Loss of heterozygosity on chromosome 11q in breast cancer

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Abstract

Aims—Chromosome 11q23 seems to be a site of frequent mutation in cancer. It also contains loci such as *ataxia telangiectasia* with possible importance in the pathogenesis of breast tumours. The short arm of chromosome 11 has been studied extensively in breast cancer, but the long arm, in particular the distal part, has been studied less frequently. Cytogenetic analysis has shown possible involvement of chromosome 11q in breast tumours. Chromosome transfer experiments have also implicated chromosome 11q in breast cancer. A high frequency of mutations might therefore be expected to occur on chromosome 11q in breast cancers.

Methods—Using restriction fragment analysis, the primary tumours of 41 patients with breast cancer were screened for mutations at five loci on chromosome 11q (D11Z1, INT2 (FGF3), DRD2, NCAM, and D11S29).

Results—Allelic loss occurred at a high frequency (59%) at D11S29. At NCAM, novel alleles were frequently seen on autoradiographs. Relatively low frequencies of mutation were detected at the other loci. Allelic loss at D11S29 was associated with the presence of lymph node metastases, but this may be a chance association.

Conclusions—The frequency of allelic loss at the D11S29 locus is high. The significance of novel alleles at NCAM and their relation to allelic loss at D11S29 are unclear. The results presented here do not permit fine mapping of a region of allelic loss, but suggest that the region of greatest loss lies distal to DRD2. The results provide further evidence for the importance of gene(s) near 11q23 in the pathogenesis of breast cancer, and of tumours in general.

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Many cancers have mutations at or near chromosome band 11q23. Particular examples include haematological malignancies, colorectal tumours,¹ ovarian cancer,² and malignant melanoma.³ Chromosome 11q23 also carries several genes, such as the ataxia telangiectasia (AT) complementation groups and Fanconi anaemia (FA) complementation group D,⁴ that may be involved in cancer predisposition.

In breast cancer chromosome 11p has been studied extensively, with mapping of a putative tumour suppressor gene to band 11p15.5.⁵ The long arm of chromosome 11 has been analysed less frequently, especially the distal part. Cytogenetic analysis, however, has shown possible involvement of chromosome 11q in breast tumours, especially in patients with metastatic disease.⁶⁻⁸ Chromosome transfer experiments have also implicated 11q in breast cancer.⁹

We have used restriction fragment analysis to search for mutations in this region of the genome in 41 cases of sporadic breast carcinoma. We report the frequency of mutations at the NCAM, DRD2, INT2 (FGF3), D11S29, ETS1, and D11Z1 loci (table 1). Each patient's age at presentation was known, and tumour stage, grade and origin were determined histologically. Associations between mutations and the clinical data have also been determined.

Methods

DNA was extracted by standard techniques from the peripheral blood and primary tumours of each patient, providing paired samples of constitutional and tumour DNA. For each locus, 8 µg of each DNA was digested with the restriction endonuclease shown in table 1. Restricted DNAs were electrophoresed on a 0.8–1.0% agarose gel for 24–48 hours to ensure complete separation of fragments. DNA loading was estimated by visual inspection of ethidium bromide stained agarose gels, or by the use of control probes when studying the INT2 and ETS1 oncogene loci. DNA was transferred

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Table 1 Loci studied and gene probes used

Map position	11 cen	11q13	11q23-1	11q23-1	11q23-3	11q23-3
Locus symbol	D11Z1	INT2 (FGF3)	DRD2	NCAM	D11S29	ETS1
Locus name	Centromeric repeat sequence	Murine mammary tumour virus integration site 2 homologue	Dopamine receptor D2	Neural cell adhesion molecule	Anonymous	Avian erythroblastosis virus E26 oncogene homologue
Probe	D11Z1	SS6	HD2G1	pM1.3	L7	pHE5.4
Source	Oncor Inc.	G. Peters, C. Dickson	ATCC	F. Walsh	ATCC	ATCC
Restriction enzyme	XbaI	BamHI	TaqI	BamHI	TaqI	BamHI
Constant bands (kb)	Multiple	None	10.5, 2.3	2.4	None	0.5, 10.0
Polymorphic bands (kb)	Multiple	8.4 [0.63]	6.6 [0.24]	9.8 [0.77]	13.9 [0.24]	None
[and reported frequency]		5.6, 2.8 [0.37]	3.7, 2.9 [0.76]	6.0, 3.8 [0.23]	10.9 [0.76]	

Loci shown in map order; kb, kilobase.

