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Original Paper

Identification of a New Commonly Deleted Region within a 2-cM Interval of Chromosome 11p11 in Breast Cancers

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Allelic loss has been observed on the short arm of chromosome 11 in a variety of human cancers. We have examined 184 breast cancers for allelic loss anywhere in chromosome 11p, using 15 well-spaced microsatellite markers. Allelic loss was observed in 86 cases (47%) and a new commonly deleted region 2-cM in length was identified at 11p11 between loci *D11S986* and *D11S1313*, in addition to a 12-cM region of a common deletion at 11p15.5. A significant association was found between allelic loss on 11p15.5 and LOH on 11p11 and the loss of progesterone receptors. © **1998 Elsevier Science Ltd. All rights reserved.**

Key words: breast cancer, loss of heterozygosity, tumour suppressor gene, chromosome 11p, progesterone receptor
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INTRODUCTION

BREAST CANCER is the most frequent neoplasm to occur among women in industrialised countries. Although 5–10% of breast cancer cases show familial clustering, most appear to be sporadic. Loss of heterozygosity (LOH) at specific chromosomal loci in tumours are considered to signal the presence of tumour suppressor genes in the affected regions. Frequent allelic losses detected on 1p, 3p, 6q, 13q, 16q, 17 and 18q in breast cancers [1–4] have suggested that a variety of tumour suppressor genes are involved in this type of cancer.

The 11p15.5 region around the c-H-ras-1 locus is frequently affected by LOH in breast cancers [5–11] and also in other neoplasms including lung cancers, Wilms' tumours, hepatoblastomas, adrenal adenomas, ovarian carcinomas, rhabdomyosarcomas, gliomas and gastric cancers [13–18]. Frequent LOH at 11q22-23 on the long arm of chromosome 11 in cancers of breast, ovary, colon, skin and uterine cervix suggest the presence of yet another tumour suppressor gene [10–12, 19–22].

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Although the 11p15.5 and 11q22-23 regions have been investigated intensively, genetic alterations in other parts of chromosome 11 have not been examined thoroughly. Therefore, we examined breast cancers for LOH throughout chromosome 11p, using 15 polymorphic microsatellite markers distributed on this chromosome arm and identified a new target region at 11p11.

MATERIALS AND METHODS

Samples and DNA preparation

Tumours and corresponding noncancerous tissues were obtained from 184 patients undergoing surgery for primary breast cancers at the Cancer Institute Hospital. Tissues were frozen immediately and stored at −80°C. DNAs were extracted from frozen tissues according to methods described previously [4].

LOH analysis

DNAs were examined for LOH using the 15 polymorphic microsatellite markers along 11p reported in the CEPH/Généthon linkage map [23]. The linear order of these markers is (tel) - *D11S922-D11S1318-D11S1338-D11S1349-D11S902-D11S1359-D11S929-D11S1324-D11S907-D11S1360-*

D11S905-D11S986-D11S1344-D11S1313-(cen)*-D11S1357* [23]. Microsatellite polymorphisms were amplified by the polymerase chain reaction (PCR) using 20 ng of genomic DNA, 30 mM of Tris-HCl (pH8.8), 50 mM of KCl, 2 mM of $MgCl_2$, 5 mM of 2-mercaptoethanol, 100 {micro} M of dNTPs, 1.6 pmol each of [γ-^{32}P]ATP-end-labelled primer and non-labelled primer and 0.25 units of Taq polymerase in a total volume of 10 µl. Cycle conditions were 94°C for 4 min, then 30 cycles of 94°C for 30 sec, 55–64°C for 30 sec and 72°C for 30 sec, with a final extension step of 5 min at 72°C in a Gene Amp PCR 9600 System (Perkin Elmer Cetus, Foster City, California, U.S.A.). PCR products were electrophoresed in 0.2 mm-thick denaturing 6% polyacrylamide gels containing 36% formamide and 8 M urea, at 2000 volts for 2–4 hours. Gels were transferred to filters, dried at 80°C and exposed to autoradiographic film at room temperature for 16–20 hours.

Definition of LOH

Signal intensities of polymorphic alleles were quantified by a Hoefer GS-300 scanning densitometer; peak areas corresponding to each signal were calculated by electric integration using the GS-370 elelctrophoresis data system (Hoefer Scientific Instruments, San Francisco, California, U.S.A.). The signal intensities of alleles of tumour-tissue DNAs were compared with those of normal tissue DNAs. We judged a reduction in signal intensity > 50% to be loss of heterozygosity. We distinguished LOH from chromosome multiplication by normalising each signal to the signal obtained when the same DNA was analysed with markers for loci on other chromosomes.

