ONLINE MUTATION REPORT

Sensitivity of *BRCA1/2* mutation testing in 466 breast/ovarian cancer families

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J Med Genet 2003;40:e107 (<http://www.jmedgenet.com/cgi/content/full/40/9/e107>)

Affected individuals from 431 families gave blood for mutational analysis in *BRCA1* and *BRCA2* 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.^{1,2} 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 were two research projects where population based cases of breast cancer before the age of 31 years³ and sporadic breast cancer before the age of 36 years⁴ 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*.⁵

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/ovary families originating from east of the Pennines were also excluded. Of the non large-scale rearrangement mutations, 26/78 (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.⁵ These results would suggest high sensitivity for the techniques, particularly for *BRCA2*. 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*.⁶ 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.^{7,8} PTT detected all 15 deleterious mutations in one study, but SSCP only detected 10.⁸ 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

Key points

- Partial or targeted mutation screening of *BRCA1/2* in non-founder populations of limited value.
- Whole gene screening adding a deletion strategy such as multiplex ligation dependent probe amplification will give good negative predictive value particularly in breast only families where risks of ovarian cancer can be substantially downgraded.
- The 546→T mutation in exon 7 *BRCA1* is missed by most non-sequencing techniques and appears to be a common UK mutation, which has spread to North America.
- Samples from affected family members from 431 non-Jewish families with a history of breast/ovarian cancer, which had been obtained from a consultee in northwest England, were screened for the presence of *BRCA1* mutations. We also screened 284 such families for *BRCA2* mutations.
- In total, 466 families were tested for mutations in one or other gene and 318 for both genes. Both *BRCA1* and *BRCA2* were screened by whole gene screening techniques; 83 (19%) and 50 (18%) pathogenic mutations were identified in *BRCA1* and *BRCA2*, respectively.

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 (59%–80%) of high risk families^{9,10}, 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* 995del5, accounts for most familial breast cancer.¹¹ Populations that are more outbred, such as the UK, have larger numbers of mutations, and founder mutations occur at lower frequencies. Even the exon 13 duplication, a known UK founder¹², is

Abbreviations: PTT, protein truncation test(ing); SSCP, single strand conformational polymorphism

Table 1 Mutations in *BRCA1/2* outside the commonly screened exons in the UK

Gene	Exon	Mutation	Codon	Effect	Number of families
BRCA1	5	278delC	68	I68X	1
BRCA1	5	331+1G→C	NA	Splice site	1
BRCA1	5	331-11 T→G	NA	Splice site	2
BRCA1	IVS6	421-2delA	NA	Splice site	1
BRCA1	7	546 G→T	143	Stop143	6
BRCA1	8	666+1G→T	NA	Splice site	1
BRCA1	IVS10	790-1G→A	NA	Splice site	1
BRCA1	13	4446C→T	1443	R1443X	1
BRCA1	13	Exon 13 duplication	NA	NA	3
BRCA1	15	4604-2 A→G	NA	Splice site	1
BRCA1	15	4744delCT	1542	Stop 1572	2
BRCA1	16	5061delA	1648	Stop1657	1
BRCA1	18	5271+4 A→G	NA	Splice site	1
BRCA1	21	5427delG	1792	Stop1792	1
BRCA1	22	5464G→A	1782	W1782X	1
BRCA1	24	5622C→T	1835	R1835X	2
BRCA1	Deletion exons 3-15	NA	NA	NA	1
BRCA1	Deletion exons 14-20	NA	NA	NA	1
BRCA2	2	253delC	9	STOP24	1
BRCA2	7	767 insAT	180	Stop185	1
BRCA2	7	800delAT	266	Stop204	1
BRCA2	IVS7	IVS7+2 T→G	NA	Splice site	2
BRCA2	8	860G→A	NA	Splice site	1
BRCA2	9	983delACAG	252	Stop275	2
BRCA2	16	7990delATA	2588	Stop 2647	1
BRCA2	17	insTT 8205-1 G→C	NA	Splice site	1
BRCA2	18	8525delC	2766	Stop 2776	2
BRCA2	22	9132delC	2968	Stop2975	1
BRCA2	22	9179C→G	2984	Stop2984	1
BRCA2	23	9318insA	3030	Stop 3047	1

relatively rare outside Yorkshire and Trent (east of the Pennines) as demonstrated by our low frequency of 1/95 (1%) in *BRCA1* negative breast/ovary families. Nevertheless, many laboratories in the UK have tried to develop a targeted approach to screening, concentrating on the large exons (exon 11 in both genes and 10 in *BRCA2*) and the smaller exons commonly reported to be involved, such as 2 and 20 in *BRCA1*. This cuts down the number of PCR assays using PTT to as few as five for *BRCA1* and four for *BRCA2*. However, failure to use another technique such as SSCP at the beginning and end of the large exons would potentially miss our two most common mutations, 2157 delG in *BRCA2* (nine families) and 4184 del TTAC in *BRCA1* (12 families). These mutations are not reliably detected by PTT as they are at the extreme ends of exon 11 in both genes.¹³ This would therefore add four PCR assays to *BRCA2* testing and two to *BRCA1*. Even with these additional assays, sensitivity for the whole gene is boosted from only around 33% to 44–46% (table 2). The utility of a negative test in this situation is negligible. However, once sensitivity rises beyond 66% the chances of a *BRCA1/2* mutation being present in the family at least halves using Bayes' theorem. Thus in a family with 4–5 breast cancers before the age of 60 years (no ovarian or male breast cancer), which has around a 33% chance of being due to *BRCA1/2*,⁶ a negative test would substantially reduce the estimate of ovarian cancer risk for family members. Taking an average risk for *BRCA1/2* of 30% lifetime chance for ovarian cancer, the risk estimate for an affected woman with breast cancer could be as much as an extra 10% prior to

Table 2 Sensitivity of mutation screening techniques for *BRCA1/2*

Technique	Proportion of mutations detected	
	<i>BRCA1</i>	<i>BRCA2</i>
1(a) PTT large exons+2, 20 <i>BRCA1</i> (A) – Assumes 66% sensitivity for whole gene approach	40/78 (51%)	26/50(52%)
1(b) Add deletion test (MLPA/MAPH)	39% for whole gene	?
2(a) A+SSCP ends of large exons. Sensitivity for whole gene approach 66%	33% for whole gene	33% for whole gene
2(b) Add deletion test	52/78 (67%)	35/50 (70%)
3(a) SSCP+PTT for whole gene	50% for whole gene	?
3(b) Add deletion test	44% for whole gene	46% for whole gene
4(a) Sequencing whole gene or similar very high sensitivity strategy (eg DHPLC)	65–73%	65–85%
4(b) Add deletion test	71–79%	?
	85%	85–90%
A-PTT large exons+2/20 <i>BRCA1</i> .	91%	

testing. 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)¹⁴ 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.¹⁵ 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.¹⁶

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 20. The finding of six exon 7 546 G→T mutations is of particular significance. We were initially unable to cover the whole of this exon 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 website¹⁷ 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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