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Germline *BRCA2* mutations in men with breast cancer

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Summary Breast cancer in men is rare and is clearly due in some cases to an inherited predisposition. A total of 28 male breast cancer patients were tested for *BRCA2* mutations; two frameshifts and one putative missense mutation were identified. One of the frameshifts was detected in the same position as a mutation estimated to be responsible for 40% of all male breast cancer cases in Iceland.

Keywords: *BRCA2*; male breast cancer; mutation

It is estimated that 5–10% of female breast cancer cases are due to inheritance of autosomal dominant susceptibility genes (Claus et al, 1991). Two such genes, *BRCA1* and *BRCA2*, localized to 17q and 13q12–13 respectively, have recently been identified (Miki et al, 1994; Wooster et al, 1995). Germline mutations in either of the two genes confers an 80–90% lifetime risk of breast cancer in women and an increased risk of ovarian cancer (Wooster et al, 1994). *BRCA1* is estimated to account for approximately one-half of all highly penetrant dominant breast cancer families and the majority of families with cases of female breast and ovarian cancer (Easton et al, 1993) but, according to both linkage and mutation studies, does not account for a significant proportion of families with cases of male breast cancer (Stratton et al, 1994; Strewing et al, 1995*a*). Unlike *BRCA1*, a considerable number of *BRCA2* mutations have been reported in heritable cases of male breast cancer (Wooster et al, 1995; Couch et al, 1996; Phelan et al, 1996; Tavtigian et al, 1996; Thorlacius et al, 1996; Friedman et al, 1997).

To determine the frequency of *BRCA2* germline mutations in men with breast cancer, we have screened DNA from 26 affected men plus two women with early-onset breast cancer who had an affected male relative.

MATERIALS AND METHODS

DNA samples

Blood samples were drawn from breast cancer-affected individuals from a case–control study of male breast cancer conducted in the Yorkshire Trent and North-West regions of the UK. Genomic DNA was extracted from blood using standard procedures. DNA samples were available from 26 male patients and two affected female patients who had male relatives with breast cancer.

BRCA1 185delAG mutation screen

The DNA samples were tested for the 185delAG mutation in the *BRCA1* gene by polymerase chain reaction (PCR) amplification. PCR fragments were run on a 6% non-denaturing acrylamide gel and visualized by UV transillumination following ethidium bromide staining.

Polymerase chain reaction (PCR), single - strand conformation polymorphism (SSCP) analysis and sequence analysis

PCR amplification of 200 to 300-bp DNA fragments covering the entire *BRCA2* coding region was performed with a set of 62 pairs of primers designed by Dr Richard Wooster (personal communication; primer sequences available on request). Six of the 62 *BRCA2* PCR products from each individual were pooled and run on 0.5 × MDE (Flowgen) gels in 0.6 × TBE. After electrophoresis, fragments were transferred to a nylon membrane (HybondN+, Amersham) and hybridized with radioactively end-labelled PCR primers. After hybridization, SSCP conformers were detected by autoradiography. All variant products identified were reamplified from genomic DNA, and sequenced using a 377 DNA sequencer (Applied Biosystems).

RESULTS

Before *BRCA2* screening, all 28 individuals were tested for 185delAG in *BRCA1*, as this mutation has been identified in three breast and breast/ovarian cancer families from the same geographical area (northern England). It should be noted that, although this mutation is common in Ashkenazi Jews (Struewing et al, 1995*b*), haplotype analysis suggests that none of the three families mentioned here are of Ashkenazi Jewish descent (D Kelsell, personal communication). All individuals in this study were found to be negative for this deletion (data not shown) and subsequently were screened for *BRCA2* mutations. *BRCA2* consists of 26 exons spanning 10 254 bp (Tavtigian et al, 1996). Three mutations were detected (Figure 1). DNA from individual MB1 has a single A insertion in a run of seven As in exon 24. In individual MB2,