In formal deletion mapping, a common region of deletion is defined as a minimally overlapping region of consistent loss among involved tumours; determination of a deleted region in each tumour and the compilation of those data in multiple tumours leads to the determination of a common region of deletion.

Clinicopathologic parameters

The panel of parameters studied included the following: histological type, tumour size and infiltration, lymph node metastasis and hormone receptor status. Oestrogen receptor (ER) and progesterone receptor (PgR) were measured by radioreceptor assay according to a standard dextran-coated charcoal (DCC) method, using [^{125}I]-oestradiol as the labelled ligand on homogenates of fresh-frozen tissue (Otsuka Pharmaceutical, Tokushima, Japan). All samples containing > 5 fmol of ER or PgR per mg protein were considered receptor-positive. The chi-square test and Fisher's exact test were used for statistical analysis of the results. One-tailed *P* values of less than 0.05 were considered statistically significant.

RESULTS

LOH was detected in 86 (47%) of 184 breast tumours that were informative with at least one of the 15 microsatellite markers on the short arm of chromosome 11. The marker loci and their frequencies of LOH are listed in Table 1, in descending order from the telomere based on a CEPH/Généthon genetic map [23]. The frequencies of LOH at each locus ranged from 25–39%. Although a large portion of tumours with LOH exhibited extended deletions showing LOH at most of the informative markers, 20 tumours showed partial deletions around the 11p15.5 or 11p11 region and were crucial in defining common region of deletions. The deletion map of each of these 20 tumours are displayed in Figure 1.

Table 1. *Frequency of LOH on* 11*p in* 184 *sporadic breast cancers*

Marker	Number of tumours Informative (%)	LOH (%)
D11S922	130 (71)	50 (38)
D11S1318	96 (52)	37 (39)
D11S1338	105 (57)	38 (36)
D11S1349	115 (63)	42 (37)
D11S902	114 (62)	36 (32)
D11S1359	112 (61)	37 (33)
D11S929	102 (55)	36 (35)
D11S1324	121 (66)	35 (29)
D11S907	108 (59)	40 (37)
D11S1360	112 (61)	33 (29)
D11S905	102 (55)	34 (33)
D11S986	56 (30)	18 (32)
D11S1344	57 (31)	18 (32)
D11S1313	116 (63)	31 (27)
D11S1357	95 (52)	24 (25)
Total	184 (100)	86 (47)

Autoradiographs of two representative cases that revealed partial deletions in the 11p15.5 region are shown in Figure 2(a). Tumor 180 showed LOH at *D11S1349* while retaining heterozygosity at *D11S922* and *D11S1324*. Tumour 760 retained heterozygosity at *11S1338* but showed LOH at *D11S922* and *D11S1318*. LOH data from 10 tumours that had partial deletions around 11p15.5 are summarised as deletion maps in Figure 2(a). Analysis of the LOH patterns in these tumours revealed a common region of deletion at 11p15.5 in a 12-cM interval flanked by *D11S922* and *D11S1338*.

Representative autoradiographs of cases that revealed partial deletions in the 11p11 region are shown in Figure 1. Tumour 824 showed LOH at *D11S986* while retaining heterozygosity at *D11S905* and *D11S1313*. Tumour 468 retained heterozygosity at *11S986*, but showed LOH at *D11S1313* and *D11S1357*. LOH data from 10 tumours that had partial deletions at 11p11 are summarised as deletion maps in Figure 1(b). Analysis of the LOH patterns in these tumours revealed a common region of deletion within a 2-cM interval flanked by *D11S986* and *D11S1313*.

We found significant correlations of 11p15.5 LOH, 11p11 LOH, respectively, with progesterone receptor (PgR) status. PgR-negative status was more frequent in tumours that had lost one allele of 11p15.5 (27 of 47, 57%) than in tumours that retained both alleles (37 of 110, 34%) ($P = 0.0054$). PgR-negative status was also more frequent in tumours that had lost one allele of 11p11 (23 of 47, 49%) than in tumours that retained both alles (31 of 110, 28%) ($P = 0.0122$) (Table 2). ER-negative status on 11p15.5 was more frequent in tumours that had lost one allele (34 of 67, 51%) than in tumours that retained both alleles (32 of 90, 36%), but this did not reach statistical significance ($P = 0.0565$). We found no significant correlation between the frequency of LOH on 11p15.5 or 11p11 and histological type, tumour size and infiltration, or lymph node metastasis.

