

## ELECTRONIC LETTER

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.<sup>1,2</sup> The frequency of *BRCA1* and *BRCA2* mutations in the general population ranges from 1-500 to 1-800.<sup>2,3</sup> In a recent population study,<sup>4</sup> 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.<sup>2,3</sup> 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<sup>5</sup>; this explains why there are multiple reports of double heterozygotes for these mutations. There have been reports of families with two mutations,<sup>6</sup> subjects with two mutations,<sup>7-9</sup> and families with three mutations in the *BRCA* genes,<sup>10</sup> 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*<sup>11</sup> or one *BRCA1* and one *BRCA2* mutation.<sup>10,12-14</sup> 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.<sup>15</sup>

## 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

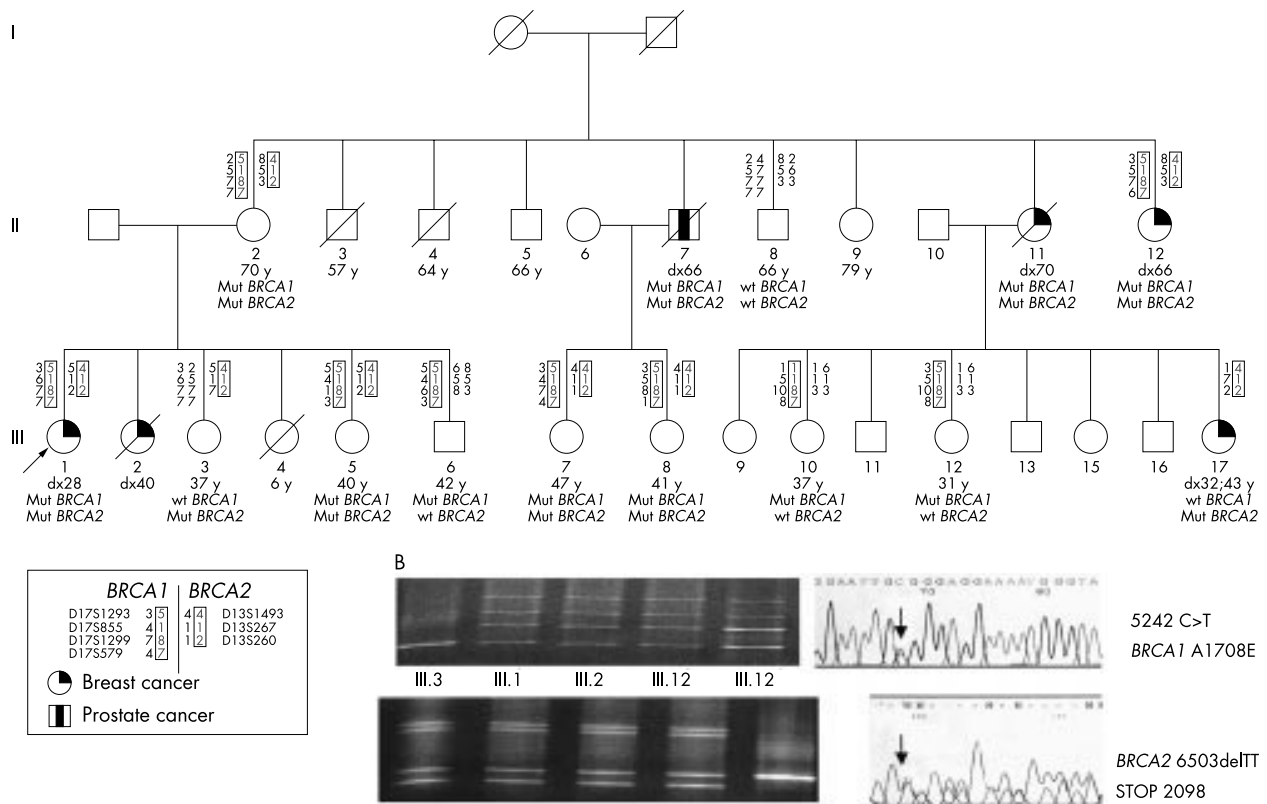
Family F121 displays features compatible with a major monogenic, dominantly inherited susceptibility to breast cancer (fig 1). The index case, who is indicated by an arrow in pedigree F121, was of Spanish origin. She was diagnosed with breast cancer (grade II adenocarcinoma) at the age of 28 years. After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

