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MUTATION IN BRIEF

Identification of a Novel Splice-Site Mutation of the *BRCA1* Gene in Two Breast Cancer Families: Screening Reveals Low Frequency in Icelandic Breast Cancer Patients

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INTRODUCTION

Germ line transmission of mutated BRCA1 alleles is believed to explain approximately half of the breast cancer incidence in Western countries caused by inherited predisposition. Positive linkage to the BRCA1 region is also evident in the majority of families affected by the breast-ovary cancer syndrome (Easton et al., 1993). Since the BRCA1 gene was cloned in 1994 (Miki et al.), over 100 germ line mutations have been described (Breast Cancer Information Core Database http://www.nchgr.nih.gov//Intramural_research/Lab_transfer/Bic/). Most of the mutations identified so far lead to premature termination of protein translation, e.g., small deletions and insertions creating frameshifts or point mutations that produce stop codons (Shattuck-Eidens et al., 1995).

Analysis of seven high-risk breast cancer families in Iceland previously identified one with high probability of linkage to the BRCA1 region (Arason et al., 1993). Additionally, the results of genetic analyses involving a group of sisters affected with breast cancer suggested only a minor contribution of BRCA1 to hereditary breast cancer in Iceland. A single sister pair of 42 in this series was suggestive of BRCA1 linkage. Further inspection of the shared haplotype in this sister pair suggested common ancestry with the BRCA1 linked breast cancer family mentioned above (Arason et al., sub.).

The purpose of this study was to characterise the Icelandic BRCA1 allele segregating in these breast cancer families and to estimate its significance in a population-based screening effort.

MATERIALS AND METHODS

Subjects

One Icelandic breast cancer family was previously identified with 17 q-linkage (FAM1), supported by a maximum LOD score of +2.34 (Arason et al., 1993). Clinical features of this family are described elsewhere (Arason et al., 1993). The coding region of BRCA1 was sequenced using DNA from normal tissue from affected family members.

A group of 55 sister pairs affected with breast cancer was identified in a population-based cancer registry. Of these, 42 were previously analysed for linkage to the BRCA1 and BRCA2 regions (Arason et al., sub.). All individuals in the group were diagnosed with breast cancer before the age of 60; 103 individuals from this series were screened for the germline BRCA1 mutation discovered in FAM1.

Finally, a group of 504 unselected breast cancer patients was screened for the FAM1-mutation using SSCP analyses of tumour DNA. This assembly represents ~60% of all Icelandic patients diagnosed with breast cancer in the period 1989–1994.

Pedigree data was obtained from the Icelandic Genetic Council. Verification of cancer incidence in

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pedigrees was obtained with aid of the Icelandic Cancer registry.

PCR

Thermal cycling was carried out in 30 µl volumes containing: 0.3 U Dynazyme polymerase, the reaction buffer provided with Dynazyme, 100 µM each dNTP, 30 ng genomic DNA, 50 ng of each primer. Cycling conditions were 35 cycles of 95°C, 30s, 55°C, 30s, and 72°C for 1 min.

Solid Phase Sequencing

The primers for PCR and sequencing of genomic DNA by the solid-phase method were obtained from Dr. Nigel Spurr at ICRF (Clare Hall Laboratories, UK). All reverse primers were biotinylated. The PCR products were immobilised on solid support using streptavidin beads (M-280/Dynal) and denatured with alkaline. The DNA template was sequenced using sequenase and α^{33}dATP (Amersham/USB). Amplification for the sequencing and SSCP analyses of exon 17 was done using the following primers: Forward 5´-TGCTCGTGTACAAGTTTGC-3´; Reverse, 5´-TCGCCTCATGTGGTTTTA-3´.

SSCP

The 146 bp product was denatured in a formamide buffer, loaded onto acrylamide gel (MDE solution/FMC bioproducts), and electrophoresed at 6 W for 6 hr. The PCR products were transferred to a nylon membrane (Hybond N+, Amersham) and fixed in a baking oven at 80°C for 3 hr. Hybridisation and visualisation of the PCR products were as described by Barkardottir et al. (1995), using nonradioactive procedure (ECL system, Amersham/USB).

RESULTS AND DISCUSSION

Sequence analyses identified a germ line mutation in FAM1 that consistently segregated with the disease haplotype. Also, tumours from carriers exhibiting wild-type allele loss displayed increased intensity of the mutant band in the sequencing reactions (Figure 1). The mutation is a base substitution in nucleotide 5193 resulting in a shift in codon 1692 from GAT to AAT. The transition predicts an amino acid change [Asp → Asn], but since it affects a conserved guanine of the splice donor consensus, it may be expected to have more drastic effects. A probable consequence of this change would be total omission of exon 17 in the transcript (exon-skipping). Activation of a cryptic 5´ splice site is the second most common outcome of mutational events of this type (Krawczak et al., 1992). Unfortunately, the mRNA could not be assessed for splice defects since it was not available from mutation carriers.

