ONLINE MUTATION REPORT

Haplotype and cancer risk analysis of two common mutations, *BRCA1* 4184del4 and *BRCA2* 2157delG, in high risk northwest England breast/ovarian families

D G R Evans, S L Neuhausen, M Bulman, K Young, D Gokhale, F Lalloo

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The prevalence of *BRCA1* and *BRCA2* mutations in families with breast and ovarian cancers depends on the type of cancer found, the number of cases, and the ethnic background of the family.[1] The proportion of breast cancers attributable to *BRCA1* or *BRCA2* may also depend on the ethnic origin of families. Several mutations have been identified that are found only in specific countries or ethnic groups, suggesting that they are founder mutations. Individuals with the same founder mutation will also share the same alleles at polymorphic markers within the gene or adjacent to the gene. Some common mutations do not segregate with the same alleles and are therefore recurrent mutations. They occur at 'hot spots' for mutation; unstable parts of the gene. 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, which are found in 2% of the Ashkenazi Jewish population.[2] Population studies have shown that the 185delAG mutation predates the separation of the Sephardi and Ashkenazi Jewish populations and is probably 2000 years old.[3]

BRCA1 4184DEL4 AND *BRCA2* 2157DELG

Full gene screening for mutations in the northwest of England has identified two recurrent mutations.[4] *BRCA1* 4182delAATC (4184del TCAA or 4184del4) is a frameshift mutation that results in a stop at codon 1364, causing a truncated protein product. This mutation has been reported 75 times in the Breast Cancer Information Core (BIC) website (www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/).[5] In one study, it was identified in breast cancer cases in Britain, France, and the United States.[6] The mutation was associated with three different haplotypes, indicating that it is a recurrent rather than a founder mutation. *BRCA2* 2157delG is also a frameshift mutation resulting in a stop at codon 659, causing a truncated protein product. It was reported in a British study in 1997,[7] and has also been listed 17 times on the BIC website.

Owing to the high frequency of these two mutations in our regional population, we developed an amplification refractory mutation system (ARMS) test to pre-screen families at risk. This enabled a widespread analysis of breast/ovary families in our area. We sought to determine the frequencies of the mutations in a much less highly selected group of families (with breast cancer only) and to determine the relative risks of breast and ovarian cancer. We also undertook haplotype analyses to determine whether *BRCA2* 2157delG and *BRCA1* 4184del4 are founder mutations in our population.

METHODS

Subjects

Affected individuals with breast and/or ovarian cancer, with a family history of breast or ovarian cancer, were ascertained from attendees at Cancer Genetics Clinics in the Manchester based region of northwest England (population 4.1 million). These were either affected individuals seeking genetic counselling or those identified through their unaffected family members at risk. Affected individuals gave blood for mutational analysis in *BRCA1* and *BRCA2*, mainly to develop genetic tests for their family. Each family had at least a 50% genetic risk estimated using the CASH dataset[8 9] (for

Key points

- From genetic testing of 700 breast/ovarian cancer families in the northwest of England, we have identified two common mutations, *BRCA1* 4184del4 and *BRCA2* 2157delG.
- The 4184del4 mutation has been found in 13 unrelated families and accounts for 15% of identified *BRCA* related breast/ovarian cancer families.
- The ratio of breast to ovarian cancer of 1.55:1 is consistent with estimates for this region of the *BRCA1* gene. It occurs on at least two haplotypic backgrounds and is likely to have occurred independently on several occasions, as it has been reported many times throughout the world.
- The *BRCA2* 2157delG mutation has been identified nine times and is present on only one haplotypic background. This is likely to represent a founder mutation for our regional population, and the mutation is observed in North America and beyond.
- Breast and ovarian cancer incidence is consistent with estimates for the non-ovarian cancer cluster region of *BRCA2*. Despite testing of families with much lower incidence and older age at diagnosis of breast cancer to enrich our screen, penetrance estimates for both mutations are high for breast cancer, with ovarian cancer lifetime risk for 4184del4 at 40% or above.
- Although these mutations can be used as a pre-screen test in our population, the relatively low frequency overall reflects the relatively outbred nature of the UK and other populations, and means that whole gene screening techniques are needed to have any negative predictive value.

