Advances in Brief

Different Tumor Types from BRCA2 Carriers Show Wild-Type Chromosome Deletions on 13q12–q13[1]

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Abstract

In this study we examined loss of heterozygosity (LOH) on chromosome 13q12–13 in 50 tumors from BRCA2 carriers in five families showing strong evidence of linkage to BRCA2. In addition to high frequency of LOH in female breast cancer, LOH was observed in tumors of the prostate, ovary, cervix, colon, male breast, and ureter. All detected losses involved the wild-type chromosome. These results suggest that BRCA2 is a tumor suppressor gene and may be involved in the tumorigenesis of several cancer types in addition to breast cancer.

Introduction

Segregation analyses have suggested that 5–10% of all breast cancer may be due to inheritance of genes with high penetrance (1). One of these breast cancer susceptibility genes, BRCA2, was recently localized by linkage analysis to chromosome 13q12–13. BRCA2 mutations may account for a substantial proportion of the high-risk breast cancer families (2). Most of the genes known to be of importance in the pathogenesis of human familial cancers are tumor suppressor genes (3). Tumor suppressor genes are thought to be inactivated by a "two-hit" mechanism, originally proposed by Knudson (4), to explain the tumorigenesis of retinoblastoma. In hereditary cancer the first hit would be a germline mutation in a specific cancer gene. The second hit would be a mutation in or loss of the second copy of that gene in the somatic cell. Recently reported results from LOH[3] studies on tumors from gene carriers have suggested that BRCA2 is a tumor suppressor gene (5). In this study, we examined LOH in tumors from members of five different families showing strong evidence of linkage to the BRCA2 locus.[4] Beside multiple cases of breast cancer in these families, there have been frequent occurrences of malignancy in other organs (Ref. 6; Table 1). To investigate the possible involvement of the BRCA2 gene in the development of different tumor types, all available tumor samples from the families were analyzed for LOH.

Materials and Methods

Cancer Patients from High-Risk Breast Cancer Families. In this study LOH analyses were performed on all available tumor samples from cancer patients of five different high-risk breast cancer families. All the families showed convincing evidence of linkage to the BRCA2 locus at chromosome 13q. The maximum Lod scores calculated for the families with markers at 13q

are as follows: family 2, 1.9 with D13S260; family 4, 1.8 with D13S267; family 5, 3.2 with D13S267; family 6, 2.3 with D13S267; and family 7, 1.06 with D13S267. Clinical characteristics of the families have been reported previously (6, 7). Information about affected organs and number of primary tumors in each family are summarized in Table 1.

Tissue Samples. Blood was used if available; otherwise normal tissue was obtained from formalin-fixed tissue embedded in paraffin. Tumor samples were in all cases derived from paraffin blocks. By using a microscope, either tumor cell-rich or normal cell-rich areas were marked on stained slides and the corresponding areas then cut out from unstained 20–30-μm thick sections.

LOH Analyses. Peripheral blood and archive material was processed as described (6, 8). DNA was amplified with PCR. The markers used were: D13S263, D13S219, D13S220, D13S267, D13S171, D13S260, afm238, and D13S217 (9). PCR reactions were carried out in a volume of 25 μl containing either 25 ng of DNA from blood or 1–2-μl aliquots of DNA prepared from archive material, 0.5 units of DynaZyme polymerase (Finnzymes Oy), and the 10× buffer supplied with the polymerase. Samples were subjected to 35 cycles of amplification, consisting of 40 s at 94°C, 40 s, at 55°C, and 30 s at 72°C, followed by a final extension for 10 min at 72°C. The PCR products were separated on 6.5% acrylamide sequencing gels. After contact blotting and probing with nonradioactive probes (7, 10), autoradiographic results were inspected visually. A deletion was scored if one allele was absent or considerably weaker.

Results and Discussion

Tumor samples were available from 50 of the 58 tumors occurring in cancer patients verified as BRCA2 carriers by haplotype analysis (Table 1). LOH was detected in 28 of 33 breast tumors. Three of the five breast tumors not showing LOH were from patients with bilateral breast cancer, and in all three patients the tumor in the opposite breast showed allelic loss. In addition to female breast cancer, seven prostate tumors, six ovarian, one colon, one cervical, one male breast, and one ureter tumor from BRCA2 carriers were analyzed for LOH. Of these 17 tumors, wild-type chromosome losses were detected in all except 1 ovary and 1 prostate tumor, which retained heterozygosity at all informative markers (Table 2). Examples of allele losses are shown in Fig. 1 A and B. Tumor samples for LOH studies from family members without suspected BRCA2 haplotype were available from three prostate, and two breast tumors. In all five tumors heterozygosity was retained at all informative markers tested.

One of the breast tumors occurring in a BRCA2 carrier showed partial deletion (Fig. 2), implying that BRCA2 is located telomeric to D13S260. Deletions seen in other tumors always extended beyond the chromosomal region of interest and did not provide information for further narrowing down the localization of the gene.