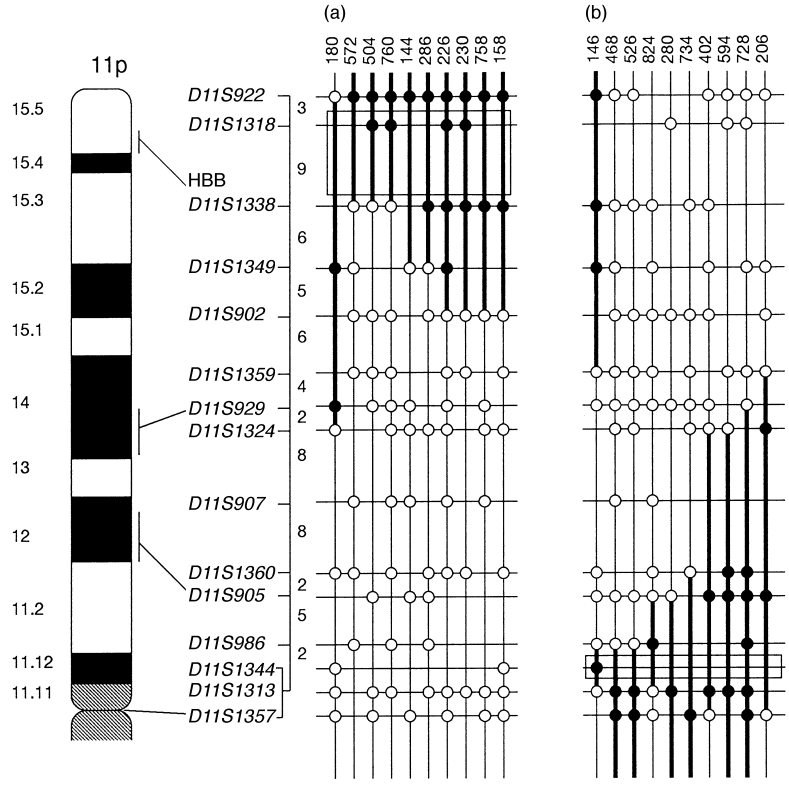

Figure 1. Schematic representation of deletion mapping in tumours that showed partial deletions on chromosome 11p in breast cancers. Locations and linear order of the microsatellite markers were derived from published linkage information [23]; genetic distances between markers are shown along the genetic map of 11p in centimorgans. (a) and (b): summary of allelic losses which defined the commonly deleted regions at 11p15.5 (a) and 11p11 (b). Tumour identification numbers are at the top of each column. White circles indicate retention of heterozygosity, black circles indicate LOH and gaps reflect uninformativeness for specific markers. Common regions of deletion are outlined by rectangles.