Abbreviations: ARMS, amplification refractory mutation system; BIC, Breast Cancer Information Core; OCCR, ovarian cancer cluster region; PTT, protein truncation test; SSCP, single strand conformation polymorphism

example, two affected relatives with an average age at diagnosis <50 years).

Genetic analysis

DNA samples from affected women from 700 breast cancer families were screened for the two mutations, *BRCA1* 4184del4 and *BRCA2* 2157delG. Initially, we screened DNA samples using single strand conformation polymorphism (SSCP) analysis and a protein truncation test (PTT) of exon 11 in each gene. After identifying two common mutations, *BRCA1* 4184del4 and *BRCA2* 2157delG, we developed an ARMS test to pre-screen samples for these two mutations.[4] The numbers tested by each method are shown in table 1. The samples chosen for ARMS testing were women without an identified mutation in *BRCA1* or *BRCA2*, who had not had mutation screening of both genes. In particular we wanted to avoid any bias in pre-selection for screening each gene.

Blood samples were obtained from all patients requesting DNA screening. On arrival at the lab, automated DNA extraction was performed using the standard phenol–chloroform method.

PCR amplification for a 10 μL PCR reaction volume was used. The PCR master mix consisted of 5.0 μL of PCR Reddymix buffer, 3.0 μL of water and 1.0 μL of primer mix. To this, 1.0 μL of DNA was added to make the final reaction volume.

The PCR was run with an initial denaturation at 94℃ for 5 minutes, followed by 35 cycles with denaturing at 94℃ for 30 seconds, annealing at 58℃ for 30 seconds and extension at 72℃ for 1 minute. This was followed by a further 10 minutes' extension at 72℃. After this the samples were kept at room temperature until run on either an acrylamide or an agarose gel.

Primers

The primers were made up to a 100 μmol/l solution. A primer master mix was then made by diluting five parts of each primer to 85 parts of water.

Primers for *BRCA1* 4184del4 were: common reverse primer: 5′-AATTTTATAAATTTATATTTTTAAAATTCATGCTTT-GCTCTTCTTGATT-3′; mutant reverse primer: 5′-CCGGATGCTTTGCTCTTCATA-3′; common forward primer: 5′-CTGCTAGCTTGTTTTCTTCACAGT-3′, giving product sizes of 161 bp for normal and 145 bp for mutant.

Primers for *BRCA2* 2157delG were: common reverse primer: 5′-CCTCAGAATTGTCCCAAAAGAG-3′ Normal forward primer for *BRCA2* 2157delG: 5′-TTTATTAATTTAAATATAGG-TTTATTATTGCATTCTTCTGCG-3′ Mutant forward primer for *BRCA2* 2157delG: 5′-CGTAGGTTTATTTGCATTCTTCTGCA-3′, giving product sizes of 113 bp for normal and 99 bp for mutant.

Haplotype analysis

DNA samples from 30 individuals in 10 families with the *BRCA1* 4182del4 mutation were genotyped at seven short tandem repeat (STR) markers in or adjacent to *BRCA1*. DNA samples from 19 individuals in 8 families with the *BRCA2* 2157delG mutation were genotyped with 6 STRs surrounding *BRCA2*. Standard PCR protocols were used. The assumed map order and distances and the markers used are shown in table 2. Where possible, haplotypes associated with each mutation were inferred from multiple samples of related individuals within each kindred known to have the same mutation; otherwise, multi-locus genotypes are shown. Alleles are given as numbers rather than actual base pairs.