A



**Figure 1** Pedigree of family F121. (A) This pedigree shows the incidence of cancer, the results of genotyping, and the *BRCA1* and *BRCA2* mutation carrier status. Age at diagnoses (dx) and age at the present time are reported. The index case, III.1, had unilateral breast cancer at the age of 28 (marked with an arrow) and she is a carrier of both *BRCA1* and *BRCA2* mutations. The carrier status and cancer type of other subjects are indicated on the pedigree. The result of the genotyping is represented beside each subject. The boxed haplotypes are associated with the mutations (on the left of the figure the individual markers are shown). Wt = unaltered *BRCA* allele; Mut = altered *BRCA* allele. (B) DGGE (denaturing gradient gel electrophoresis) and electropherogram for *BRCA1* and *BRCA2* mutations. Homozygosity and heterozygosity patterns of III.3, III.1, II.2, II.12, and III.12 are shown. The arrow in the electropherogram indicates the heterozygous germline *BRCA1* A1708E and *BRCA2* 6503delTT mutations.

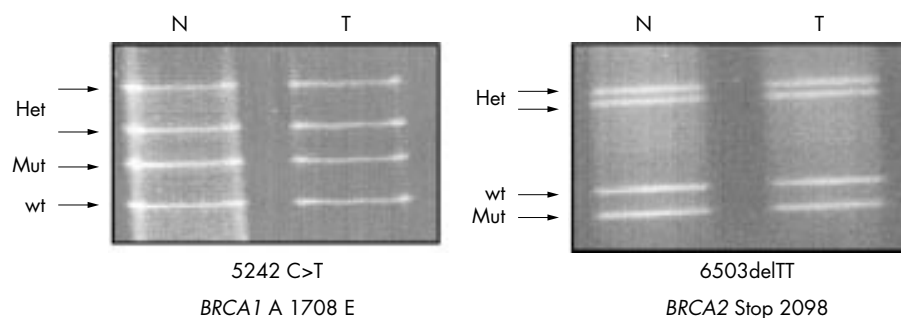
search of DNA variants for all coding exons and intron/exon boundaries of the *BRCA1* and *BRCA2* genes by PCR-DGGE analysis. The sample exhibited abnormal migration patterns for exon 18 of *BRCA1* and exon 11 of *BRCA2*. Direct sequencing showed *BRCA1* A1708E as a missense mutation and *BRCA2* 6503 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.<sup>16,17</sup> The *BRCA2* 6503 del TT mutation was a novel mutation not previously reported in the BIC database.

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.<sup>18</sup> 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) harbouring 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 alterations 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.17, 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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*Electronic Database Information.* URLs for data in this article are as follows: Cooperative Human Linkage Center, <http://lpg.nci.nih.gov/CHLC/> (for (databases of microsatellite markers). Breast cancer information core database, [http://www.nhgri.gov/Intramural\\_research/Lab\\_transfer/Bic/](http://www.nhgri.gov/Intramural_research/Lab_transfer/Bic/). Genome Database, <http://www.gdb.org/>

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#### REFERENCES

- Miki Y, Swensen J, Shattick-Eidens D, Futreal AP, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett ML, Ding W, Bell R, Rosenthal J, Hussey C, Tran T, McClure M, Frye C, Hattier T, Phelps R, Haugen-Strano A, Katcher H, Yakumo K, Gholami Z, Shaffer D, Stone S, Bayer S, Wray C, Bogden R, Dayananth P, Ward J, Tonin P, Narod S, Bristow PK, Norris FH, Helvering L, Morrison P, Rosteck P, Lai M, Barret JC, Lewis C,

