A</td>
<td>60</td>
<td>1.19</td>
<td>1.02</td>
<td>0.88</td>
<td>16</td>
<td>48</td>
<td>36</td>
<td>0.86</td>
<td>100</td>
</tr>
</tbody>
</table>

Figure 1. Consort diagram—prospective analysis.
negative for the family mutation. Ninety-five breast cancers occurred in FDRs after the family ascertainment date and 21 (22%) of these tested negative. Of those who underwent predictive testing for the family mutation as unaffected individuals but who have subsequently developed breast cancer, 8 of 42 (19%) have tested negative. There were only two ovarian cancer phenocopies ages 66 and 71 years, both in BRCA1 families, and these were not evaluated further.

The proportion of women with breast cancer with a negative test increased with age (Table 2) but leveled off after age 50 years, although 25% of tests were negative above this age. The percentage of retrospective phenocopies is similar in both BRCA1 and BRCA2—16.3% and 17.5%, respectively (Table 2). In each age range (apart from <30 years where there were only 11 cases), there were approximately twice as many cases of breast cancer (phenocopies) than would have been expected in the general population. This proportion may be an underestimate as only recent population incident rates were utilized. Twenty percent of phenocopies (10/49) were diagnosed prior to 1990 when the incidence of breast cancer in the population was lower.

**Prospective analysis**

In total, 279 female FDRs tested negative for the family BRCA1 mutation and 251 for BRCA2 (Fig. 1). Two women (BRCA1) who had undergone bilateral risk-reducing breast surgery prior to ascertainment in the genetics service were excluded from the analysis as were 27 women who had developed breast cancer prior to family ascertainment, and one woman who died prior to family ascertainment (BRCA2). Thus, 17 breast cancers from BRCA1 families and 13 from BRCA2 families were excluded from the prospective analysis. Since family ascertainment, 7 (2.5%) cases of breast cancer occurred in the remaining 262 women testing negative for the familial BRCA1 mutation and 14 (5.9%) in 238 women testing negative for their familial BRCA2 mutation (Table 3).

Using a pragmatic recent date of follow-up (June 30, 2011), assuming notification of breast cancers, the rates

<table>
<thead>
<tr>
<th>Age range</th>
<th>BRCA1</th>
<th>BRCA2</th>
<th>BRCA1 and BRCA2</th>
<th>BRCA1/2 proportion of carriers developing their first breast cancer for each decade in age group</th>
<th>Population breast cancer rates per decade in age group</th>
<th>Ratio of rates in age group compared with population</th>
<th>Proportion of 50% expected to test negative if no other factors involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>18–29</td>
<td>0/8</td>
<td>0/3</td>
<td>0/11</td>
<td>3%</td>
<td>0.05%</td>
<td>600:1</td>
<td>0.016%</td>
</tr>
<tr>
<td>30–39</td>
<td>2/43</td>
<td>3/40</td>
<td>5/83 (6%)</td>
<td>15%</td>
<td>0.5%</td>
<td>30:1</td>
<td>3.2%</td>
</tr>
<tr>
<td>40–49</td>
<td>6/46</td>
<td>8/48</td>
<td>14/94 (15%)</td>
<td>30%</td>
<td>1.5%</td>
<td>20:1</td>
<td>4.8%</td>
</tr>
<tr>
<td>50–59</td>
<td>10/29</td>
<td>9/37</td>
<td>19/66 (28.8%)</td>
<td>15%</td>
<td>2.7%</td>
<td>6:1</td>
<td>15%</td>
</tr>
<tr>
<td>60–80</td>
<td>5/15</td>
<td>7/21</td>
<td>12/36 (33%)</td>
<td>25%</td>
<td>5.8%</td>
<td>4:1</td>
<td>18.9%</td>
</tr>
<tr>
<td>Total</td>
<td>23/141(16.3%)</td>
<td>26/149(17.5%)</td>
<td>49/290</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2. Proportion of first-degree relatives with breast cancer of a proven BRCA1 or BRCA2 mutation carrier who test negative for the family mutation, by age group**