RESULTS

In total, 700 distinct separate families with breast and/or ovarian cancers and a minimum heterozygosity likelihood of 50% were screened for the two common mutations either as part of a full gene screen with SSCP or using the ARMS test (table 1). Thirteen (13/700) families were shown to have the *BRCA1* 4184del4 mutation and nine (9/660) to have the *BRCA2* 2157delG mutation.

Table 1 Detection rates for the common mutations

Gene	Full gene testing	Detected using ARMs pre-screen	BC/OC families	Female BC only families	Male BC families	Total
BRCA1	11/420 (2.6%)	2/280 (0.7%)	11/198 (5.5%)	2/471 (0.4%)	0/31	13/700 (1.85%)
BRCA2	5/284 (1.8%)	4/376 (1%)	3/198 (1.5%)	4/431 (0.9%)	2/31 (6%)	9/660 (1.4%)

BC, breast cancer; OC, ovarian cancer

In our population, 100 pathogenic *BRCA1* mutations were found, including 80 from the above families and 20 from Jewish multiplex testing (19 with 185delAG, 1 with 5382 insC). *BRCA1* 4184del4 accounted for 16% (11/67) of the *BRCA1* mutations found in breast/ovary families, 16% (13/80) of those in non-Jewish *BRCA1* families and 13% of those in all *BRCA1* mutation families.

In the 284 fully screened samples, 41 other *BRCA2* mutations were identified, and ten 6174delT mutations were detected on Jewish multiplex testing. It was not possible to screen 40 of the breast only families screened for the *BRCA1* mutation for 2157delG. This mutation accounted for 18% (9/50) of the *BRCA2* non-Jewish families and 15% of all the *BRCA2* mutation positive families.

Predictive and confirmatory testing of affected individuals was carried out in 16/22 families. Breast cancer phenocopies were identified in three families verified by repeat testing, resampling of all individuals and sequencing. In two families, a first cousin of the affected proband does not carry the deleterious mutation (fig 1). Two women with a phenocopy had breast cancer aged 32 and 49 years, respectively. For the *BRCA2* mutation, this result was unexpected as both intervening female family members had early onset breast cancer and the mutation has been shown to segregate on that side of the family. The phenocopy in the family with the *BRCA1* mutation was in a branch of the family with a potentially less significant aggregation of breast cancer. The third occurred in a 56 year old affected maternal aunt (mother had ovarian cancer) of a woman affected with breast cancer at 38 years identified with the 2157delG mutation.

Haplotype analysis

Among the 9 of 13 families tested with the *BRCA1* 4184del4 mutation, only 1 sample was available for each of 4 families (table 1). There appear to be two primary genotypes, with modifications for each. Families 10, 15, and 16 share the same haplotype of 4–6–10–6–3–4 from D17s1320 to D17S1183 (table 2), with family 10 having a 3 instead of a 2 allele at the most centromeric marker of D17s1185. The second common shared haplotype is in families 9, 11, 12, 13, 14, and 18 from D17s855 (in *BRCA1*) telomeric to D17s1325, which is 25–11–2–11. Four of the families have a 4 allele at D17s1183 (the most telomeric marker), whereas families 14 and 18 have a 1 allele. Families 11, 13, 14, and 18 have a 5 allele at D17s1320, whereas families 9 and 12 have a 4 allele. The haplotype at the most centromeric marker, D17s1185, could not be determined, with a genotype of 2/3 in those six families. Thus, families 11, 13, 14, and 18 are identical except at D17S1183, and families 9 and 12 differ from them at marker D17s1320 (table 2).

