A breast cancer family from Spain with germline mutations in both the BRCA1 and BRCA2 genes

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There are two major cancer susceptibility genes, BRCA1 and BRCA2, mutations in which predispose to early onset breast and ovarian cancer.1,2 The frequency of BRCA1 and BRCA2 mutations in the general population ranges from 1-500 to 1-800.1 In a recent population study,3 the authors showed that the estimated population frequencies for BRCA1 and BRCA2 mutations were similar under both recessive and polygenic models, 0.024 and 0.041%, respectively. These frequencies are lower than the frequencies found before.3 Therefore, the prior probability of finding any person or family with mutations in both the BRCA1 and BRCA2 genes is very low. In the Ashkenazi Jewish population, however, the likelihood of being a carrier of one of the three common BRCA1 or BRCA2 mutations is as high as 1 in 50; this explains why there are multiple reports of double heterozygotes for these mutations. There have been reports of families with two mutations,4 subjects with two mutations,5,6 and families with three mutations in the BRCA genes,7 mostly in the Ashkenazi Jewish population. To date, only a few subjects or families have been reported to have more than one non-Ashkenazi BRCA mutation. There are five reports describing families that harbour two BRCA1 or one BRCA1 and one BRCA2 mutation.8,9,10,11,12 We report the first Spanish breast cancer family where two independent mutations, one in BRCA1 and the second in BRCA2, are present in multiple members of a single sibship. The two mutations were found in many subjects. Analysis of the pedigree showed a spectrum of cancer phenotypes associated with one or two mutations, as well as different ages of onset of the cancer.

MATERIALS AND METHODS

Patients

The family was selected through the clinic for familial cancer at the San Carlos Hospital in Madrid, Spain. Informed consent was obtained from each participant. Personal and cancer histories were obtained from the proband and participating relatives, and cancer diagnoses and deaths were confirmed by reviewing the medical records, pathology reports, or death certificates.

Mutation analysis

Genomic DNA was isolated from peripheral blood lymphocytes according to standard protocols. The entire BRCA1 and BRCA2 coding region and the splice junctions were amplified from genomic DNA using 37 and 72 primers sets respectively (primer sequences and PCR protocols are available upon request). All amplicons were subjected to denaturing gradient gel electrophoresis analysis (DGGE). DNA fragments that displayed an abnormal DGGE pattern were analysed by cycle sequencing with the ABI Prism dRhodamine Terminator Sequencing Kit in the ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, CA). The mutations were confirmed by two independent sequencing PCR reactions and sequenced in both directions.

Loss of heterozygosity analysis (LOH)

In addition to mutation analysis, we used DGGE to perform DGGE-LOH analysis in the tumour DNA of the index case. The tumour tissue was sectioned and subjected to haematoxylin-eosin staining to determine the proportion of tumour cells. Only samples with >95% tumour cells were selected. DNA was obtained from microdissected tumour cells and DGGE-LOH analysis performed as previously described.13

Genetic markers

The family included in this study has been typed for markers flanking BRCA1 (D17S1293, D17S855, D17S1299, and D17S579) and markers flanking BRCA2 (D13S1493, D13S267, and D13S260) by ABI PRISM Fluorescent Genotyping (Applied Biosystems, Foster City, CA), according to the manufacturer’s protocol.

RESULTS AND DISCUSSION

Family F121 displays features compatible with a major monogenic, dominantly inherited susceptibility to breast cancer (fig 1). The index case, who is identified by an arrow in pedigree F121, was of Spanish origin. She was diagnosed with breast cancer (grade II adenocarcinoma) at the age of 28 years. After informed consent was obtained, blood DNA was screened in

Key points

- Germline mutations in the BRCA1 and BRCA2 genes predispose people to early onset breast and ovarian cancer. The frequency of mutations in these genes in the general population is very low. Therefore, the probability of finding any family with mutations in both genes is even lower.
- This study reports the presence of two mutations, one in BRCA1 and a second in BRCA2, with variable expression. The BRCA1 mutation, A1708E, was identified first in the proband. Analysis on a related family member with early onset breast cancer for the same mutation was negative. Further analysis on the second gene, BRCA2, led to the identification of the germline mutation STOP2098 in this family.
- Without the knowledge of the second mutation in this family, some members would have been given a negative test result and been falsely reassured. This study shows the importance and benefit of testing for both the BRCA1 and BRCA2 genes in order to obtain an accurate result for genetic counselling.

