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BRCA1 and *BRCA2* in breast cancer families from Wales: moderate mutation frequency and two recurrent mutations in *BRCA1*

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Summary Mutations in the *BRCA1/BRCA2* genes account for varying proportions of breast cancer families studied, and demonstrate considerable variation in mutational spectra coincident with ethnic and geographical diversity. We have screened for mutations in 17 families from Wales with two or more cases of breast cancer under age 50 and/or ovarian cancer. Eight out of 17 (47%) families had demonstrable mutations. Six out of 17 (35%) carried *BRCA1* mutations and 2 out of 17 (12%) carried *BRCA2* mutations. Two recurrent mutations in *BRCA1* were identified, which appear to represent founder mutations in this population. These data support the existence of additional breast and ovarian cancer susceptibility genes.

Keywords: *BRCA1*; *BRCA2*; breast and ovarian cancer; Wales

Two genes, *BRCA1* and *BRCA2*, have been identified as being causative in familial breast/ovarian cancer (Miki et al, 1994; Wooster et al, 1995). Carriers of germline mutations in these genes are at increased risk of developing breast and ovarian cancers. Analysis of *BRCA1/BRCA2* in high-risk families has demonstrated considerable heterogeneity in inactivating mutations, with a lower proportion of families attributable to mutations in either gene than had previously been predicted by statistical analyses (Castilla et al, 1994; Phelan et al, 1996; Serova et al, 1997). We have screened affected individuals from 17 high-risk Welsh breast and breast/ovarian cancer families for mutations in both *BRCA1* and *BRCA2* to determine the proportion of families attributable to defects in these genes. Recent experience with recurrent *BRCA1* and *BRCA2* mutations in the Ashkenazi Jewish and Icelandic populations (Neuhausen et al, 1996*a*; Offit et al, 1996; Thorlacius et al, 1996), coupled with studies of Welsh cystic fibrosis patients (Ashley, 1986; Cheadle et al, 1993; Lucotte and Hazout, 1995), suggests that screening for common genetic alterations in patients with familial breast and ovarian cancer in the Welsh population may prove a productive approach to the study of susceptibility in this group.

PATIENTS AND METHODS

Seventeen families with two or more individuals diagnosed with breast cancer before age 50 years and/or ovarian cancer were selected under informed consent from patients attending the Department of Medical Genetics at the University Hospital of Wales, Cardiff, UK. All families were resident in Wales, with all obviously non-UK names excluded. Pedigrees were obtained by a registered nurse in a face-to-face interview with the patient. Verification of diagnosis was carried out by patient chart and pathology report review wherever possible. All families fulfilling inclusion criteria, who agreed to participate, were reported in the study. All coding exons for *BRCA1* and *BRCA2* were analysed by single-stranded conformation analysis (SSCA) in fragments of 200–300 bp (Miki et al, 1994; Lancaster et al, 1996*a*). Genomic DNA was amplified by the polymerase chain reaction (PCR) in a Perkin-Elmer 9600 Thermocycler (Perkin-Elmer, CA, USA). Reaction products were then electrophoresed on 0.5 × MDE (FMC, ME, USA) gels, dried and autoradiographed. Additionally, *BRCA1* exon 11 and *BRCA2* exons 10, 11 and 27 were screened for frameshift and nonsense mutations (producing premature stop codons) using the protein truncation test (PTT) (Lancaster et al, 1996*a*, 1996*b*). Exon 11 (*BRCA1*) and exons 10 and 27 (*BRCA2*) were amplified by PCR in one fragment, and exon 11 (*BRCA2*) in two fragments, in the presence of forward primers containing a T7 promoter and transcription/translation initiation sequence (primer sequences available from jmlanc@acpub.duke.edu). The PCR product was then subjected to an in vitro transcription/translation reaction (Promega, Madison, WI, USA), electrophoresed on a 10–20% sodium dodecyl sulphate (SDS)-polyacrylamide Ready-Gel (Biorad, Malvern, PA, USA), fixed, dried and autoradiographed. Sequencing templates were produced for samples showing aberrant mobility on SSCA or PTT and sequenced using a PRISM DyeDeoxy Terminator Cycle Sequencing Kit and an ABI PRIZM 377 Automated Fluorescent Sequencer (Applied BioSystems, CA, USA), according to manufacturer's instructions. Genotyping was carried out using fluorescent dye-labelled primer pairs analysed on the ABI377 with the Genescan software package. Sequences of primers are available from the Genome