Of the nine families with the *BRCA2* 2157delG mutation, there was DNA from only one family member for three of

Table 2 Haplotypes of families with *BRCA1* 4182del4

Marker	Approximate position (kb)	Family 9*	10	11*	12*	13*	14	15	16	18
D17s1185	−1500	3/2	3	3/2	3/2	3/2	3/2	2	2	–
D17s1320	−500	4/7	4	4/5	4	5	5	4	4	5/3
D17s855	Intron 20 *BRCA1*	6/25	6	5/25	6/25	25/4	25	6	6	25
D17s1327	150	11/8	11/10	11/25	11/6	11	11	10	10	11
D17s1326	200	2/7	6	2/7	2/7	2	2	6	6	2
D17s1325	400	11/9	3	11/10	11/10	11	11	3	3	11
D17s1183	1700	4/1	4	4/24	4/2	4	1	4	4	1

*Only one sample available, which is a from a mutation carrier

them. Eight of the BRCA2 families (1, 2, 3, 4, 5, 7, 8, and 19) appear to share an 8–2–1–2–4–2 haplotype from D13s1700 to D13s310 (table 3). Family 6 has a different haplotype, and only appears to share alleles telomeric to *BRCA2*.

Incidence of female breast cancer

In total, there were 29 cases of female breast cancer in the *BRCA1* group, but one of these had a phenocopy at the age of 49 years (table 4, fig 1). Forty nine women were designated mutation carriers, either because they were obligate or tested carriers, or because they had developed breast or ovarian cancer, and 20 were at 50% risk of carrying the mutation (a possible 10 further carriers). Twelve women at 50% risk were excluded as carriers by mutation testing. If the risk of breast cancer for a mutation carrier is taken as 60–80%, then in the projected carrier population (n = 59) 35–47 women would be expected to develop breast cancer in their lifetimes. The number of breast cancers was between 60 and 80% of that expected over a full life expectancy.

There were 31 cases of female breast cancer in the *BRCA2* group, including two phenocopies at the ages of 32 and 56 years respectively (table 5, fig 1). Thirty seven women were designated mutation carriers, and 22 were at 50 % risk of being mutation carriers. Eight women at 50% risk have been found mutation negative on predictive testing. From this population, 48 carriers would be expected if half those at 50% risk tested positive (n = 11). If 60–80% of women were to develop breast cancer in their lifetime, 29–38 women would be expected to develop cancer. The number of cancers was therefore between 76 and 100% of that expected over a lifetime.

The mean age at diagnosis of breast cancer was 41 years for the *BRCA1* group and 43.1 years for the *BRCA2* group. A Mann-Whitney U test showed no statistically significant difference between these results.

Incidence of ovarian cancer

There was at least one case of ovarian cancer in each of 11 families with the *BRCA1* mutation and 3 with the *BRCA2* mutation. In the *BRCA1* group, two families (16 and 18) had two cases of ovarian cancer each, and families 17 and 21 had four and three cases respectively. No *BRCA2* family had more than one case of ovarian cancer. A χ^2 test was performed comparing the ratios of breast with ovarian cancer; he ratios were 28:18 (1.55:1) for *BRCA1* and 29:3 (9.7:1) for *BRCA2*, a significant difference ($p<0.01$).

The likelihood of a *BRCA1* mutation carrier developing ovarian cancer has been estimated as between 20 and 40%. In this population, between 12 and 24 women would be expected to develop ovarian cancer; in fact, 18 (75–100% of expected) have done so. The likelihood of a *BRCA2* mutation carrier developing ovarian cancer is estimated between 10 and 20%. Thus, in our population, between 5 and 10 women would be expected to develop ovarian cancer; there are 3 cases (30–60% of expected). As with the breast cancer cases, some of these women have not lived through their risk period for ovarian cancer, and may go on to develop the disease. The mean age at diagnosis in the *BRCA1* group was 49.1 years, and the mean age at diagnosis in the *BRCA2* group was 49 years.