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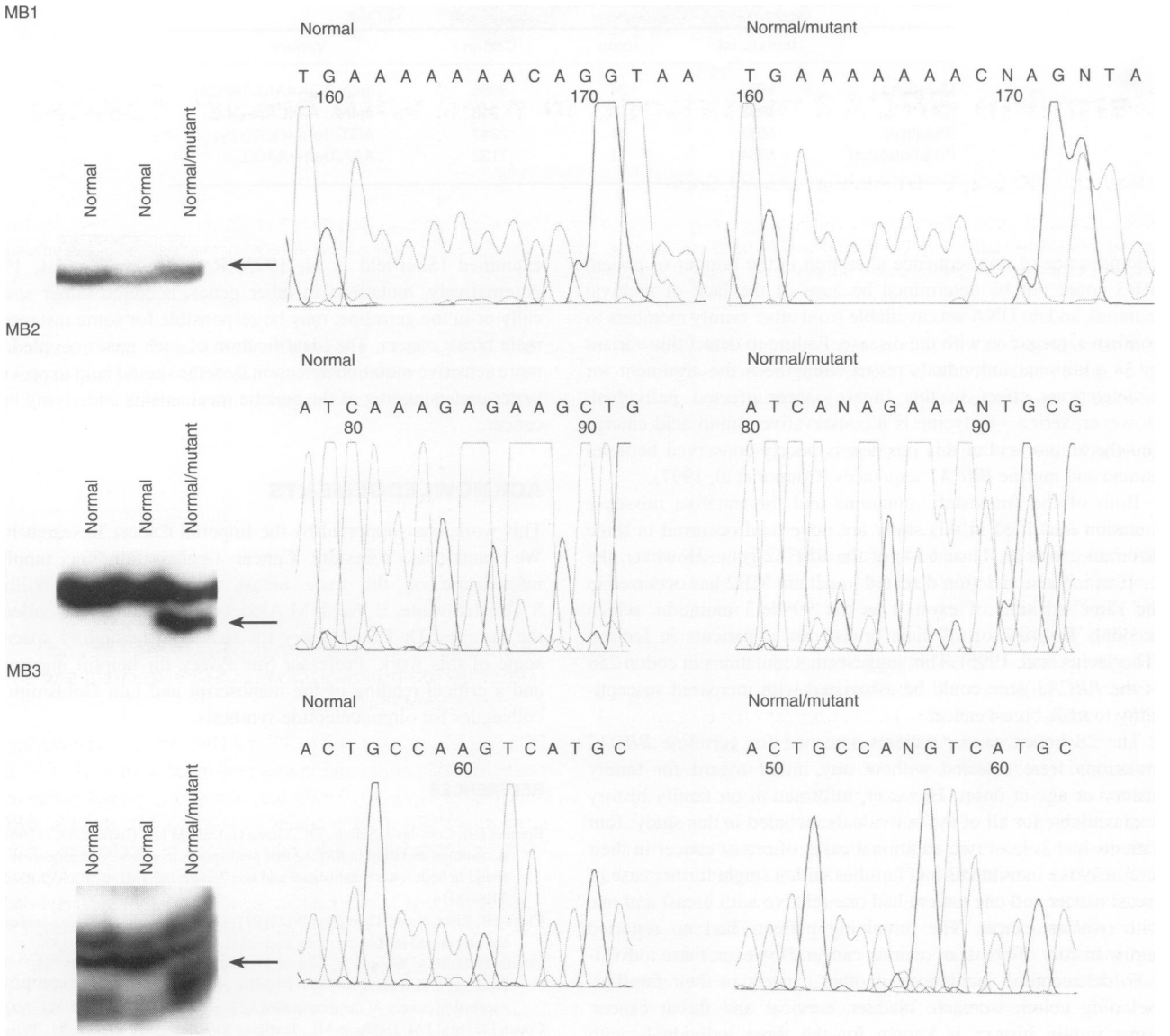

Figure 1 Germline *BRCA2* mutations in breast cancer-affected men. SSCP profiles are shown on the left, with band shifts due to the mutant alleles arrowed. Two normal profiles are shown in each case for comparison. Corresponding sequence electropherogram traces are shown on the right. Individuals MB1 and MB2 have frame-shift mutations due to an 'A' insertion and an 'AA' deletion respectively. Individual MB3 has an A→G transition.

