Pergamon

European Journal of Cancer Vol. 33, No. 4, pp. 683–686, 1997

Printed in Great Britain
0959-8049/97 $17.00 + 0.00

PII: S0959-8049(96)00499-6

Short Communication

BRCA1 Mutations Found in Archived Early Onset Breast Tumours

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Inherited mutations in the *BRCA1* gene are thought to account for approximately 5% of breast cancers in women under the age of 45 years. In order to determine whether mutations could be found at the expected frequency, 60% of the protein coding region of *BRCA1* was screened in 75 archived early-onset breast tumours, taken from women under 45 years of age. Two of the 75 tumours (2.7%) had detectable mutations, in close agreement to that predicted. Since *BRCA1* mutations found in breast tumours are invariably germline, two immediate consequences are apparent. Firstly, family members of affected patients are likely to carry mutations as well, and should be considered for *BRCA1* screening; and secondly, persons harbouring a germline *BRCA1* mutation should be examined frequently and indefinitely for new primary tumours in remaining breast tissue.

Key words: breast cancer, tumour suppressor genes, genetic screening, neoplasm DNA, PCR

Eur J Cancer, Vol. 33, No. 4, pp. 683–686, 1997

INTRODUCTION

THE MOST common cancer among occidental women is breast cancer, affecting approximately 1 in 8 women [1]. The strongest risk factor is a family history of the disease and recently a breast cancer susceptibility gene, *BRCA1*, has been isolated [2]. Women with mutations in *BRCA1* have a 95% risk of breast/ovarian cancer by the age of 70 years [3]. *BRCA1* mutations are predicted to account for approximately 5% of breast tumours that develop under the age of 45 years [4], a prediction that can be tested by analysing the *BRCA1* gene in such tumours.

The *BRCA1* gene encodes a large protein of 1863 amino acids, and mutations predisposing to disease have been identified throughout the gene, making mutation detection tedious by standard techniques. Fortunately, approximately 60% of *BRCA1* mutations are found in exon 11 [5], which encodes 60% of the protein, and 86% of the mutations reported to date result in a truncated protein product due to a frameshift or a nonsense mutation [5]. Hence, by screening for truncated protein products encoded by exon 11, approximately 52% of all mutations in *BRCA1* should be detected. The protein truncation test (PTT) lends itself to *BRCA1* screening as truncating mutations in the coding sequence can be rapidly identified. Overlapping segments of continuous coding sequence (that is, sequence contained within a single exon) can be PCR amplified directly from genomic DNA. The PCR products are used in a coupled transcription/translation reaction and the size of the protein products are determined by denaturing gel electrophoresis. Although the PTT has previously been used to detect mutations in the *BRCA1* gene using genomic DNA as template for exon 11 [6], it cannot be used efficiently to screen the numerous smaller exons of *BRCA1* using genomic DNA as template. However, by screening the 60% of *BRCA1* encoded by exon 11 from the 75 patients, a large amount of coding sequence (257 025 base pairs) could be examined to determine if archived breast tumours have the predicted frequency of *BRCA1* mutations.

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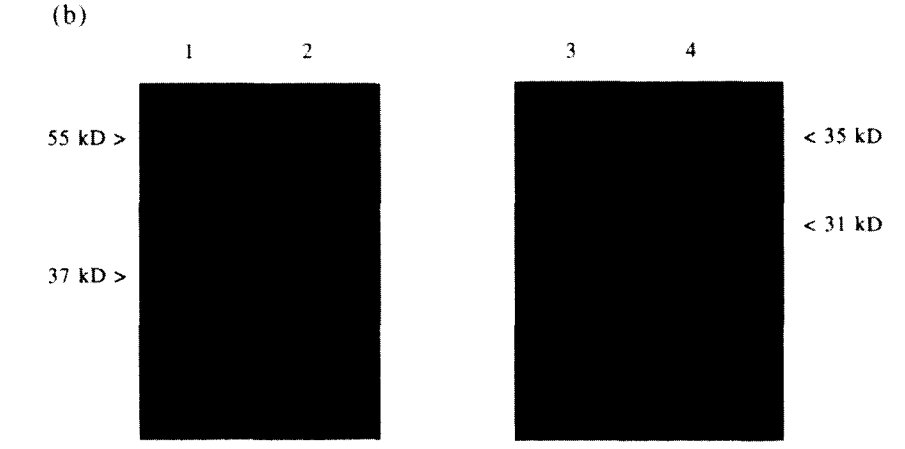
Received 10 Aug. 1996; revised 10 Sep. 1996; accepted 1 Oct. 1996.