DISCUSSION

We have analysed 700 families with breast/ovarian cancer from the northwest of England for the presence of two common mutations in *BRCA1* and *BRCA2*. Although individually these mutations are only detected in 1.8% and 1.4% of tested samples (combined 3.2%) they account for around 1 in 8 of every mutation identified in each gene. They also account for a more substantial proportion of the high risk families. In our region, 20% of male breast cancer families with *BRCA2* mutations have the 2157delG mutation,[10] and 16% of identified *BRCA1* mutations in breast/ovarian cancer families are accounted for by 4184del4. The low number of mutations in lower risk families (three or fewer female breast cancer only) and the age at diagnosis of cancers suggests that these are highly penetrant mutations in our population. This is also reflected in the ratios of negative to positive predictive tests in women at 50% risk (12:4 for *BRCA1* (table 4) and 8:3 for *BRCA2* (table 5)). Although the selection criterion for testing was at least a 50% probability of there being a genetic basis to the familial aggregation, it is known that in smaller breast cancer families *BRCA1/2* account for less than one third of the hereditary element.[1] The low detection rate in the breast cancer only families is therefore not surprising.

There has been little evidence in the UK for frequent founder or recurrent mutations. The exon 13 duplication has been found around the world,[11] but is not very common outside the Yorkshire and northeast Midlands area of the UK. Indeed, we have only found the mutation in 1/95 northwest breast/ovarian cancer families previously testing negative for a full *BRCA1* screen. The 4184del4 mutation is clearly a more significant cause of ovarian cancer in our area, which is only 30 miles (48 km) over a mountain range from a region in which the exon 13 duplication is commonly found in the population. In concordance with other studies, we have found the 4184del4 mutation in more than one haplotypic background.[12] Given its large number of reports in BIC (75), it is likely to be a recurrent mutation, although many of the mutations probably date back many generations. The 4184del4 mutation has been found recurrently in all parts of the UK: southern England,[13] Scotland,[14] and Wales.[17] However, haplotype analysis was not undertaken. We have found the 4184del4 mutation in at least two clear haplotypic background, implying that the mutation has occurred independently on at least two occasions in our population. Indeed, the confusion over the nomenclature (4182delAATC or 4184delTCAA) indicates the repeat sequences, which may make this a warm spot for mutation. The 2157delG mutation in *BRCA2* could be under-represented, as it may be missed by the PTT unless the 5′ end of exon 11 is screened by another