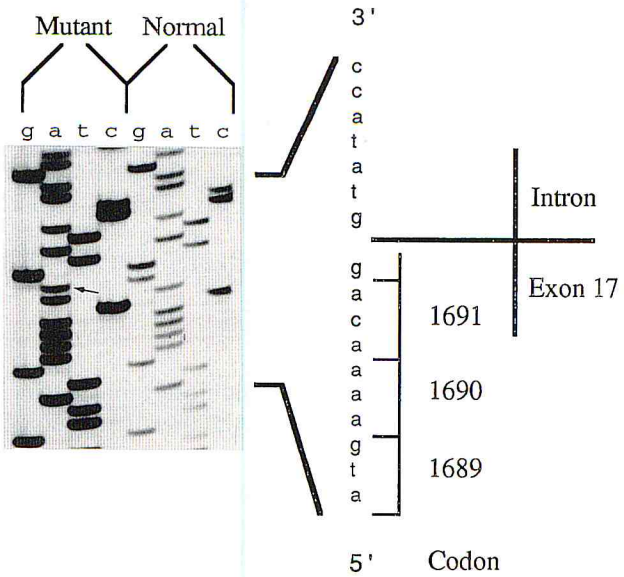

FIGURE 1. **Solid-phase sequence analysis of the region around nucleotide 5193 in the BRCA1 gene. Samples are from normal individual and ovary tumour of a mutation carrier. An arrow points to the aberrant A in position 5193 in the tumour. The wild-type G in this position is completely lost from the tumour sequence as a result of chromosome deletion.**

Mutation screening using SSCP analyses on the 42 sister pairs revealed one pair carrying the mutation. This family [SP17] was previously identified as the only BRCA1 candidate in the series, on basis of linkage/LOH analyses of tumours (Arason et al., sub.). The mutation is present in two affected sisters in family SP17. A third sister that also was affected with breast cancer is not a mutation carrier. The BRCA1 haplotype shared by the two mutation carriers in this family was identical to the disease haplotype observed in FAM1. Breast or ovarian cancer is not noticeable in relatives of the affected sisters in family SP17.

Screening of the 504 breast tumours with SSCP (Fig. 2) revealed one positive sample from a patient diagnosed with breast cancer at age 71. This patient had a mother affected with ovarian cancer, but further inspections of her relatives revealed low incidence of breast and ovary cancer.

From this data, it can be deduced that <100 Icelanders (the population counts 267,000 individuals) are carriers of the BRCA1 defect described here. This estimation was based on the assumption that mutation penetrance is high, in accordance with Claus et al. (1991). Despite low frequency estimate, this mu-

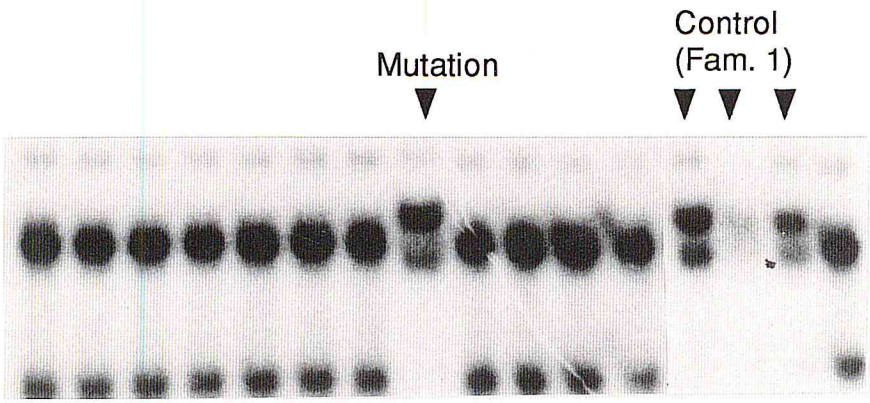

FIGURE 2. **SSCP analysis of BRCA1 exon 17. Band patterns from sporadic tumours and mutation carriers from FAM 1.**

tation may represent the most common cause of BRCA1-linked breast cancers in the Icelandic population. This is concluded from the results of linkage studies on large breast cancer families and affected sib-pairs. However, we cannot exclude altogether the possibility of other low frequency BRCA1 defects, or the existence of low penetrance alleles in Iceland.

The majority of hereditary breast cancers in Iceland is explained by defects in the recently identified BRCA2 gene (Wooster et al., 1995; Gudmundsson et al., 1996). This is concluded from linkage studies that confirmed positive BRCA2 linkage in five large breast cancer families of seven (Gudmundsson et al., 1996). An identical BRCA2 haplotype segregates in these five families indicating a common ancestral origin. The same BRCA2 haplotype was also apparent in 14 sister pairs with positive BRCA2 linkage of the 42 pairs assessed by Arason et al. (sub.). The predominance of a common BRCA2 haplotype in familial breast cancer patients as well as low incidence of BRCA1 linkage in Iceland may be explained by founder effect and genetic drift. The Icelandic population formed about 1,000 years ago by Norse and Celtic settlers and is particularly prone to founder effect since the number of primary settlers was small.

Identification of this novel BRCA1 mutation adds to the growing body of information on BRCA1 in hereditary breast cancer and may have future implications for the families involved.

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