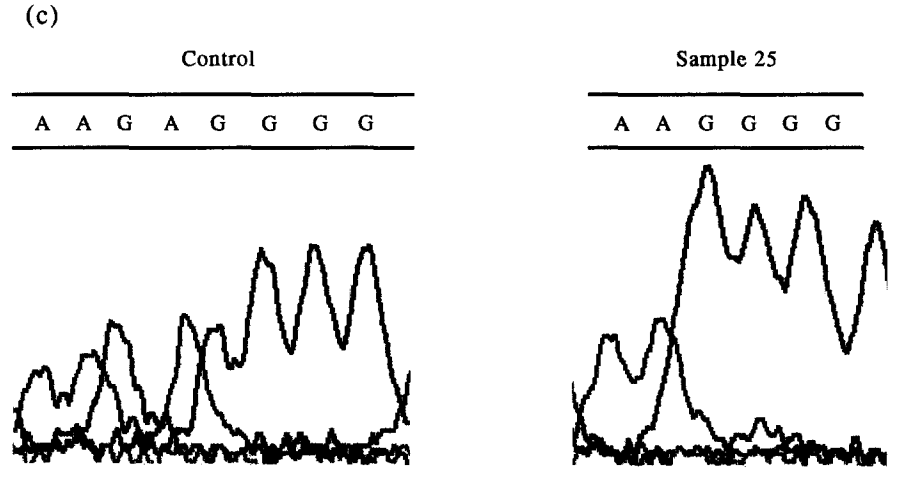
MATERIALS AND METHODS

Genomic DNA was isolated from 75 breast tumours taken from patients under the age of 45 years (median 38.8, range 22.9–44.8) who underwent surgical procedures for removal of tumour at various clinics in Switzerland. The

(a)

11a5' T7–GCTTGTGAATTTTCTGAGAC (792–811)
11a3' TCATCACTTGACCATTCTGCTCC (1736–1758)
11b5' T7–TTTGGGAAAACCTATCGGAA (1500–1519)
11b3' CGTTGCCTCTGAACTGAGATGATAG (2979–3003)
11c5' T7–CGCCAGTCATTTGCTCCGTTTTC (2715–2737)
11c3' AAGATACTAGTTTTGCTGAA (3601–3620)





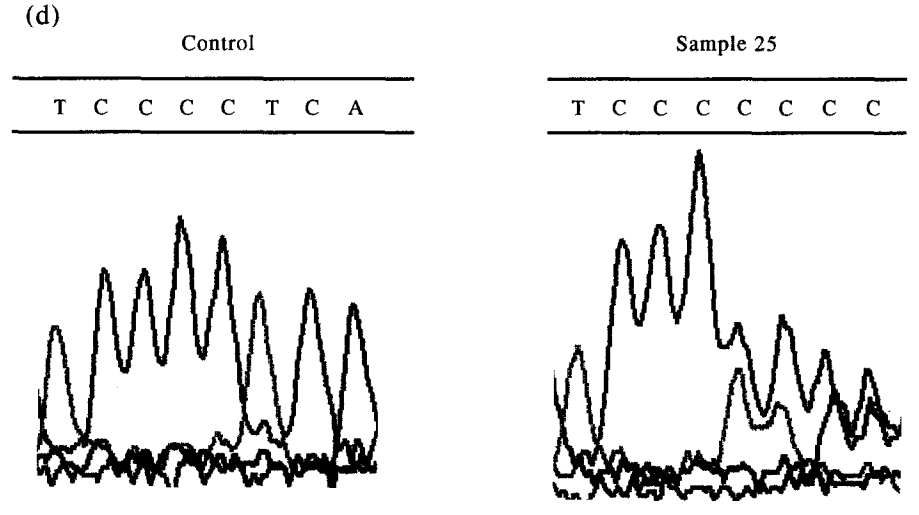


Figure 1. (a) Oligonucleotide primers used for PCR and sequencing. T7 = the T7 promoter and Kozak initiation sequence GGATCCTAATACGACTCACTATAGGGAGACCACCATG. The numbers adjacent to each sequence refer to its position within *BRCA1* as given in Genbank accession number U14680. The 3′ primers are the reverse compliments of the *BRCA1* sequence. (b) PTT of *BRCA1* sequences amplified from archived tumours. Segment 11c from a control (lane 1) and sample 25 (lane 2). Segment 11a from a control (lane 3) and sample 48 (lane 4). (c) Sequence showing the deletion of adenine at position 3731 in a control and sample 25 and (d) the deletion of cytosine at position 1611 in a control and sample 48. The ambiguous sequence was observed after the cytosine deletion for sample 48 immediately after the deletion, whereas it only appeared after five base pairs into the sequence for sample 25 due to there being a contiguous stretch of 5 Gs (data not shown).

