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BRCA1/2 mutation analysis in male breast cancer families from North West England

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Abstract 64 families with a history of male breast cancer aged 60 or less or with a family history of male and female breast cancer were screened for the presence of *BRCA1* and *BRCA2* mutations. Seventeen pathogenic *BRCA2* and four *BRCA1* mutations were identified (34%) in samples from an affected family member. All but one of the mutations segregated with disease where samples were available and pedigree structure permitted. Despite high sensitivity of mutation testing only 64% of families fulfilling BCLC criteria had an identifiable pathogenic mutation. It is possible that at least some of these families may have mutations in other genes, although we found no involvement of *CHEK2* 1100delC.

Keywords Breast cancer · Male · BRCA2 · BRCA1 · Ovarian cancer · Manchester score

Introduction

Male breast cancer (MBC) is relatively rare, affecting around 1 in 1,000 men in their lifetime. MBC may occur as part of rare syndromes which give rise to androgen deficiency such as Klinefelter's and Reifenstein syndromes. However, it is more commonly due to an inherited mutation in the predisposing gene, *BRCA2*. Depending on the population studied between 4–40% of MBC is due to mutations in *BRCA2* [1–7]. The highest frequency of *BRCA2* mutations are present in the Icelandic and Jewish populations [2, 3, 5]. Whilst the Breast Cancer Linkage Consortium (BCLC) estimates that 76% of families with four or more breast cancers (female < 60 years, male any age) are caused by *BRCA2* [8] and most other similarly affected families are due to mutations in *BRCA1*, very few studies of unselected series or MBC families have identified *BRCA1* mutations. Due to the current cost of *BRCA1*/2 analysis and the large demand for it, samples in most countries have to be prioritised to those most likely to carry mutations [9]. We have undertaken analysis of MBC families to determine the likelihood of detecting mutations dependent on family history. This updates our previous analysis of 33 families, which were tested only for mutations in *BRCA2* [10].

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Patients and methods

MBC families with living affected individuals were ascertained from referrals to the Regional Genetics service in Manchester covering a population of 4.5 million in North West England. From more than 14,000 referrals (>9,000 families) mainly of female unaffected relatives with a family history of breast cancer, over 80 families with a reported history of MBC have been ascertained. Three-generation pedigrees have been collected and cancers verified where possible from cancer registries, hospital notes and death certificates. All cases of MBC and the majority of female breast cancers were confirmed. Blood samples have been obtained from living affected (breast cancer) patients in 64 families. DNA from the male patient if available or the closest affected female was tested for mutations in both the *BRCA1* and *BRCA2* genes. In the great majority of instances (Table 1) samples were

Table 1 Details of families with and without BRCA2 mutations (Mutations have been named according to Human Genome Variation Society guidelines using the Genbank reference sequences as follows BRCA1 – U14680; BRCA2 – U43746)