a 2-bp amino acid deletion was detected in exon 9. These mutations cause frameshifts predicted to result in premature termination of translation. A putative missense mutation was detected in DNA from individual MB3. This consists of an A→G transition in codon 2247 of exon 11 causing the amino acid substitution serine to glycine. The functional significance of this amino acid change is unknown. To determine if this variant is simply a common polymorphism, 34 further individuals were tested for this transition by SSCP analysis and no positives were identified. None of these three *BRCA2* mutations had previously been reported (Wooster et al, 1995; Couch et al, 1996; Foster et al, 1996; Goggins et al, 1996; Lancaster et al, 1996; Miki et al, 1996; Neuhausen et al, 1996; Phelan et al, 1996; Takahashi et al, 1996; Tavtigian et al, 1996; Teng et al, 1996; Thorlacius et al, 1996; Weber et al, 1996; Gayther et al, 1997; the BIC database). No further frameshift or missense mutations were identified; however, a polymorphic SSCP conformer was detected in 12 of the 28 individuals. Direct sequencing characterized it as a silent A→G base substitution in codon 1132 of exon 11. This polymorphism has been reported previously (Phelan et al, 1996). These sequence variants are summarized in Table 1.

DISCUSSION

In this study, four variant conformers were identified, one of which is clearly a polymorphism. Of the remaining alterations detected, two cause a frameshift predicted to result in a truncated protein. The third sequence variant, consisting of an A→G transition in codon 2247 of exon 11, causes the amino acid substitution serine to glycine, which is of unknown functional significance.

Table 1 Germline *BRCA2* mutations in men with breast cancers

	Individual	Exon	Codon	Variant
Mutations	MB1	24	3085	insA: TGAAAAAAA^CA
	MB2	9	258	delAA: AATCAaaGAG
(Putative)	MB3	11	2247	AGT(Ser)→GGT(Gly)
Polymorphism	MB4	11	1132	AAA(Lys)→AAG(Lys)

The presence of this sequence alteration in the tumour of patient MB3 could not be determined because of the lack of archival material, and no DNA was available from other family members to confirm segregation with the disease. Failure to detect this variant in 34 additional individuals tested strengthens the argument for a deleterious effect in this breast cancer-affected individual. However, serine → glycine is a conservative amino acid change and the amino acid at this position is poorly conserved between human and murine *BRCA2* sequences (Connor et al, 1997).

Both of the frameshift mutations and the putative missense mutation identified in this study are novel and occurred in three different exons (9, 11 and 24) of the *BRCA2* gene. However, the 2-bp amino acid deletion detected in patient MB2 has occurred in the same position of exon 9 as the 999del5 mutation, which accounts for 40% of all male breast cancer patients in Iceland (Thorlacius et al, 1996). This suggests that mutations in codon 258 of the *BRCA2* gene could be associated with increased susceptibility to male breast cancer.

The 28 breast cancer patients screened for germline *BRCA2* mutations were selected without any initial regard for family history or age at onset. However, information on family history was available for all of the individuals included in this study: four patients had at least two additional cases of breast cancer in their families, five individuals had families with a single further case of breast cancer and one patient had one relative with breast and one with ovarian cancer. The remaining patients had no reported family history of breast or ovarian cancer. However, these individuals had recorded incidences of other cancers in their families, including colon, stomach, bladder, cervical and throat cancer. Some family history is known for the three individuals with *BRCA2* mutations. Individual MB2 developed breast cancer at the age of 68 and has two male and two female affected relatives. Patients MB1 and MB3, with an age of incidence of 65 and 64 years respectively, have no known family history of breast or ovarian cancer. However, other malignancies have been reported in these two families, including sarcoma, cervical and stomach cancer. Our data, together with previous reports, suggest that *BRCA2* could be involved in other forms of cancer in addition to breast cancer (Goggins et al, 1996; Berman et al, 1996; Couch et al, 1996; Phelan et al, 1996; Thorlacius et al, 1996). To investigate this possibility, mutation analyses on tumour samples from other malignancies in families with germlime *BRCA2* mutations are required.

The frequency of *BRCA2* mutations detected (2 or 3 out of 28, or 7–11%) is in good agreement with a previous study (Couch et al, 1996). The low number of mutations identified could be explained by the existence of non-coding mutations that are not covered by this approach. Furthermore, some mutations may remain undetected by SSCP. Although the sensitivity of this method is high, it depends largely on the electrophoretic conditions, the gel concentration and the size of the DNA fragments examined (Sheffield et al, 1993; Ravnik-Glavac et al, 1994). Alternatively, mutations in other genes, acquired either somatically or in the germline, may be responsible for some instances of male breast cancer. The identification of such genes coupled with more sensitive mutation detection systems should help to provide a better understanding of the genetic mechanisms underlying breast cancer.

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