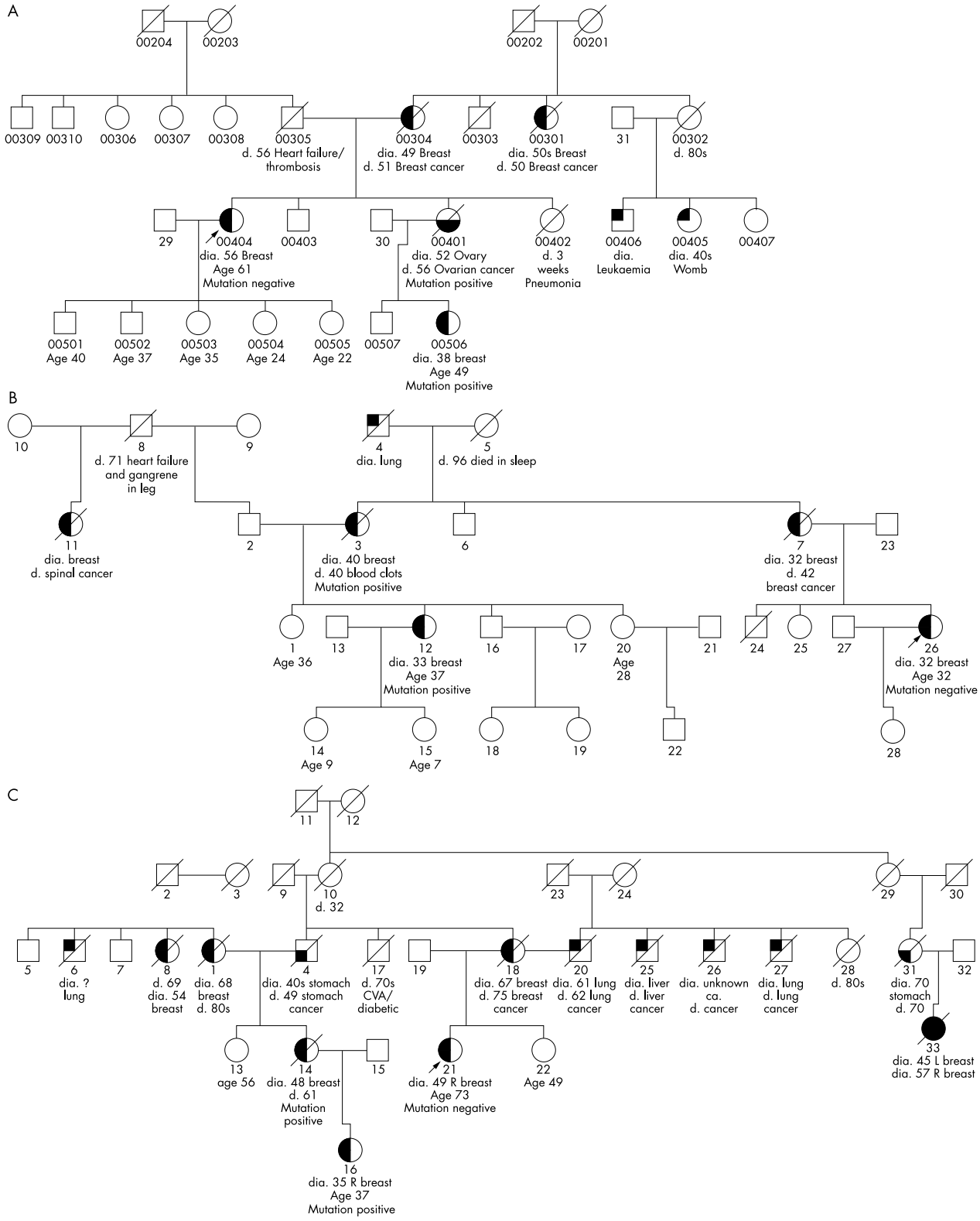

Figure 1 Pedigrees of three families containing a breast cancer phenocopy. Arrows point to the phenocopy.

technique.[4] Even so, there are a number of reports in BIC (16 times) from North America to suggest that this is a fairly frequent mutation outside the northwest of England. We have only found one clear haplotypic background for this mutation and it may thus have originated from the northwest of England on one occasion and spread to North America by migration. It may nevertheless be common in other regions of the UK. A recent report from Scotland and Northern Ireland[14] has highlighted the fact that two different mutations (one in each gene) accounted for 25/107 (23%) mutations identified. In *BRCA1*, the 2800delAA mutation comprised 13 of 61 (21%) mutations and 6503del TT 12 of 46

Table 3 Haplotypes of families with *BRCA2* 2157G mutation

Marker	Approximate position (kb)	Family 1*	2	3	4*	5	6	7*	8	19
D13s1700	−650	8/3	8	8	8/3	8	9	8/3	8	8
D13s260	−550	2/7	2/6	2	2/4	2	5	2	2	2
D13s1699	−440	1	1	1	1	1	1/2	1/2	1	1
BRCA2	0									
D13s171	260	2/3	2	2	–	2	2	2	2	2
D13s1695	450	4/2	4	4	4/3	4	4	4	4	4
D13s310	890	2	2	2/3	2/1	2	1	–	1	1