Family	Female breast cancer no (ages)	MBC no (age)	Ovarian cancer no (age)	Prostate cancer no (age)	Manchester score	Mutation
1 R2330	24 (28–75)[#]	1* (65)	0	1 (70)	>100[BCLC]	*BRCA2* c.6591_6592delTC
2 R2371	4 (30*,31,34,56/56[+]*)	1* (64)	0	0	42 [BCLC]	*BRCA2* c.1929delG
3 G49767	3 (42*,42*,46)	1[+]* (70)	1* (57)	0	41 [BCLC]	*BRCA2* c.537_538dup
4 R2595	4 (51*[+2],45,45,33)	1 (68)	1 (50)	0	43 [BCLC]	*BRCA2* c.631 + 2T > C
5 R2301	4 (39,45,52,60)	1*[+] (65)	0	0	30 [BCLC]	*BRCA2* c.1929delG
6 R2597	3 (31/31*[+2],47*)	1* (48)	0	0	35 [BCLC]	*BRCA2* c.26delC
7 G38206	3 (48/48*[+2],29/48)	1 (48)	?1 (23)	0	55 [BCLC]	*BRCA2* c.6275_6276delTT
8 G47897	3 (35*[+2],37,38)	3 (46*,61,62)	0	0	57 [BCLC]	*BRCA2* c.5682C > G (p.Tyr1894X)
9 C0148	2 (45*,50*)	3(42/68*[+],70)	0	0	43 [BCLC]	*BRCA2* c.6079dup
11 R2506	1* (29)	1*[+] (40)	0	0	24	*BRCA2* 9318 ins A
12 G49767	2 (38,59)	1*[+] (67)	0	1 (78)	33	*BRCA2* c.6275_6276delTT
34 C1097	3 (52*,53,50,29)	1 (63)	0	1 (41)	35 [BCLC]	*BRCA2* del exons 1–2
35 R2855	1 (49*)	1 (54*[+])	0	0	19	*BRCA2* c.755_758delACAG
36 C0806	3 (70,39[–])	1*[+] (79)	0	0	20	*BRCA2* c.6275_6276delTT
37 C046075	4 (33/42*,42/50*)	1*[+] (67)	0	0	35 [BCLC]	*BRCA2* c.8020_8022delAA
38 G47897	8 (42*[2+],37,48,40,30, 42,65,65)	1 (58)	0	0	57 [BCLC]	*BRCA2* c.7166delG
39 C1528	2 (37/46*[+])	1 (65)	1 (53*)	0	39 [BCLC]	*BRCA2* 7172delTAAA
13 C0285	3 (35,45/47)	1*[+] (69)	0	0	30 [BCLC]	*BRCA1* c.4065_4068del
39 FH4155	3 (35,45)	1*[+] (48)	2 (34,56)	0	53~ [BCLC]	*BRCA1* c.68_69del
40 G41245	1 (47/53*[+2], 37, 34)	1 (77)	1 (50)	1 (77)	47 [BCLC]	*BRCA1* del exons 21–24
41 C2211	1 (42*[2+],69,40)	1 (57)	0	0	27	*BRCA1* del exons 14–20
Uvs						
10	1*[+] (50)	1* (58)	0	0	17	*BRCA2* c.9976A > T (p.Lys3326X) $
42	3 (39[+],66*,29,50)	1 (66)	0	0	35 [BCLC]	*BRCA2* c.6725A > T (p.Asp224Val)
43 C0658	5 (31[+]*,31,31,49,69)	1 (74)	2(41,69)	0	65 [BCLC]	*BRCA2* c.1395A > T (p.Val465Val)
Nil						
(15)	3 (44[+],48,49)	1[+] (36)	0	0	31 [BCLC]	Nil on sequencing/MLPA
(16)	3 (30/30,55[+])	1 (35)	0	0	33 [BCLC]	Nil on SSCP/PTT/MLPA
(20)	3 (35,43 [+ 2],55)	1 (72)	0	0	28 [BCLC]	Nil on SSCP/PTT/MLPA
44	3 (41/41[+],53/55)	1 (54)	0	0	33 [BCLC]	Nil on sequencing/MLPA
45	3 (38/39,74)	1 (39)	0	0	31	Nil on SSCP/PTT/MLPA Sequencing BRCA1
46	3 (35,49,63,50 [+ 2])	1 (65)	0	1 (65)	31 [BCLC]	Nil on SSCP/PTT/MLPA Sequencing BRCA1

Table 1 continued

Family	Female breast cancer no (ages)	MBC no (age)	Ovarian cancer no (age)	Prostate cancer no (age)	Manchester score	Mutation
47	1 (52)	3 (64^{+},54,55)	0	0	40 ^{BCLC}	Nil on sequencing/MLPA
48	3 (50,54,38)	1 (74^{+})	0	0	28 ^{BCLC}	Nil on sequencing/MLPA

+ patient tested for mutations; * Proven carrier of mutation, by testing or by obligate status as an intervening relative between proven mutation carriers; $^{-}$ tested negative for family mutation; 28/38 bilateral breast cancer aged 28 and 38 years; # nearly all affected cases proven carriers; ^{2} second degree relative tested; ~ Ashkenazi Jewish sample; families 1–13 have been previously reported [10]; ^{BCLC} BCLC criteria met; $ previously reported as pathogenic, but now thought to be a polymorphic stop codon [11]

available from the MBC case or their affected first-degree relative.