Table 2 Allelic loss, amplification and rearrangement at the six loci studied

Locus	No. cases studied	No. heterozygotes	Heterozygotes with LOH n(%)	Heterozygotes with rearrangements n(%)	No. homozygotes	Homozygotes with rearrangements n(%)	Cases with amplification n(%)	Cases with rearrangements n(%)
D11S29	41	17	10(59)	0(0)	24	0(0)	—	—
NCAM	36	14	2(14)	3(21)	22	3(14)	—	—
DRD2	33	10	2(20)	0(0)	23	1(4)	—	—
D11ZI	13	4	1(25)	0(0)	9	1(11)	—	—
INT2	34	—	—	—	—	—	9(26)	0(0)
ETS1	32	—	—	—	—	—	3 (9)	0(0)

LOH, loss of heterozygosity, allelic loss.

Figure 1 A: Loss of the 10.9 kilobase allele at the D11S29 locus. Sizes of the bands (in kilobases (kb)) are shown. Allelic loss is arrowed. B: Each of the six cases with allelic rearrangements at the NCAM locus. Sizes of the bands (kb) are shown in all cases. Novel alleles are arrowed. (i) Novel allele of about 8 kb in constitutional heterozygote. Note the loss of the 3.8 kb band here, seen very faintly in the tumour, suggesting that the new allele arose from this band by duplication, translocation or loss of a restriction site. (ii) Novel alleles of about 1.5 kb and 5.5 kb in constitutional heterozygote, together with loss of the 3.8 kb and 6.0 kb bands. It is likely that the new alleles arose from the chromosome carrying the 3.8/6.0 kb bands. (iii) Novel alleles of about 1.5 kb and 5.0 kb in constitutional heterozygote, together with loss of the 3.8 kb and 6.0 kb bands. Again, it is probable that the new alleles arose from the chromosome carrying the 3.8/6.0 kb bands. (iv) Novel alleles of about 5 kb in two constitutional homozygotes and novel allele of about 4.5 kb in another constitutional homozygote (plus a probable further novel allele, slightly larger than the 5.0 kb band and just visible). A normal heterozygote is shown in the centre in order to permit comparison between the sizes of the novel alleles seen in the tumours and the normal 6.0 kb and 3.8 kb polymorphic alleles (seen faintly).

to Hybond nylon membranes (Amersham, Little Chalfont, UK) in 25 mM phosphate buffer and bound by ultraviolet cross-linking (1200 mJ).

Gene probes to each locus of interest were obtained from the sources shown in table 1 and isolated from their vectors. Using the random priming method, probes were labelled with

Table 3 Association between LOH at D11S29 and tumour stage

	No. patients node +ve	No. patients node -ve	Total
LOH at D11S29	10	0	10
No LOH at D11S29	1	6	7
Total	11	6	17

LOH, loss of heterozygosity; $\chi^2 = 10.1$ (Yates' correction), $p < 0.002$, concordance = 94%.

[$\alpha^{32}\text{P}$ -dCTP] to activities of around 2×10^9 dpm/ μg . Membranes were pre-hybridised overnight at 42°C in 50% formamide, $10 \times$ Denhardt's solution, $1.5 \times$ SSPE, 0.1% SDS, and 0.5 mg/ml denatured sheared herring sperm and hybridised overnight under the same conditions, with the addition of 5% dextran sulphate and the appropriate labelled probe. Membranes were washed twice for 10 minutes at 22°C in $2 \times$ SSPE/0.2% SDS and once for five to 20 minutes at 65°C in $0.2 \times$ SSPE/0.2% SDS, and were then exposed to x ray film for up to one week. Autoradiographs were scored by three workers, using a visual comparison between constitutional and tumour DNAs.

Mutations in tumours were scored in different ways, depending on the loci involved and on the basis of previous studies. At the oncogene loci INT2 and ETS1, mutations that changed the intensity of autoradiograph bands (relative to those detected by control probes) were classed as amplification, irrespective of constitutional genotype. At the D11S29, NCAM, DRD2, and D11Z1 loci, mutations that changed the intensity of one autoradiograph band relative to its allele were scored as loss of heterozygosity (allele loss) in constitutional heterozygotes (informative cases) only. At all loci, tumours showing novel alleles were classed as allelic rearrangements, both for constitutional homozygotes and heterozygotes. The χ^2 and t tests were used to determine the associations between the presence of mutations and the three clinical variables.

