**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, 1996a; 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, 1996a). 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, 1996a, 1996b). 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 jmnlanc@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
**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 | Two bilateral BrCa cases |
| | CDF7 | 4184delTCAA | ter1364 | 4 | 4 | 0 | One bilateral BrCa case |
| | CDF10 | 2011insT | ter615 | 3 | 3 | 0 | 'abdominal' cancer |
| | CDF13 | 2594delC | ter845 | 1 | 1 | 3 | Uterus, testicle, lung |
| | CDF128 | 2594delC | ter845 | 6 | 4 | 0 | |
| | 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 |

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.ncbi.nlm.nih.gov/dsr/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.

**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 and to our data from Wales adds a third (Neuhausen et al, 1996d; 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 asessing 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 6503delTTT mutation in BRCA2 that has been seen frequently in British families (Mazoyer et al, 1996). These data support the observation that although the overall proportion of BRCA1/BRCA2 to familial breast cancer may be the same among study groups, the distribution of mutations is importantly 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 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 families 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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