Mutation analysis

Blood lymphocyte DNA from affected patients from each family were analysed for all exons of *BRCA1* and *BRCA2* by either Single Strand Conformation Polymorphism (SSCP) and the protein truncation test (PTT) [10, 11] or by direct sequencing. To assess the possibility of whole exon deletions or duplications, Multiplex Ligation Dependant Probe Amplification (MLPA) was also performed.

Results

Excluding the one contralateral MBC (family 9) the mean age of the 68 MBC patients in the families was 62.5 years. Seventeen pathogenic *BRCA2* mutations were identified (27%) in samples from an affected family member. The mean age of onset of MBC in mutation positive families was 62.1 years compared to 62.9 years in the mutation negative families. Four mutations were identified in *BRCA1* (mean age 63.75). There was a single Jewish family containing the 185delAG founder mutation. MLPA analysis detected two *BRCA1* and one *BRCA2* mutations. Two unclassified variants (UVs) of unknown significance in *BRCA2* were also identified. These have not been reported before and segregated with disease in samples that were obtained from the extended family. The exon 24 nonsense mutation in family 10, which we previously reported [10] is now considered as a polymorphic stop codon mutation deleting the last 24 amino acids of the *BRCA2* protein [12]. The history of female breast cancer, MBC, ovarian and prostate cancer in the 24 mutation positive families are outlined in Table 1. Mutations were shown to segregate with disease in all but one of the 13 families where blood was available on more than one affected relative. Of the 25 families fulfilling BCLC criteria (see above and table 1), 16 (64%) had pathogenic mutations; 13 (52%) in *BRCA2* and 3 (12%) in *BRCA1*, two of the remaining nine had UVs mentioned previously. In contrast only 5/38 (13%) of MBC families with less significant female breast cancer family history had a mutation. 4/7 (57%) of families with reported ovarian cancer (a fifth case had a UV) and three of four (75%) families with multiple MBC cases had *BRCA2* mutations, the remaining two (29%) ovarian cancer families contained a *BRCA1* mutation. Of the nine families in which a second-degree relative was tested 7/9 had mutations. Using the Manchester scoring system [13] 1/24 (4%) families with combined scores <20 had pathogenic mutations, this rises to 4/17 (24%) of those with scores of 20–29;

6/12 (50%;1/12 had a UV) of families with scores of 30–39 and 9/11 (82%) of scores >39 (1/11 had a UV). Of the six families with no identified mutation with scores >30, three had mutation testing of *BRCA2* with SSCP/PTT and a coding sequence mutation may have been missed. No *CHEK2* c.1100delC mutations were identified in our cohort of 64 families.

Discussion

Our clinic-based study has detected a high rate (34%) of *BRCA1/2* mutations in MBC families. Whilst we have only tested one patient per family (except family 15) it is unlikely we have missed mutations by testing the "wrong" individual as 7/9 families in which a second degree relative to the MBC case was tested had a mutation, and similar proportions of male patients were initially tested in mutation proven and mutation negative families. Indeed in the 21 families with *BRCA1/2* mutations 15/21 (71%) have a proven MBC mutation carrier. The 64% detection rate in 25 BCLC families with our mutation detection strategy suggests a fairly high sensitivity. The ratio of *BRCA2* to *BRCA1* mutations is consistent with a 3.5:1 ratio, but not the 76% *BRCA2* or overall 94% level predicted by Linkage consortium data [8]. The two families with UVs which have limited supporting segregation data could have an unidentified pathogenic mutation on the same allele. This is supported by family 37, in which a synonymous change in *BRCA2* was shown to segregate, before application of more sensitive techniques identified the pathogenic change on the same allele. An alternative explanation would be that the missense variants could be pathogenic, although these are rare in *BRCA2*. Despite the comprehensive scan of the coding sequence of the gene there remains a substantial chance of a missed *BRCA2* mutation, which may be a splicing mutation deep within an intron or a mutation affecting the promotor. Alternatively given the reasonably high sensitivity of our techniques and the sample size of 25 being almost as high as the BCLC (26) it could indicate that other genes may be the cause in a minority of these high risk MBC clusters.