The most likely explanation of the high frequency of loss of the wild-type chromosome observed in the tumors of BRCA2 carriers is a strong selection of tumor cells with both alleles of the BRCA2 gene being mutated. It suggests that BRCA2 is a tumor suppressor gene. The reason why deletions at the BRCA2 locus were not found in all tumors analyzed from the BRCA2 carriers could be that the wild- type copy of the gene was inactivated by point mutations or

Table 1 *Affected organs and number of primary tumors in BRCA2 carriers from five high-risk breast cancer families showing linkage to the BRCA2 locus on chromosome 13q*

Site of primary tumor	Family no.				
	2	4[a]	5	6	7
Breast	6 (1)[b]	4 (1)	7	6 (4)	3 (2)
Bilateral breast	1	2		4	1
Breast and ovary		1	1	1	
Ovary			3		
Male breast				1	
Prostate	3 (1)	2 (3)	3		2 (2)
Colon				2 (2)	
Cervical			2 (1)		
Ureter		1			
Kidney		1			
Testis			1		

[a] In family 4, the kidney and the ureter tumors occurred in the same individual. This same individual was also diagnosed with breast cancer.

[b] Numbers in parentheses, number of tumors occurring in family members not carrying the suspected haplotype.

small internal deletions not detected by the markers used in the study. Other possible interpretations are genomic imprinting or that another tumor suppressor gene that is located close to the *BRCA2* is the target of LOH. Genomic imprinting can be ruled out because LOH observed in the 43 tumors of *BRCA2* carriers involved alleles inherited both from the mother and the father. Furthermore, LOH breakpoints between *RB1* and *BRCA2* seen in some of the samples exclude the tumor suppressor gene *RB1* from being the target of the deletion.

The results reported here, regarding LOH in breast cancer, are in line with those recently published by Collins *et al.* (5) who found that seven of eight breast tumors from *BRCA2* carriers showed allele loss on the wild-type chromosome. Data on LOH in other tumor types from *BRCA2* carriers have not been reported previously. The losses seen in the ovarian and male breast tumors support linkage data suggesting the involvement of *BRCA2* in the tumor development of these two cancer types (2).[4] Epidemiological studies have indicated a link between breast and prostate cancer. (11–13) In 4 of the 5 families analyzed here 16 males had developed prostate cancer (Table 1). Of these 16 cases, 10 were *BRCA2* carriers and 6 were noncarriers. The deletion frequency seen in the prostate tumors from *BRCA2* carriers was 6 of 7 and the losses always involved the wild-type alleles. The probability of obtaining these results if either chromosome was af-

Table 2 *Deletion frequency at the BRCA2 locus on chromosome 13q12–13 in tumors from BRCA2 carriers*

Tumor type	Deletions in tumors from BRCA2 carriers[a]
Breast cancer	28/33 (85%)
Prostate cancer	6/7 (86 %)
Ovarian cancer	5/6 (83 %)
Cervical cancer	1/1
Colon cancer	1/1
Male breast cancer	1/1
Ureter cancer	1/1

[a]The number of tumors manifesting deletion for at least three markers within the *BRCA2* region of the total number of tumors available for deletion analysis in each tumor type.
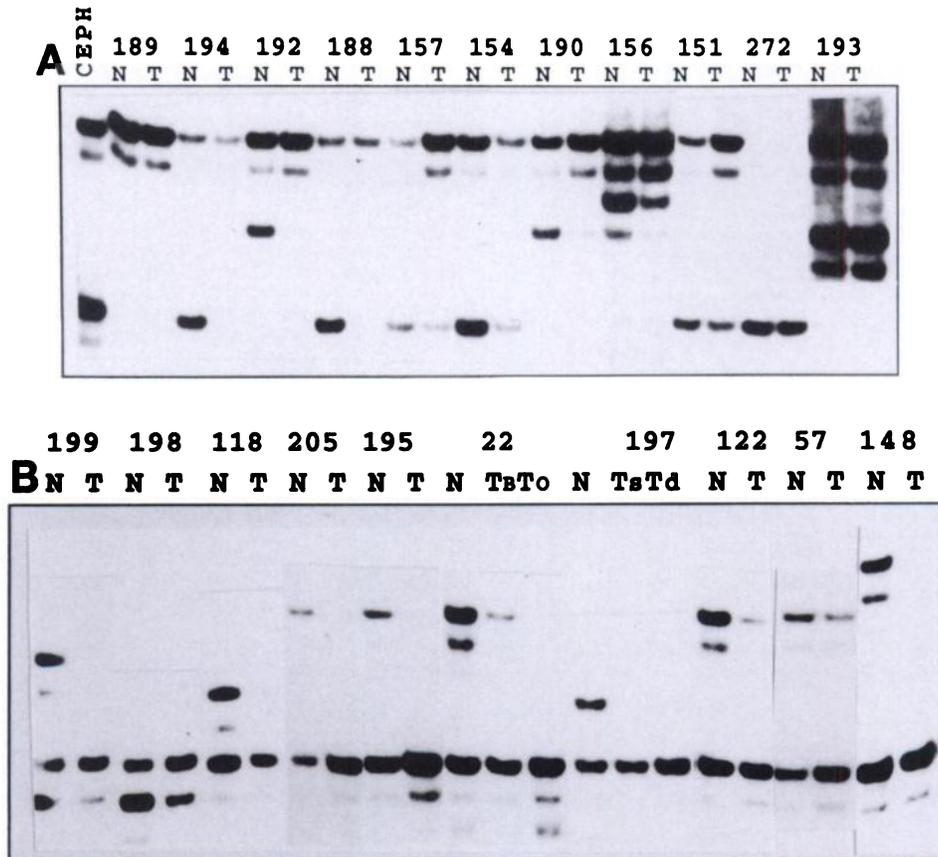