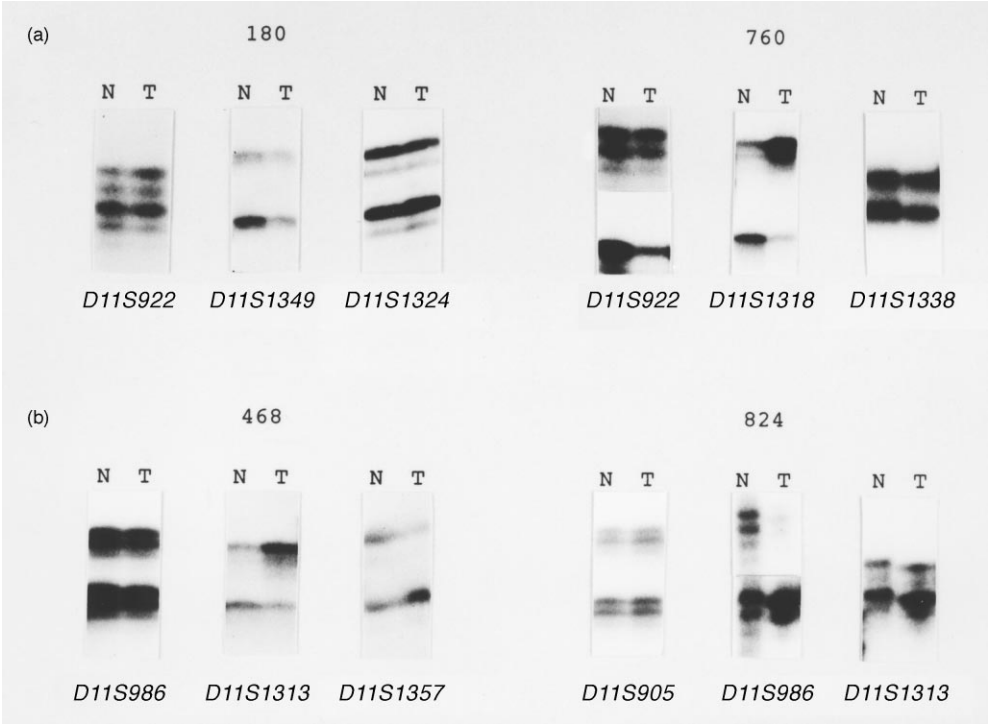

Figure 2. Representative autoradiographs of LOH that determined the commonly deleted regions at 11p15.5 (a) and 11p11 (b). Tumour identification numbers are shown at the top of each autoradiograph. N and T indicate normal and tumour DNA from the same patient.

Table 2. Correlations of 11p15.5 LOH, 11p11 LOH with progesterone receptor in sporadic breast cancers

		PgR (−)	PgR (+)	Statistical significance
11p15.5	LOH	27	37	$P = 0.0054$
	Retention	20	73	
11p11	LOH	23	31	$P = 0.0122$
	Retention	24	79	

PgR(−): progesterone receptor level below 5 fmol/mg protein
PgR(+): progesterone receptor level above 5 fmol/mg protein

DISCUSSION

Using 15 polymorphic microsatellite markers to examine the entire short arm of chromosome 11, we identified a new target region of allelic loss in a 2-cM interval of 11p11 near the centromere, in addition to the 12-cM target region at 11p15.5.

Frequent LOH (47%) observed on 11p in the present study must reflect non-random genetic alterations associated with breast carcinogenesis, since we observed LOH at frequencies less than 10% in the same panel of breast cancers when we studied it with markers from several other chromosomal regions including 1q21-32, 2q13-21, 7p11-15, 9q33-34, 19q13, 20p12-13 (unpublished data).

In a microcell-mediated chromosome transfer experiment, Negrini and colleagues showed that transfer of a normal human chromosome 11 suppressed tumorigenicity in an MCF-7 breast cancer cell line [24]. Those results indicated that the human chromosome 11 contains one or more genes that can act as a suppressor of breast cancer. Observation of frequent allelic losses on 11p in breast cancers, by ourselves and others, support this notion. Coleman and colleagues [28] reported that the transfer of an 11p11.2-12 fragment of human chromosome 11 to a rat liver epithelial tumour cell line suppressed the tumorigenic phenotype and suggested that this region may contain a gene that can suppress tumours of the liver in humans.

In an experiment when fragmented human chromosome 11 was transferred to a rat prostate cancer cell line AT6.1, Ichikawa and colleagues [25] showed that the 11p11.2-13 region could suppress metastasis of this cancer cell. A gene termed *KAI1*, which is capable of suppressing metastasis, was recently identified from this chromosomal region [26]. The *KAI1* gene, whose product belongs to a family of leucocyte surface glycoproteins, codes for a polypeptide of 267 amino acids with four hydrophobic transmembrane domains and a large extracellular hydrophilic domain. Expression of the *KAI1* gene was reduced in cell lines derived from metastatic prostate cancers. Recently, an elevated level of *KAI1* expression in tumours was found to correlate with good prognosis in patients with non-small cell lung cancers [27]. Since the new target region of allelic loss we defined at 11p11 in the present study and the *KAI1* gene maps to the same chromosomal band, it is tempting to speculate that *KAI1* is the target gene of allelic loss in breast cancers. Fine-scale mapping of *KAI1* in relation to the genetic markers used in the present study will eventually resolve this point.

Some researchers have observed a correlation between LOH on 11p and the loss of hormone receptors (ER and PgR) [5–9], but others have failed to observe this [10, 11]. Our analysis, using a large panel of breast cancers and highly informative markers, has demonstrated significant associations of 11p15.5 LOH and 11p11 LOH, each with loss of PgR. Although 11p15.5 LOH status showed a similar tendency with loss of ER, it did not reach statistical significance. Since LOH on 11p loci are not correlated with clinicopathological parameters related to tumour progression, i.e. tumour size and infiltration, lymph node metastasis, or histological type, it is tempting to contemplate that the 11p15.5 LOH and 11p11 LOH may reflect genetic alteration that may influence the hormonal deregulation that occurs during breast carcinogenesis.

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