Affected individuals from 431 families gave blood for mutation analysis in BRCA1 and BRCA2 (table 1), mainly to develop genetic tests for their family. Individuals were eligible if there was at least a 50% chance of a gene predisposing to breast cancer (not necessarily BRCA1/2) in their family. Assessment was made using the Cancer and Steroid Hormone (CASH) dataset and the Claus curves. A minimal requirement was two close relatives with breast cancer before the age of 50 years, but combinations of male and female breast cancer, and breast and ovarian cancer were particularly identified. An exception to this was two research projects where population based cases of breast cancer before the age of 31 years and sporadic breast cancer before the age of 36 years were screened for both genes. Male breast cancer (MBC) families presenting to the clinic with at least one MBC before the age of 60 years or at any age if female breast cancer had occurred were screened for BRCA2.

Initial screening for mutations involved a whole gene assessment using single strand conformational polymorphism (SSCP) analysis and protein truncation testing (PTT) of exon 11 in each gene. All mutations were confirmed, in both orientations, by direct fluorescent sequencing of the appropriate exon. We excluded one exon 13 duplication and two exonic deletions detected on screening 95 BRCA1 negative breast/ovarian families. A further two exon 13 duplications in five subsequent BRCA1 negative breast/ovarian families originating from east of the Pennines were also excluded. Of the 26 non-large-scale rearrangement mutations, 27/26 (33%) were detected outside the commonly screened regions of BRCA1 (exons 2, 11, 20) in the UK (table 1). Similarly 15/50 (30%) BRCA2 mutations were detected outside exons 10 and 11.

In an attempt to assess the sensitivity of our techniques, we studied the outcome of testing in families with two or more confirmed ovarian cancers, which also had a total of at least four breast/ovarian cancers (breast cancer before the age of 60 years) and male breast cancer families with a similar four or more breast/ovarian cancers in total. Of breast/ovarian families fulfilling the above criteria, 25/38 (66%) had pathogenic BRCA1 mutations (five had BRCA2 mutations and one had a BRCA1 deletion) and 9/14 (64%) male breast cancer families had pathogenic BRCA2 mutations. These results would suggest high sensitivity for the techniques, particularly for BRCA1, from Breast Cancer Linkage Consortium data, 90% of such breast-ovarian families were linked to BRCA1, and 76% of male breast cancer families were linked to BRCA2. This would suggest sensitivity of close to 85% for BRCA2 and 73% for BRCA1. However, previous attempts to validate SSCP have shown that only 65–72% of BRCA1 mutations are correctly ascribed by SSCP. Protein truncation testing detected all 15 deleterious mutations in one study, but SSCP only detected 10. Nevertheless sensitivity is not just dependent on the proportion of different mutations detected, but on the frequency of each in a particular population. Taking all these factors into account we have estimated that our whole gene testing technique (without testing for large deletions or duplications) would have a sensitivity of at least 66% (see table 2), and that about 6% (4/66) of our breast/ovarian families have deletions or large-scale rearrangements.

The proportion of breast/ovarian cancers attributable to BRCA1 or BRCA2 depends on the ethnic origin of families. Many countries or ethnic groups have particular founder mutations that are not seen in other populations. In countries with a small founder population, very few mutations may account for the vast majority of breast cancer families. The Ashkenazi Jewish population have three founder mutations, 185delAG and 5382insC in BRCA1, and 6174delT in BRCA2, which are found in over 2% of the this population. At least two studies have shown that one of the three mutations is present in the majority (99–100%) of high risk families, and all three account for nearly all of the involvement of these genes in families. Another country with a small number of mutations is Iceland, where one mutation, BRCA2 99sdelS, accounts for most familial breast cancer.

Abbreviations: PTT, protein truncation test(ing); SSCP, single strand conformational polymorphism.
BRCA1/2, a negative test would substantially reduce the cancer), which has around a 33% chance of being due to using Bayes’ theorem. Thus in a family with 4–5 breast mutation being present in the family at least halves
BRCA1
However, once sensitivity rises beyond 66% the chances of a
utility of a negative test in this situation is negligible.