About 67% of the multiple MBC case families and families with ovarian cancer in addition to MBC had *BRCA2* mutations and one of the remaining two had a missense mutation. This suggests most MBC/ovarian families will have *BRCA2* rather than *BRCA1* mutations and therefore mutation testing should start with *BRCA2* if testing is carried out sequentially. Indeed the detection rate in MBC/ovarian cancer families suggests that MBC is a more sensitive indicator of a *BRCA2* mutation than ovarian cancer is to *BRCA1* despite the fact that nearly 80% of BCLC families with ovarian cancer have a *BRCA1* mutation [8].

Our analysis using MLPA has again pointed to the importance of a technique to detect large rearrangements in *BRCA1/2*. Studies of MBC including our own have been too small to give a robust estimate as to the contribution of these mutations to this phenotype. Nonetheless, the detection of three further large whole exon deletions accounting for 15% of *BRCA1/2* mutations is further evidence supporting the routine use of this type of technique in *BRCA1/2* screening. In contrast to two previous reports 2/3 of our rearrangements were in *BRCA1*, although as numbers are small this may be a chance finding. A French group selected 39 MBC families, which had already tested negative for mutations in the coding regions of *BRCA1* and *BRCA2* [14]. They developed an assay for *BRCA2* rearrangements, based on quantitative multiplex PCR of short fluorescent fragments (QMPSF). They found three rearrangements: (1) a deletion of exons 12–13; (2) a duplication of exons 1–2; and (3) a complete deletion of *BRCA2* [15]. Using MLPA analysis of *BRCA2* an Australian group found three individuals (2%) with novel genomic rearrangements in 149 families with a strong family history of breast and/or ovarian cancer that were tested [14]. These rearrangements involved a deletion of exons 14–16 in one family and the deletion of exons 1–2 in two families. All individuals that were found to harbour a genomic rearrangement of *BRCA2* were women affected by breast cancer and were members of families where a male relative was also affected by breast cancer. Three of 25 (12%) such male/female breast cancer families were found to be positive for a deletion [15]. Another French group also recently identified three families with large genomic rearrangements [16].

The age at onset of breast cancer in this study is not a reliable guide to the presence of a mutation although the mean age of breast cancer in *BRCA2* mutation positive families was marginally younger than negative families. The mean age of 62.1 years for *BRCA2* mutation carriers is a little higher than the 58.8 found in a UK population study for mutation carriers compared to 67.9 for non-carriers [7]. Whilst *BRCA2* mutations in founder populations are detected in isolated MBC [2, 3, 5, 6] the UK series [7] only demonstrated mutations in 3/76 (4%) patients without a family history of breast cancer. This suggests that isolated cases of MBC are not a priority for mutation testing on clinical grounds. However, it is worth testing families with at least a Manchester score of 17, which is achieved with one MBC and one female case if both are young (<60). All our mutations have been detected in families demonstrating high penetrance similar to those expected from the BCLC [8]. As the present study was not selected purely on the grounds of high risk it may suggest that the presence of MBC at least in the UK is an indicator of high penetrance. As these are the families routinely ascertained in clinic,

BCLC penetrance figures should be used when counselling, unless the family has only a limited history.

From our present study and other studies [7] there does not appear to be a genotype/phenotype correlation for MBC with *BRCA2*. The mutations in our series are spread throughout the gene in contrast to ovarian cancer, which appears to cluster around exon 11 [17, 18]. Most of the mutations have been reported before on the Breast Information Core (BIC) database [19], and family 1 was in the original report of the cloning of *BRCA2* [20]. Whilst the great majority of high-risk families with MBC have *BRCA2* mutations it seems likely that there are other genes predisposing to male and female breast cancer. Indeed population surveys have shown that perhaps as little as 15% of the hereditary element is explained by *BRCA1* and *BRCA2* [7]. Although recent evidence has cited the *CHEK2* gene as a contributor with an approximately 10 fold relative risk, we would only expect 4/41 of our *BRCA1/2* negative families to have a mutation and this would be unlikely to segregate with disease [21]. In fact we did not find a single case with the *CHEK2* mutation c.1100delC and given the low overall risk it is doubtful whether testing for this mutation is justified clinically. The families in our series without *BRCA1/2* mutations may be useful in identifying other genes predisposing to moderate/high risk.