<table>
<thead>
<tr>
<th>Series</th>
<th>Number</th>
<th>Years follow-up (rate per 1,000)</th>
<th>Breast cancers</th>
<th>Expected cancers</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All tested negative from family ascertainment</td>
<td>BRCA1</td>
<td>262</td>
<td>3,217 (2.17)</td>
<td>7</td>
<td>3.95</td>
</tr>
<tr>
<td></td>
<td>BRCA2</td>
<td>238</td>
<td>2,634 (5.3)</td>
<td>14</td>
<td>3.06</td>
</tr>
<tr>
<td>Family History Clinic group from family ascertainment</td>
<td>BRCA1 negative</td>
<td>111</td>
<td>1,141.8 (1.76)</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BRCA2 negative</td>
<td>134</td>
<td>1,189.9 (5.9)</td>
<td>7</td>
<td>1.45</td>
</tr>
<tr>
<td></td>
<td>BRCA2 untested</td>
<td>52</td>
<td>490.5</td>
<td>0</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>Adjusted BRCA2 including untested</td>
<td>162</td>
<td>1,454.8 (4.8)</td>
<td>7</td>
<td>1.98</td>
</tr>
<tr>
<td>From negative genetic test date</td>
<td>BRCA1</td>
<td>262</td>
<td>1,456.8 (2.7)</td>
<td>4</td>
<td>3.80</td>
</tr>
<tr>
<td></td>
<td>BRCA2</td>
<td>238</td>
<td>852 (4.7)</td>
<td>4</td>
<td>2.01</td>
</tr>
</tbody>
</table>

**Table 3. Prospective rates of breast cancer incidence and ORs in those FDRs testing negative for BRCA1/2**
were 2.17 per 1,000 (in 3,217 years) in BRCA1 and 5.3 per 1,000 (in 2,634 years) in BRCA2 noncarriers, with a statistically significant difference between these phenocopy rates ($P = 0.0536$; Table 4). Age at ascertainment was very similar between noncarriers in BRCA1 (mean age at ascertainment, 38.07 years) and BRCA2 (mean age at ascertainment, 38.24 years) families, with a $P$ value of 0.9025. Using a person years at risk analysis, 3.95 cancers would have been expected in the cohort of BRCA1 women and 3.06 in BRCA2. The observed:expected ratio was, therefore, 1.77 (95% CI, 0.71–3.65) for the BRCA1 group and 4.57 (95% CI, 2.50–7.67) for BRCA2. The difference between the observed and expected values for BRCA2 was statistically significant with $P$ value less than 0.0001. This analysis does not allow for any testing bias of those developing breast cancer. We are aware of 21 breast cancers in untested female FDRs from BRCA1 tested negative. There were 9 of 475 untested female FDRs from BRCA2 degree of breast cancer family history, and subsequently tested negative for a family-specific $BRCA1/2$ mutation (111 $BRCA1$ and 134 $BRCA2$). Confining the analysis to these 245 cases; nine breast cancers occurred in 2,330.68 women years of follow-up, of which seven were in 134 $BRCA2$ noncarriers. The rate of breast cancer in these women was 4.29 per 1,000 for women with a median age at entry of 39.5 years as compared with an age-stratified populations 10-year risks of 1.5 per 1,000 annually. The rate in $BRCA1$ noncarriers was 1.76 per 1,000 and 5.9 per 1,000 for $BRCA2$. Using population incidence rates for $BRCA2$, only 1.45 breast cancers would have been expected (RR, 4.82; 95% CI, 2.63–8.09). Due to the potential bias in the full dataset of testing those developing breast cancer, an analysis of FDRs who have not been tested was also undertaken for $BRCA2$. Fifty-two untested FDRs with an average age of 40.2 years had 490.5 years of follow-up. None of these had developed breast cancer. It is estimated that, had these women undergone predictive testing, 54% of these would have tested negative (323/600; 53.8% of presymptomatic predictive FDR genetic tests in $BRCA2$ at less than 60 years were negative). As such, just over half of 490.5 years (264.8) would be estimated to be contributed by negative tests in the FDR unknown category. Taking into account the potential testing bias, a total of seven breast cancers occurred in 162 predicted $BRCA2$ negatively tested FDRs in an adjusted rate of 4.8 per 1,000. Expected breast cancers were 1.55 for a 134 $BRCA2$ negative cohort and were 0.79 in the 52 untested FDRs. Taking the same 0.54 proportion of this figure, expected breast cancers were 1.98 compared with seven observed (OR, 3.535; 95% CI, 1.43–7.37). A final analysis was carried out on those testing negative from date of mutation test. It was only possible to ascertain a recent date (within 18 months) of follow-up with known unaffected status for 149 of 238 women testing negative. For the remainder, the last contact from the family file was used. For 132 women, verification of cancer status was possible on the NWCIS registry. For $BRCA2$, a total of four prospective breast cancers occurred in 852 women years of follow-up in the 230 women who had not had risk-reducing mastectomy prior to genetic testing. This gives a rate of 4.7 per 1,000 in women with a median age at
predictive testing of 42.37 years. Expected cancers in this cohort were 2.01 for BRCA2 (RR, 1.99; 95% CI, 0.54–5.10). There was no increase in risk seen for BRCA1 families (Table 2).