Abbreviations: DGGE, denaturing gradient gel electrophoresis; LOH, loss of heterozygosity
search of DNA variants for all coding exons and intron/exon boundaries of the BRCA1 and BRCA2 genes by PCR-DGGE analysis. The sample exhibited abnormal migration patterns for exon 18 of BRCA1 and exon 11 of BRCA2. Direct sequencing showed BRCA1 A1708E as a missense mutation and BRCA2 6503 del TT as a frameshift mutation that produces a stop codon at position 2098 (fig 1B). Both mutations were confirmed by two independent sequencing PCR reactions and sequenced in both forward and reverse directions. The BRCA1 A1708E mutation has been reported previously in the Breast Cancer Information Core (BIC) database and is one of the very few BRCA1 missense mutations known to have a pathogenic role.

The index case has five sibs (four sisters and one brother) and a maternal history of breast cancer (fig 1A). One of the sisters had been diagnosed with breast cancer at the age of 40 years and she died at 42 years. DNA samples were available from her parents. It was therefore possible to determine that both mutations were inherited from her mother. Interestingly, her mother was not diagnosed with breast cancer until then. The knowledge of the mutation status in the proband motivated a number of family members to request testing. Informed consent was signed by each person tested. The maternal side contained two cases of postmenopausal breast cancer (II.11 and II.12) diagnosed at 71 and 66 years of age. One of them (II.11) died at the age of 72 years and she was an obligate carrier of both BRCA1 and BRCA2 mutations because two of her daughters (III.10 and III.12) are carriers of BRCA1 A1708E and another daughter (III.17) is a carrier of BRCA2 6503 del TT. The other postmenopausal breast cancer case, II.12, is a carrier of both BRCA1 and BRCA2 mutations. There was one case of prostate cancer (II.7) diagnosed at the age of 66 years who died at 68 years; he was also an obligate carrier of both mutations because his two daughters are carriers of both BRCA1 A1708E and BRCA2 6503 del TT. There was also one case of early onset breast cancer (III.17) diagnosed at the age of 32 years who is a carrier of only the BRCA2 6503 del TT mutation. Other results of mutation analysis are indicated on the pedigree (fig 1A).

In order to document the involvement of either BRCA1 or BRCA2 in pedigree 121, genetic linkage analysis was performed with markers for BRCA1 (D17S579, D17S1299, D17S855, and D17S1293) and markers for BRCA2 (D13S260, D13S267, and D13S1493). The segregation of BRCA1 and BRCA2 haplotypes is shown in fig 1A. All carriers of BRCA1 and BRCA2 mutations share the same allele at each marker, which is consistent with the presence of a common haplotype. Given the diversity of phenotype among the double mutation carriers in our family, we decided to study which of the two genes was causing the disease in subjects who carry both mutations. Although not definitive, the best way to address this issue is by examining the tumours in order to characterise the LOH patterns, since the vast majority of BRCA tumours exhibit loss of the wild type allele.

The LOH study for both mutations A1708E in BRCA1 and STOP2098 in BRCA2 in the tumour DNA of the index case (fig 2) showed that the wild type allele and the mutant allele were equally retained in
Figure 2. DGGE-LOH analysis of different BRCA1 and BRCA2 exons amplified from both tumour DNA (lane T) and peripheral blood lymphocyte DNA (lane N). Analysis of the index case III.1 in the pedigree harbours the missense mutation A1708E in BRCA1 and the in frame mutation STOP2098 in BRCA2. Bands corresponding to wild type (wt) and mutant (Mut) alleles and the heteroduplex (Het) are indicated. The wild type and mutant alleles are retained in the tumour DNA for both mutations A1708E in BRCA1 and STOP2098 in BRCA2.

the tumour DNA. The lack of LOH suggests an alternative pathway of causing the disease; this way could include alternations in others genes and/or environmental factors associated with modification of breast cancer risk, which might explain the low incidence of breast cancer in the family.

This case is the first example to date of a double heterozygote for the high penetrance breast cancer susceptibility genes, BRCA1 and BRCA2, in a family from Spain. In this family, in the early onset breast cancer case III.1, the prior probability of being a mutation carrier based on her age at onset and the family history is similar to the early onset breast cancer case III.1, but if we had not studied both the BRCA1 and BRCA2 genes in the index case (III.1) we would never have found the BRCA2 mutation in case III.17. The study shows the importance and benefit of testing for both BRCA1 and BRCA2 in order to obtain an accurate result for genetic counselling.

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