Fig. 1. *A*, LOH for marker *D13S267* in tumors from members of one of the families with incidence of prostate cancer in *BRCA2* carriers (family 2). Samples 188, 157, 154, and 272 are from patients with prostate cancer. Other samples are from breast cancer cases. Samples 272 and 193 are from individuals not sharing the affected haplotype. All samples from *BRCA2* carriers that were informative showed loss of the wild-type allele. The sample marked CEPH (derived from individual 134702 in CEPH family 1347) was used for allele size determination (9). *B*, autoradiographs showing allelic loss for marker *D13S267* in representative tumor samples from *BRCA2* carriers in families 4 and 5. Samples 199, 198, and 118 were from individuals with prostate cancer; samples 205 and 195 were from individuals with ovarian cancer; patient 22 had both breast (T_B) and ovarian (T_O) cancer; patient 197 had bilateral breast cancer (T_s and T_d, left and right breast, respectively); samples 122 and 148 were from individuals with breast cancer. Sample 57 is from a male breast cancer case. *N*, normal tissue; *T*, tumor.
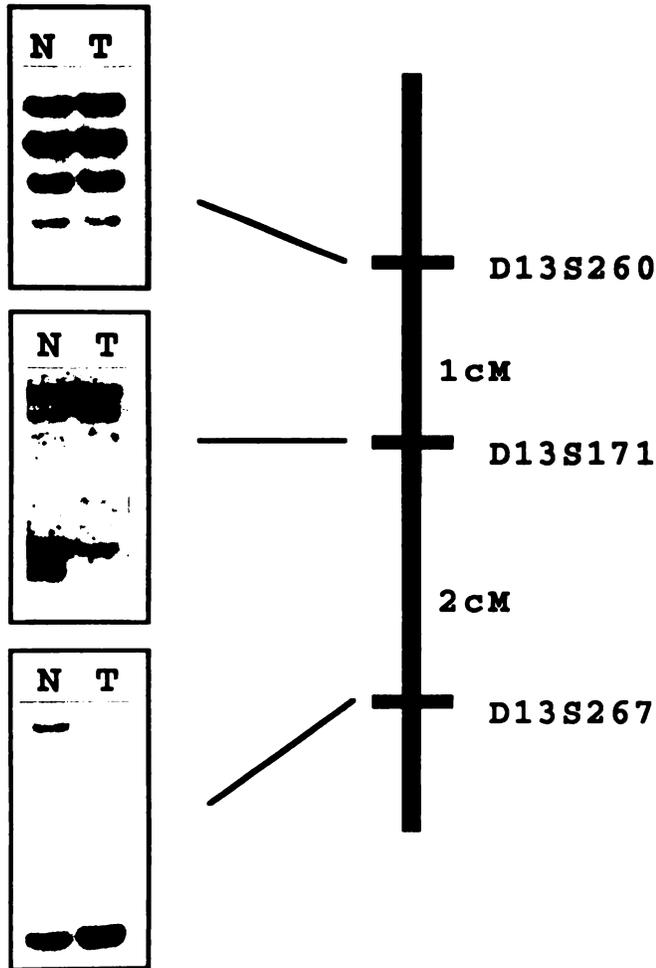
Fig. 2. The figure shows a part of chromosome 13q, relative position of the three markers, and the distance between them (9). The autoradiographs show LOH results for sample 216 where a partial deletion was found only to involve markers distal to *D13S260*. The markers *D13S267* and *D13S171* showed allelic loss, but heterozygosity was retained at *D13S260*. This implies that the target region of the deletion is telomeric to *D13S260*, thereby indicating that the *BRCA2* gene is located distal to that marker. Recombinant events described earlier localized the gene to 6-cM region between markers *D13S267* and *D13S289* (2). The data presented here shorten this region to 3 cM. *N*, normal tissue; *T*, tumor.

fected at random is 1 in 64. The losses of wild-type alleles seen in tumor of the prostate, colon, ureter, and cervix imply that *BRCA2* may be involved in the tumor development of these cancer types. *BRCA2* carriers were seen to develop kidney and testical cancers in addition to those included in this study (Table 1), but the LOH analyses of

these tumors were not possible because tumor material was not available. From the number of different cancers seen in *BRCA2* carriers and the consistent loss of the wild-type allele in the tumors, it appears that certain mutations in the *BRCA2* gene can influence cancer development in several different organs in addition to the breast.

Acknowledgments

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