*Only one sample available, which is a mutation carrier

(26%) *BRCA2* mutations. The *BRCA1* 2800delAA has been previously shown by haplotype analysis to be a Scottish founder that has spread into North America and beyond.[16] Interestingly, our two common mutations accounted for only 5% (4184del4) of *BRCA1* and 2% (2157delG) of *BRCA2* mutations. The converse relationship is that 2/100 *BRCA1* mutations (2%) in the northwest were 2800delAA, and 4/60 *BRCA2* mutations were 6503delTT (6.7%). Although there has been little migration from England into Scotland or Northern Ireland, and a fair amount in the reverse direction, the relative frequencies between the two areas are similar. However, the differences between regions within the UK are so great (we have not found in our population the *BRCA1* 3875 mutation that has been found 5/17 times in Southampton)[13] thus development of pre-screens based on even a relatively close region are probably ill advised. Even in Scotland and Northern Ireland, where five mutations in each gene account for nearly half of all detected mutations, the situation comes nowhere near that in the Jewish population where >95% of *BRCA1/2* involvement is due to just three mutations.[17] Thus, a pre-screen of a Jewish family has very good positive and negative predictive value, whereas even in Scotland screening for 10 mutations would still hardly reduce the chances of *BRCA1/2* involvement. Indeed pre-screens in the UK are probably only justified because there is often a long delay to full mutation testing, and pre-screening at least gives the family some information.

It is likely that there are at least two subsets of *BRCA1* mutation; one causing predominantly an ovarian cancer risk and the other only a relatively low risk of ovarian involvement.[18 19] Although the basis of the ovarian cancer cluster region (OCCR) in *BRCA2*[20] remains controversial, risks in this region are likely to be the highest of any for *BRCA2*.[21] As the 2157delG mutation resides just outside the OCCR and 4184del4 is in the highest risk region for *BRCA1*,[21] the comparisons from this study are interesting. There is clearly a much higher incidence of ovarian cancer for the *BRCA1* mutation, with a ratio of breast to ovarian cancer of 1.5 to 1 compared with the *BRCA2* ratio of nearly 10 to 1. This ratio is very similar to that for the non-OCCR region calculated in a recent study.[21] The age at diagnosis of ovarian cancer was young, at a mean of <50 years for both genes. Despite the fact that many women had not lived a full life expectancy, 18 of 59 (30%) probable 4184del4 carriers had already developed ovarian cancer. This would suggest lifetime risks of closer to 40% for ovarian cancer.[18] The number of ovarian cancers was much smaller for *BRCA2* and extrapolations thus more difficult.

Phenocopies such as the three described in this study are problematic in genetic counselling and testing, and may also reflect the presence of other predisposing genes in the family. Care should be taken to ensure that everything has been done to verify in as many of the affected relatives as possible in the indicated lineage that the identified mutation has caused the

Table 4 Ages at diagnosis and numbers of breast and ovarian cancers occurring in the families with *BRCA1* 4184del4

Family	Gene	Breast cancers (years)	Ovarian cancers (years)	Unaffected carriers	Unaffected women at 50% risk >30 years	Women at 50% risk mutation negative
9	*BRCA1*	3 (39*, 52, 56)	1 (44)	0	2 (56, 52)	0
10	*BRCA1*	3 (36*, 47*, 48)	1 (59*)	0	3 (36,45,47)	0
11	*BRCA1*	1 (39/41*)	1 (48*)	0	0	1 (85)
12	*BRCA1*	3 (29*, 42, 45)	0	0	2 54, 32)	0
13	*BRCA1*	3 (29*, 43*, 34)	1 (46)	2 (39§, 45)	2 (80,56)	3 (56,56, 43)
14	*BRCA1*	5 (35*, 48*, 58, 49†, 60)	0	0	1 (55)	0
15	*BRCA1*	3 (30*, 69*, 43*)	1 (49*)	2 (48, 33)	2 (35, 37)	3 (43, 45, 48)
16	*BRCA1*	1 (32*)	2 (30*, 43*)	1 (41)	3 (32, 47, 30)	1 (38)
17	*BRCA1*	1 (39*‡)	4 (57*, 48*, 39*‡, 30*)	0	3 (68, 35, 33)	1 (71)
18	*BRCA1*	1 (51)	2 (52*, 54*)	0	0	3 (76, 73, 40)
20	*BRCA1*	4 (33*, 50, 49, 50)	1 (50)	0	1 (45)	0
21	*BRCA1*	0	3 (49*, 64, 64)	0		0
22	*BRCA1*	1 (48/50*‡)	1 (56*‡)	0	0	0
	Total	16*+12 untested = 28 Mean age 41 years; 1†	13*+5 = 18 Mean age 49.1 years	5 (Mean 41 years 1§	20 (Mean 47.1 years)	12 (Mean 57 years)