The Manchester score for the family before mutation screening was calculated to assess the strength of breast cancer history in the full dataset of 500 noncarriers and was dichotomized to scores 11 or more or less than 11 (Table 4). There were no prospective cases of breast cancers diagnosed in mutation-negative women in the BRCA1 cohort with scores less than 11. The cancer diagnosis rates were similar at 3.2 and 2.9 per 1,000 in the BRCA2 mutation–negative women from families with a Manchester score less than 11 and BRCA1 families with a score of 11 or more. However, the highest rate of 6.48 per 1,000 was found in the BRCA2 cohort with the highest Manchester scores (Table 4). The observed:expected ratio for the three breast cancers in BRCA2 carriers with scores less than 11 was 2.03 (expected, 1.47; 95% CI, 0.41–5.93) compared with 4.22 for the 11 BRCA2 noncarrier cancers with family scores of more than 10 (expected, 2.61; 95% CI, 2.10–7.55). A further analysis was carried out on BRCA2 mutation–negative tested individuals to assess whether those with increased degree of breast cancer family history had a higher rate of breast cancer diagnosis. A total of 126 individuals met the degree of breast cancer family history criteria and 10 developed breast cancer in 1448 years of follow up (6.9 per 1000) and 4 of 112 who did not meet the criteria developed breast cancer in 1204 years of follow up (3.3 per 1000) from family ascertainment. The observed:expected ratio for the degree of breast cancer family history group was 7.04 (expected, 1.57; 95% CI, 0.69–6.52).

There was no censoring for oophorectomy; in fact, 12 (4.8%) BRCA2 noncarrier FDRs and 10 (3.6%) BRCA1 noncarrier FDRs underwent oophorectomy with three BRCA2 and two BRCA1 noncarriers subsequently developed breast cancer.

**DNA testing for SNPs**

Sufficient DNA was available to test 36 FDR phenocopies for the 18 validated SNPs. In addition, testing was carried out on 445 BRCA2 mutation carriers (280 affected with breast cancer, 165 unaffected) and 462 BRCA1 carriers (268 affected, 194 unaffected), 185 family history breast cancers testing negative for BRCA1/2 mutations, and 421 population female controls from the NHS breast screening program in the PROCAS trial (19). The mean RR for the 18 SNPs was 1.27 for 22 FDR BRCA2 phenocopies (10 before ascertainment and 12 after; range, 0.82–3.17; median, 1.18; 1.31 for 18 diagnosed age less than 60 years) and 1.24 (range, 0.41–2.63; median, 0.96) BRCA1 for 14 FDRs phenocopies (1.13 for 12 at <60 years). In the 280 affected BRCA2 mutation carriers, the mean RR was 1.165 (range, 0.29–5.09; median, 1.01), with a RR of 0.993(0.33–3.36; median, 0.86) for the 165 unaffected carriers. In the 268 affected BRCA1 mutation carriers, the mean RR was 1.07 (range, 0.24–4.35; median, 0.93), with a RR of 1.11 (0.265–3.79; median, 0.956) for the 194 unaffected individuals. Among 185 family history–positive breast cancers without BRCA1/2 mutations, the RR was 1.24 (range, 0.37–4.62; median, 1.10). The mean score in a series of 421 control samples from the general female population was 1.04 (range, 0.24–4.3; median, 0.93).