tumours were initially shock-frozen in liquid nitrogen and were confirmed to be primary breast tumours by histological inspection. Samples had been stored at −70°C for as long as 3 years. The samples were irreversibly randomised by removing all identification from the sample tubes before DNA extraction, since unfortunately informed consent had not been obtained when the tissues were archived. A similar problem was encountered by Struewing and associates who received permission to screen archived DNA for the *BRCA1* 185delAG mutation without informed consent because the study was made anonymous by stripping the samples of all individual identifiers [17]. Tissue (100–500 mg) was homogenised with a tissue grinder (Janke and Kunkel, Staufen, Germany) in 5 ml of a buffer containing 10 mM Tris pH 8.0, 0.5% sodium dodecyl sulphate (SDS), 20 μg/ml RNase and 100 μg/ml proteinase K. After shaking overnight at 37°C, the DNA was purified by phenol/chloroform extraction and ethanol precipitation. Exon 11 was divided into three overlapping segments of coding sequence (11a, 11b, 11c) and the segments were polymerase chain reaction (PCR) amplified from genomic DNA using the primers given in Figure 1a. One hundred nanograms of DNA were used per 25 μl PCR reaction under standard conditions. Cycling parameters were 94°C 34 sec, 55°C 30, 7°C 2 min, 35 cycles. One microlitre of PCR product was used in a 5 μl coupled transcription/translation reaction and analysed according to the manufacturer's protocol (Promega, Madison, Wisconsin, U.S.A.). DNA sequencing reactions were performed with Thermosequenase (Amersham International, U.K.) using PCR products as template which were analysed in a LICOR model 4000L automated sequencer (LiCoR, Lincoln, Nebraska, U.S.A.).

RESULTS

We detected 2 *BRCA1* mutations in the 75 tumour samples that were screened. Sample 25 had a 344 amino acid (37 kDa) segment 11c protein product (lane 2, Figure 1b), 156 amino acids smaller than the 500 residue full-length protein (lane 1, Figure 1b). The truncation was due to a frameshift caused by the deletion of adenine 3731 (Figure 1c). Sample 48 had a truncated segment 11a protein of 279 amino acids (30 kDa) (lane 4, Figure 1b), 43 residues smaller than the 322 amino acid full-length product (Figure 1c, lane 3). The truncation was due to the deletion of cytosine 1611 (Figure 1d). In both cases, the full-length protein was detected along with the truncated product of the size predicted from the DNA sequence, but at less than the stoichiometric levels expected of heterozygous cells, suggesting some loss of the wild-type *BRCA1* allele in these two samples. This notion is supported by the finding that when chromosome loss at the *BRCA1* locus is observed in tumour tissue from *BRCA1* carriers, it is invariably the wild type *BRCA1* allele that is missing [8].

DISCUSSION

The *BRCA1* carrier frequency is estimated to be 1 in 833 in the general population, and *BRCA1* is predicted to account for approximately 5% of the breast cancer cases under the age of 45 years [4]. Since only approximately 52% of *BRCA1* mutations are detectable by PTT of exon 11, only 2.6% of the tumours we tested would be expected to produce truncated proteins, and in fact this corresponds closely with our observed frequency of 2 in 75 (2.7%). Recently, population-based *BRCA1* screening of blood samples from women with early-onset breast cancer revealed a similar mutation frequency—2 of 30 (6.7%) patients under the age of 30 years had truncating mutations in exon 11 [9], while 2 of 80 (2.5%) women under 35 years of age had mutations in exon 11, generating prematurely terminated proteins [10].

Since mutations found in breast tumours are invariably germline [11], patients found to have a *BRCA1* mutation in their tumour tissue will almost certainly harbour the mutation in their healthy breast tissue. Currently, the non-existence of sporadic *BRCA1* tumours cannot be ruled out so therefore, any mutation detected in breast tumour tissue should be verified in normal tissue obtained from the patient. Normal breast tissue surrounding the tumour should not be used for testing as it remains difficult to determine when *BRCA1* mutations may occur during disease development. At the time of surgery, or shortly thereafter, a blood sample should be taken and used for this analysis.

If such a patient has undergone lumpectomy or monolateral mastectomy, the remaining breast tissue is still at high risk of developing a second primary tumour and should be monitored indefinitely. Since the family members of patients with known *BRCA1* mutations are at risk of harbouring the same mutation, they should be considered for *BRCA1* screening. Presymptomatic carriers detected in this way may benefit from increased surveillance and early intervention.

The mutation in sample 48 (1611delC) was also found independently in another *BRCA1* study we performed on DNA and cDNA from blood samples of Swiss breast cancer patients fulfilling rigorous criteria for selection [12], and most likely came from the same patient or a first- or second-degree relative. This finding suggests that *BRCA1* screening of age-restricted tumour samples may be useful for screening patients in the absence of any strong family history especially if women under 40 years of age are screened. It appears, even from this limited study, that family history remains the single most important factor in determining the likelihood that a woman harbours a *BRCA1* germline mutation. In order to give patients and their families the opportunity to benefit from genetic diagnosis, we recommend that informed consent for genetic testing be obtained at the time tumour tissues are collected.

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Acknowledgements—This work was supported by grants AKT90 and AKT463 from the Swiss Cancer League and by the Swiss National Fund No. 3200-042558.94