References

1. Couch FJ, Farid LM, Deshano ML et al (1996) BRCA2 germline mutations in male breast cancer cases and breast cancer families. Nat Genet 13:123–125
2. Thorlacius S, Olafsdottir G, Trygvaddottir L et al (1996) A single BRCA2 mutation in male and female breast cancer families from Iceland with varied cancer phenotypes. Nat Genet 13:117–119
3. Friedman LS, Gayther SA, Kurosaki T et al (1997) Mutation anlysis of BRCA1 and BRCA2 in a male breast cancer population. Am J Hum Genet 60:313–319
4. Mavraki E, Gray IC, Bishop T et al (1997) Germline BRCA2 mutations in men with breast cancer. Br J Cancer 76:1428–1431
5. Haraldsson K, Loman N, Zhang OX et al (1998) BRCA2 germline mutations are frequent in male breast cancer patients without a family history of the disease. Cancer Res 58:1367–1371
6. Csokay B, Udvarhelyi N, Sulyok Z et al (1999) High frequency of germline BRCA2 mutations among Hungarian male breast cancer patients without a family history. Cancer Res 59:995–998
7. Basham VM, Lipscombe JM, Ward JM et al (2001) BRCA1 and BRCA2 mutations in a population based study of male breast cancer. Breast Cancer Res 4:1–5
8. Ford D, Easton M, Stratton S et al (1998) Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. Am J Hum Genet 62:676–689
9. Davies JF, Redmond EK, Cox MC, Lalloo FI, Elles R, Evans DGR (2000) 2157delG: a frequent mutation in BRCA2 missed by PTT. J Med Genet 37:e42
10. Evans DGR, Bulman M, Gokhale D, Lalloo F (2002) High detection rate for BRCA2 mutations in male breast cancer families from North West England. Fam Cancer 3:131–133
11. Evans DGR, Bulman M, Young K, Gokhale D, Lalloo F (2003) Sensitivity of BRCA1/2 mutation testing in 466 breast/ovarian cancer families. J Med Genet 40(9):e107
12. Martin ST, Matsubayashi H, Rogers CD et al (2005) Increased prevalence of the BRCA2 polymorphic stop codon K3326X among individuals with familial pancreatic cancer. Oncogene 24(22):3652–3656
13. Evans DG, Lalloo F, Wallace A, Rahman N (2005) Update on the Manchester scoring system for BRCA1 and BRCA2 testing. J Med Genet 42(7):e39
14. Tournier I, Paillerets BB, Sobol H et al (2004) Significant contribution of germline BRCA2 rearrangements in male breast cancer families. Cancer Res 64(22):8143–8147
15. Woodward AM, Davis TA, Silva AGS (2005) Large genomic rearrangements of both BRCA2 and BRCA1 are a feature of the inherited breast/ovarian cancer phenotype in selected families. J Med Genet 42(5):e31
16. Casilli F, Tournier I, Sinilnikova OM et al (2006) The contribution of germline rearrangements to the spectrum of BRCA2 mutations. J Med Genet 43(9):e49
17. Gayther SA, Mangion J, Russell P (1997) Variations of risks of breast and ovarian cancer associated with different germline mutations of the BRCA2 gene. Nat Genet 15:103–105
18. Thompson D Easton D (2001) Variation in cancer risks, by mutation position in BRCA2 carriers. Am J Hum Genet 68:410–419
19. Breast Cancer Information Core: http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/
20. Wooster R, Bignell G, Lancaster J et al (1995) Identification of the breast cancer susceptibility gene BRCA2. Nature 378:789–792
21. Meijers-Heijboer H, van den Ouweland A, Klijn J et al (2002) Low penetrance breast cancer susceptibility due to CHK2 1100delC in non-carriers of BRCA1 or BRCA2 mutations. Nat Genet 31(1):55–59