- Neuhausen S, Cannon-Albright L, Goldgar D, Wiseman R, Kamb A, Skolnick MH. A strong candidate for the breast and ovarian cancer susceptibility gene *BRCA1*. *Science* 1994;**266**:66-71.
- Wooster R, Bignel G, Lancaster J, Swift S, Seal S, Mangion J, Collins N. Identification of the breast cancer susceptibility gene *BRCA2*. *Nature* 1995;**378**:789-92.
- Ford D, Easton DF, Peto J. Estimates of the gene frequency of *BRCA1* and its contribution to breast and ovarian cancer incidence. *Am J Hum Genet* 1995;**57**:1457-62.
- Antoniou AC, Pharoah PD, McMullan G, Day NE, Ponder BA, Easton D. Evidence for further breast cancer susceptibility genes in addition to *BRCA1* and *BRCA2* in a population based study. *Genet Epidemiol* 2001;**21**:1-18.
- Hartge P, Struewing JP, Wacholder S, Brody LC, Tucker MA. The prevalence of common *BRCA1* and *BRCA2* mutations among Ashkenazi Jews. *Am J Hum Genet* 1999;**64**:963-70.
- Gershoni-Baruch R, Dagan E, Kepten I, Fried G. Co-segregation of *BRCA1* 185delAG mutation and *BRCA2* 6174delT in one single family. *Eur J Cancer* 1997;**33**:2283-4.
- Ramus SJ, Friedman LS, Gayter SA, Ponder BAJ. A breast ovarian cancer patient with germline mutations in both *BRCA1* and *BRCA2*. *Nat Genet* 1997;**15**:14-15.
- Friedman E, Bar-Sade Bruchim R, Kruglikova A, Risel S, Levy-Lahad E, Halle D, Bar-On E, Gershoni-Baruch R, Dagan E, Kepten I, Perez T, Lerer I, Wienberg N, Shushan A, Aveliovich AD. Double heterozygotes for the Ashkenazi founder mutations in *BRCA1* and *BRCA2* genes. *Am J Hum Genet* 1998;**63**:1224-27.
- Tsongalis GJ, Linfert DR, Johnson RC, Ackroyd R, Berman MM, Ricci A Jr. Double heterozygosity for mutations in the *BRCA1* and *BRCA2* genes in a breast cancer patient. *Arch Pathol Lab Med* 1998;**122**:548-50.
- Lies A, Rehal P, Vesprini D, Jack E, Abrahamson J, Narod SA. A breast cancer patient of Scottish descent with germline mutations in *BRCA1* and *BRCA2*. *Am J Hum Genet* 1998;**62**:1543-4.
- Stoppa-Lyonnet D, Fricker JP, Essioux L, Pages S, Limacher JM, Sobol H, Laurent-Puig P, Thomas G. Segregation of two *BRCA1* mutations in a single family. *Am J Hum Genet* 1996;**59**:479-81.
- Randall TC, Bell KA, Rebase BA, Rubin SC, Boyd J. Germline mutations of the *BRCA1* and *BRCA2* genes in a breast and ovarian cancer patient. *Gynecol Oncol* 1998;**70**:432-4.
- Loader S, Rowley PT. Deleterious mutations of both *BRCA1* and *BRCA2* in three siblings. *Genet Test* 1998;**2**:75-7.
- Ganguly A, Citron M, Godmilow L, Ahrens M, Ganguly T. Caucasian family with two independent mutations: 2594 delC in *BRCA1* and 5392 delAG in *BRCA2* gene. *Am J Med Genet* 2001;**110**:46-52.
- De la Hoya M, Díaz-Rubio E, Caldes T. Denaturation gradient gel electrophoresis-based analysis of loss of heterozygosity distinguishes nonobvious, deleterious *BRCA1* variants from nonpathogenic polymorphisms. *Clin Chem* 1999;**45**:2028-30.
- Chapman MS, Verma IM. Transcriptional activation by *BRCA1*. *Nature* 1996;**382**:678-9.
- Vallo-Christersson J, Cayan C, Haraldsson L, Loman N, Bergthorsson JT, Brondum-Nielsen K, Gerdes AM, Moller P, Kristofferson U, Olsson H, Borg A, Monteiro AN. Functional analysis of *BRCA1* C-terminal missense mutations identified in breast and ovarian cancer families. *Hum Mol Genet* 2001;**10**:353-60.
- De la Hoya M, Pérez-Segura, Van Orsow N, Díaz-Rubio E, Caldes T. Spanish family study on hereditary breast and/or ovarian cancer: analysis of the *BRCA1* gene. *Int J Cancer* 2001;**91**:137-40.