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Table 1 Families screened for *BRCA1* and *BRCA2* mutations

Family	Mutation	Effect	BrCa cases (*n*)	BrCa < 50 years cases (*n*)	OvCa cases (*n*)	Comments/other cancers
Families positive for *BRCA1* mutations						
CDF2	4184delTCAA	ter1364	2	2	0	
CDF7	4184delTCAA	ter1364	4	4	0	Two bilateral BrCa cases
CDF10	2011insT	ter615	3	3	0	One bilateral BrCa case 'abdominal' cancer
CDF13	2594delC	ter845	1	1	3	
CDF128	2594delC	ter845	6	4	0	Uterus, testicle, lung
CDF209	1997del4	ter610	2	2	0	
Families positive for *BRCA2* mutations						
CDF107	4075delGT	ter1284	3	2	0	BrCa maternal and paternal sides, Pancreas, prostate, cervix
CDF325	6287del4	ter2047	4	2	0	
Families screened negative for mutations						
CDF1			4	4	0	One bilateral BrCa case
CDF5			4	3	1	One dual primary BrCa/OvCa
CDF8			4	2	0	
CDF11			5	2	0	
CDF12			1	1	3	All affected < 40 years
CDF16			1	1	1	OvCa age 48 years
CDF65			7	4	0	
CDF85			4	2	2	Cancer cervix, stomach (40s)
CDF312			4	2	0	Cancer larynx

ter, termination codon; BrCa, breast cancer; OvCa, ovarian cancer; del, deletion. Readers should refer to the Breast Cancer Information Core (BIC) for full, up-to-date mutation spectra in both genes.

Data Base (http:gdbwww.gdb.org/gdb/gdbtop.html). A database of updated *BRCA1* and *BRCA2* mutations is catalogued by the Breast Cancer Information Core Database at the BIC web site (http://www.nchgr.nih.gov/dir/lab_transfer/bic/).

RESULTS

Inactivating mutations were identified in 8 out of 17 (47%) families – six in *BRCA1* (35%) and two in *BRCA2* (12%) (Table 1). All mutations produced frameshifts that were predicted to result in premature termination of protein synthesis. The average age at diagnosis of breast cancer in eight families carrying mutations was 40.7 years, compared with an average age of 48.6 years in nine families without mutations (not statistically significant). Seven out of eight (87%) families carrying mutations contained a very early onset case of breast cancer (< 40 years), compared with four out of nine (44%) families without mutations. Of 13 site-specific breast cancer families (no ovarian cancer), five (38%) contained *BRCA1* mutations and two (15%) *BRCA2* mutations. Of the five breast/ovarian families in the study, only one was found to be segregating a *BRCA1* mutation. No *BRCA2* mutations were observed in breast/ovarian families. Two recurrent *BRCA1* mutations were found, 4184del4 and 2594delC. DNA samples from the recurrent mutation carriers were genotyped for a series of polymorphic microsatellite markers both in the *BRCA1* gene (D17S855, D17S1322) and flanking (D17S1185, D17S1320, D17S1325) the *BRCA1* gene. Genotyping results for the recurrent mutation chromosomes are given in Table 2 and discussed below.

Table 2 Haplotypes of common *BRCA1* mutations in Wales

	Family (mutation)			
Marker	CDF2 (4184del4 – *BRCA1*)	CDF7 (4184del4 – *BRCA1*)	CDF13 (2594delC – *BRCA1*)	CDF128 (2594delC – *BRCA1*)
D17S1185	ND	ND	214	214
D17S1320	170	170	172/176	172/176
D17S855	157	157	149	149
D17S1322	121	121	121/127	121/127
D17S1325	196	196	214	214

Alleles given in basepairs. Markers are in centromeric to telomeric order. D17S855 and D17S1322 are both intragenic to *BRCA1*. ND, not done.