**Discussion**

The results of this analysis suggest higher phenocopy rates in women in families with pathogenic mutations in BRCA2 than expected in all age groupings. These data suggest that breast cancer genetic modifiers in these families may increase the breast cancer risk even in women who test negative for familial BRCA1/2 mutations. From the prospective analysis, it would appear that the risk of breast cancer is significantly greater in women who test negative for familial BRCA2 compared with BRCA1 mutations. This is consistent with data showing much wider penetrance estimates for breast cancer in BRCA2 than in BRCA1 (5, 20–26), and the greater number of SNPs shown to modify BRCA2 risk (7, 27). It is important to note that eight (six BRCA2) of the 21 cases have occurred after our previous report (4). Furthermore, the increased rates of phenocopies among women from BRCA2 mutation–positive families with high previous incidence of breast cancer as assessed by the Manchester score and degree of breast cancer family history criteria add support to the potential contribution of nonlinked genetic modifiers of breast cancer risk. In this case, unaffected women in BRCA2 mutation–positive families might be expected to have a protective profile with an SNP RR of less than 1.0. However, we did not find this, suggesting that there is a bias toward higher allele frequencies of risk SNPs in the BRCA2 mutation–positive families. This then infers that selection of families for mutation screening also selects for higher SNP scores irrespective of the subsequent BRCA2 mutation status. For BRCA1 there was little effect of the SNPs with affected carriers having a lower RR than unaffected individuals; this is perhaps unsurprising as many of the common variants influence risk of ER-positive disease. The 18 SNPs appear to contribute to the higher rate of breast cancer for those testing negative in BRCA2 families although there must be other factors involved. At present, the 18 SNPs are considered to account for no more than 15% of the familial component of breast cancer.

Several groups have assessed risk in women testing negative with varied results (10–15, 28). The most compelling case for there not being an increase in risk was provided by a study of 28 families in which 395 female relatives tested negative for the family mutation. Breast cancer incidence was assessed from family/individual ascertainment as opposed to the date of testing negative in the present study. This study reported that the
RR of breast cancer, compared with the population average, was only 0.82 overall, but 1.33 in FDRs of breast cancer cases. This study however, predominantly assessed BRCA1 carriers (322/395, 81.5%); a cohort in which fewer modifying SNPs have been validated (7). Although the investigators did not present data on BRCA2 noncarriers, we calculate that the RR in this group would be approximately 1.53 in order to balance the 0.66 found in BRCA1 noncarriers. A further prospective study from Australia found a nonsignificant increased risk of 1.29 in 442 first/second-degree relatives, but did not separate FDRs or BRCA1/BRCA2 cases and, again, had smaller numbers of women with BRCA2 mutations (15).

Kurian and colleagues (14) presented modeling data comparing cancer incidence of affected women testing negative for the familial BRCA1/2 mutations with cancer incidence in affected women from families without BRCA1/2 mutations. We have previously indicated that this was not a prospective study, but was population based, which would dilute any effect of modifier genes. In addition, this study did not compare risks with the general population (29). The lack of confirmation of cancer status of all individuals against a cancer registry in the North American/Australian studies may result in underestimating the risk of breast cancer. In the present study, 242 of 500 cases were checked against a cancer registry. Confining the analysis in this way, there is clear evidence of a difference in the phenocopy rate between families with BRCA1 and BRCA2 mutations. In addition, we have assessed all FDR women testing negative in Manchester and Birmingham, excluding those who developed breast cancer before family ascertainment. A final analysis showed that there was a nonsignificant increased rate of breast cancer in BRCA2 mutation-negative FDRs, although this analysis was based on shorter follow-up time, and current vital and cancer status could not be confirmed with certainty in approximately 37% of women.

Further recent support for the potential increased risk in FDR noncarriers has just been published. A Dutch study found 17 breast cancers among 464 noncarriers, and estimated a breast cancer risk by 50 years of age of approximately 37% of women.

A potential weakness in the present study is that breast cancer incidence could be affected by lead time bias from extra mammography compared with the general population. In addition, there may be a screening bias if women with a strong family history of breast cancer have undergone early MRI. In 71 of 242 (37 BRCA2) women testing negative for the familial mutation, extra mammography screening at less than 50 years of age is continuing. As such, this may increase breast cancer rates although only half of these women had been screened for less than 5 years. However, the effects may be expected to have had an equal effect on those testing negative for mutations in either gene, but no extra risk of breast cancer was seen in BRCA1 mutation families.

Many computer models, including BOADICEA (8) and Tyrer–Cuzick (30), give increased risks for women testing negative in the context of a strong early-onset family history of breast cancer, although this imputation has not been directly validated. We believe that genetics specialists should remain cautious about stating that an individual’s risk of breast cancer has returned to that of the general population unless they are tested negative for a family BRCA1 mutation in a family without a strong family history of breast cancer. In the context of a family BRCA2 mutation, especially when there are multiple close relatives affected with early-onset breast cancer, specialists should advise that breast cancer risks may still be increased compared with the general population. The recent discovery of further genetic loci that alter penetrance predominantly in BRCA2 carriers (27) may facilitate additional testing for multiple SNPs to accurately predict whether those women negative for the familial BRCA2 mutation still have an increased genetic risk.

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No potential conflicts of interest were disclosed.

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