*Tested mutation carrier or obligate carrier by testing of other family members; †phenocopy; 48/50, bilateral breast cancer aged 48 and 50 years; ‡breast/ovary double primary; §obligate or presumed unaffected mutation carrier (intervening unaffected individual).

Table 5 Ages of onset and numbers of breast and ovarian cancers occurring in the families with *BRCA2* 2157delG

Family	Gene	Breast cancers (years)	Ovarian cancers (years)	Unaffected carriers	Unaffected women at 50% risk >30 years	Women at 50% risk mutation negative
1	BRCA2	5 (28,44, 56, 72, 35)	1 (47*)	70§	10 (60, 65, 56, 53, 48, 45, 52, 44, 42, 40)	0
2	*BRCA2*	29*	1 (48*)	50	0	0
3	*BRCA2*	4 (39, 49, 65, 55) M65*	0	0	3 (76, 52, 60)	1 (52)
4	*BRCA2*	2 (32*, 44)	0	64§	0	0
5	*BRCA2*	4 (33*, 40*, 32, 32†)	0	1 (35)	1 (40)	0
6	*BRCA2*	4 (58*, 42*, 40, 44)	0	0	2 (44, 33)	3 (41, 44, 73)
7	*BRCA2*	3 (32*, 43, 49)	0	0	1 (35)	0
8	*BRCA2*	4 (38*, 56†, 68, 54)	1 (52*)	0	80	0
19	*BRCA2*	4 (56/56*, 41, 32*, 32*) M68	0	1 (40)	4 (55, 48, 37, 60)	4 (54, 65, 58, 57)
	Total	11*+18 untested = 29+ 2† +2M; Mean age 43.1 years	3* (Mean age 49 years)	5 (Mean age 52 years) 3+2§	22 (Mean age 50.9 years)	8 (Mean age 55.5 years)

*Tested mutation carrier or obligate carrier by testing of other family members; †phenocopy; 48/50, bilateral breast cancer aged 48 and 50 years; ‡breast/ovary double primary; §obligate or presumed unaffected mutation carrier (intervening unaffected individual). M, male breast cancer

breast/ovarian cancer. In particular, if the nearest verified mutation carrier is a cousin, testing of block material for an intervening relative would be advisable. In the absence of such testing, it would be potentially inaccurate to counsel an individual that their risk had returned to population levels on the basis of a negative mutation result. A phenocopy could be the result of another high or relatively highly penetrant gene such as is likely in family 14, or due to other factors. It is not clear yet whether the potential presence of such genes warrants changing advice to families to indicate that their risk has not returned to population levels even with a negative test. Worryingly, even excluding family 14, 2/22 women testing negative had developed breast cancer (table 5), and the mean age for these 22 was only 56 years. By this average age, only about 3–4% of women (0.66–0.88) would have been expected to develop breast cancer.

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Authors' affiliations

D G R Evans, M Bulman, K Young, D Gokhale, F Lalloo, Academic Unit of Medical Genetics, Regional Genetics Service and National Genetics Reference Laboratory, St Mary's Hospital, Manchester M13 0JH, UK
S L Neuhausen, Division of Epidemiology, Department of Medicine, University of California, IIrvine, CA, USA

Correspondence to: Professor D G R Evans, Consultant Clinical Geneticist, Academic Unit of Medical Genetics and Regional Genetic Service, St. Mary's Hospital, Hathersage Road, Manchester M13 0JH, UK; gareth.evans@cmmc.nhs.uk

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