DISCUSSION

In this study, 17 Welsh families with apparent hereditary breast/ovarian cancer were studied for *BRCA1/BRCA2* mutations. The results show that, overall, only approximately half of these families were found to be segregating either a *BRCA1* or *BRCA2* mutation. This proportion, although lower than that predicted by statistical analysis of a cohort of families collected for the linkage analysis, is in agreement with that observed in several other studies. Interestingly, recurrent mutations were observed in half of the families with mutations, all of whom appear to be unrelated. Families CDF2 and CDF7 were found to have a *BRCA1* 4184del4 mutation, whereas families CDF13 and CDF128 were found to have a *BRCA1* 2594delC mutation. DNA samples from the recurrent mutation carriers were genotyped for a series of polymorphic microsatellite markers in and flanking *BRCA1* to assess haplotype sharing which would be indicative of common founder effects for these two mutations (Table 2). A common 157-bp allele at the D17S855 intragenic marker was found in the 4184del4 families. This D17S855 allele was not detected in typing 94 chromosomes

from unaffected Welsh control subjects and appears to be quite rare in this population (unpublished results). Other studies have also reported a D17S855 157-bp allele in association with the 4184del4 mutation (Neuhausen et al, 1996). However, in those families, a 118-bp allele at the adjacent intragenic marker D17S1322 has been reported, whereas CDF2 and CDF7 share a 121-bp D17S1322 allele in disequilibrium with the D17S855 allele. The 4184del4 mutation has been reported 14 times in individuals of various ethnic backgrounds, and appears to be segregating on at least two different haplotypes to which our data from Wales adds a third (Neuhausen et al, 1996*b*; Szabo and King, 1997). Two other families, CDF13 and CDF128, were found to be carrying a common 2594delC *BRCA1* mutation. Genotyping revealed a common haplotype for this mutation as well. An additional marker centromeric to *BRCA1*, D17S1185, was also typed to confirm allele sharing proximal to the ambiguous genotype at D17S1320. This mutation has also been reported frequently in Sweden and Denmark (Johannsson et al, 1996; Hakansson et al, 1997), with those families reported to share a common allele of D17S855, and has also been reported once in a family from the south of England (Campbell et al, 1997). As is the case for the *BRCA1* 4184del4 mutation, data for the *BRCA1* 2594delC mutation indicates that it is also probably a recurrent founder mutation in the Welsh population. Full genealogical tracing is ongoing for all families with recurrent mutations. Additionally, we hope to obtain DNA from other members of family CDF128 to assess *BRCA1* mutation status in individuals affected with uterine, lung and testicular carcinoma. This study suggests that screening for the two recurrent *BRCA1* mutations described here may be a useful first step in assessing Welsh hereditary breast/ovarian cancer families.

The mutation detection rate in this cohort compares favourably with families ascertained in other studies (Phelan et al, 1996; Szabo and King, 1997). The proportion of breast cancer families attributable to *BRCA1* and *BRCA2* mutations in this cohort is roughly similar to that described elsewhere in Britain (Szabo and King, 1997). However, the finding of only one of five breast/ovarian families with *BRCA1* mutations is less than has been reported elsewhere (Narod et al, 1995). Additionally, we failed to detect any instances of the 6503delTT mutation in *BRCA2* that has been seen frequently in British families (Mazoyer et al, 1996). These data support the observation that although the overall contribution of *BRCA1/BRCA2* to familial breast cancer may be the same among study groups, the distribution of mutations is quite variable with respect to ethnogeographical differences. *BRCA1* mutation detection sensitivity using SSCA alone is approximately 80% (Lancaster et al, 1997), and our detection rate in this study is probably increased by the additional use of the protein truncation test (PTT). However, even if SSCA and PTT together fail to identify 15% of mutations, our data still provide support for the view that a significant proportion of familial breast/ovarian cancer (approximately 35–53%) is not due to mutations in either *BRCA1* or *BRCA2*, and that other dominant breast cancer susceptibility genes may exist. Linkage analysis of families negative for *BRCA1* and *BRCA2* mutations would further support this philosophy, and we are currently collecting samples from other family members wherever possible. The finding of *BRCA1* mutations in only one out of five breast/ovarian cancer families suggests additional familial ovarian cancer gene(s) also exist. Patients tested for *BRCA1* and *BRCA2* mutations and found to be negative should, thus, be informed of the potential of mutations in other as yet unidentified breast and ovarian cancer susceptibility genes contributing to continued increased cancer risk.

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