Gene Exon Mutation Codon Effect Number of families
BRCA1 5 2786C→T 68 I66X 1
BRCA1 5 3311G→C NA Splice site 1
BRCA1 5 3311T→G NA Splice site 2
BRCA1 IVS6 421→delA NA Splice site 1
BRCA1 7 546G→T 143 Stop143 6
BRCA1 8 666G→T NA Splice site 1
BRCA1 8 IVS10 790→G→A NA Splice site 1
BRCA1 13 4446C→T 1443 R1443X 1
BRCA1 13 Exon 13 NA NA 3

BRCA1 15 4604→2 A→G NA Splice site 1
BRCA1 15 4774delKT 1542 Stop1572 2
BRCA1 16 5036delA 1648 Stop1657 1
BRCA1 18 5271+4 NA Splice site 1
BRCA1 21 5427delG 1792 Stop1792 1
BRCA1 22 5486+19A 1792 W1782X 1
BRCA1 24 5622C→T 1835 R1835X 1

BRCA1 Deletion exons 3–15 NA NA NA 1

BRCA1 Deletion exons 14–20 NA NA NA 1

BRCA2 2 2536A→C 9 STOP24 1

BRCA2 7 767insAT 180 Stop185 1

BRCA2 7 800delAT 266 Stop204 1

BRCA2 IVS7 8000→A NA Splice site 2

BRCA2 8 8600→A NA Splice site 1

BRCA2 9 9831delACAG 252 Stop275 2

BRCA2 16 7994delAT 2588 Stop2647 1

BRCA2 17 8205→1 insAT 1 NA Splice site 1

BRCA2 18 8525delC 2766 Stop2776 2

BRCA2 22 9132delC 2968 Stop2975 1

BRCA2 22 9177C→G 2984 Stop2984 1

BRCA2 23 9318insA 3030 Stop3047 1

<table>
<thead>
<tr>
<th>Gene</th>
<th>Exon</th>
<th>Mutation</th>
<th>Codon</th>
<th>Effect</th>
<th>Number of families</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA1/2</td>
<td>3-15</td>
<td>NA</td>
<td>NA</td>
<td>1</td>
<td></td>
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<tr>
<td>BRCA1/2</td>
<td>14-20</td>
<td>NA</td>
<td>NA</td>
<td>1</td>
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<td>39% for whole gene</td>
<td>20% BRCA1/2</td>
<td>A-PTT</td>
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<tr>
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<td>33% for whole gene</td>
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<tr>
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<td>65–85%</td>
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<td>BRCA1/2</td>
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</table>

The utility of a negative test in this situation is negligible. However, with a negative whole gene screen this extra ovarian cancer risk will fall to below 5%, and 2.5% for at-risk unaffected relatives. Adding an exon deletion/duplication strategy such as multiplex ligation dependent probe amplification (MLPA) would add an extra 6% sensitivity in our sample (table 1), but in other populations such as the Netherlands could account for up to 27% or more of BRCA1 pathogenic mutations. As this is a single assay, it or a similar test should probably now be added to all BRCA screening strategies. As can be seen from table 2 even direct sequencing plus a technique such as MLPA does not reach 100% sensitivity. This is because mutations buried in the intron that affect splicing, or effects outside the gene that affect RNA transcription would not be detected. Indeed, chromosome translocations not affecting an exon would also be undetected. Nevertheless, many pathogenic mutations might be inappropriately labelled “undetected”, as many missense changes of uncertain pathogenic significance remain unclassified. Improvements in classifying these variants should boost mutation detection sensitivity further.

Our results would also suggest that for the UK at least exon 11 in BRCA1 does not account for more mutations than would be predicted from its size alone. Only 48/83 (58%) pathogenic BRCA1 mutations were detected in exon 11, which is equivalent to its contribution of 60% to the coding sequence. Similarly only 5/34 (14%) of the non-Jewish non-exon 11 BRCA1 mutations were in exons 2 and 20. Indeed exons 5 and 7 contribute more mutations individually than either exons 2 or 7. The finding of six exon 7 546 G→T mutations is of particular significance. We were initially unable to cover the whole of exon 7 by SSCP due to the large number of repeat sequences in the intron. It is our understanding from other groups that virtually no laboratory has been able to obtain workable non-sequencing results for exon 7. Eventually we sequenced exon 7 in 30 breast/ovarian families previously negative on BRCA1 testing. Three families tested positive for the 546 G→T mutation. We have now tested 201/354 families testing negative for other BRCA1 pathogenic changes and identified six of these mutations. Although the most likely families have been tested it is possible that at least a further two mutations would be identified on testing the remainder. It is likely this mutation is more than just a local founder.
mutation as it has been recorded 30 times on the BIC website by Myriad Genetics and only twice by other laboratories. Given the frequency of this mutation in our northwest population and Myriad it would suggest that many laboratories are missing the mutation even if they are screening exon 7 unless they are using sequencing techniques. It would also suggest that this mutation should be incorporated into even a partial gene screen in the UK.

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