Results

The frequencies of mutation detected at each of the six loci are given in table 2. Representative autoradiographs, showing mutations at D11S29 and NCAM, are shown in fig 1. The highest mutant frequency was found at D11S29, with 59% of informative cases having lost an allele. At NCAM, the frequency of allelic loss was 14%. The results at NCAM, however, are complicated by the finding of allelic rearrangements in a substantial proportion of patients (21% of informative and 14% of non-informative cases). These rearrangements were seen as one or more additional bands of less than 9.8 kilobases on the autoradiographs, present in tumours only, and of sizes different from the normal alleles detected by the gene probe used (fig 1B). Mutations at the four other loci occurred at essentially unremarkable frequencies,¹⁰⁻¹² irrespective of whether allelic loss or amplification took place (table 2). Allelic rearrangements were not detected at INT2 or ETS1, and just two solitary cases had novel alleles at D11Z1 and DRD2. Neither of these

patients had an allelic rearrangement at NCAM.

Patients presented at a mean age of 53.2 years (SD=11.1). All cases had undergone axillary node sampling: 20 cases were axillary node positive on histopathological grounds, 20 were node negative, while the nodal status of one was unknown. None of the patients had distant metastases. Thirty four patients had tumours of ductal origin: of these, two were scored as grade 1, 17 as grade 2 and 15 as grade 3. Six tumours were of lobular origin and there was one case of ductal carcinoma in situ. Unfortunately, examination of the latter carcinoma did not provide much useful information: it showed no amplification at INT2 and ETS1, did not show allelic loss at

Figure 2 Attempting to define a minimum region of loss of heterozygosity (LOH) and/or allelic rearrangement at the DRD2, NCAM and D11S29 loci. The cases shown are those useful for this purpose. They either have LOH or rearrangement at one locus and are informative at one or both of the other loci or have LOH or allelic rearrangement at more than one locus.

Table 4 Details of results at NCAM, DRD2 and D11S29 in patients with LOH at D11S29 or NCAM, or with allelic rearrangements at NCAM

Case No.	DRD2	NCAM	D11S29
1	NL	NI	LOH
3	NI	R	LOH
4	LOH	LOH	LOH
5	NI	NI	LOH
7	NI	NL	NI
8	NI	NL	L
13	NI	NI	NI
22	NI	-	LOH
23	-	R	LOH
30	NI	LOH	NI
31	NI	-	LOH
41	NI	-	LOH
73	NL	R	NI
76	NL	R	NL
79	LOH	R	LOH
87	NI	R	NL

LOH, loss of heterozygosity; R, allelic rearrangement; NL, no mutation; NI, not informative; -, not studied.

DRD2 and was non-informative at the other three loci.

With one exception, no associations were found between any mutation and the clinical data, whether age, stage, grade, or origin. The exception was a strong correlation between allelic loss at D11S29 and tumour stage ($p < 0.002$), with a concordance of nearly 95% between allelic loss and the presence of axillary node metastasis (table 3). One significant association at the 5% level would be expected by chance, given a study of six loci and three clinical variables. The possibility exists, however, that the association between allelic loss at D11S29 and tumour stage may be a genuine biological phenomenon.

Discussion

The frequency of allelic loss at the D11S29 locus is high compared with other loci in breast cancer.¹³⁻¹⁵ The only previous study of D11S29 in breast cancer¹³ did not publish explicit results at this locus. The results presented here do not permit fine mapping of a region of allelic loss (fig 2), but suggest that the region of greatest loss lies distal to DRD2, as there is a low frequency of allelic loss at this locus and one case with allelic loss at D11S29 retained heterozygosity at DRD2. The data do not show conclusively whether or not the allelic rearrangements at NCAM are separate events from allelic loss at D11S29. It is possible—for example, to speculate that chromosome breakage within or near NCAM could lead to loss of the distal part of 11q and explain the observed data at both NCAM and D11S29. In two cases, however, rearrangements were found at NCAM, yet allelic loss was not detected at D11S29. Perhaps, therefore, the region of greatest allelic loss lies proximal to D11S29. Table 4 shows detailed results from patients with allelic loss at D11S29 or NCAM, or allelic rearrangements at NCAM.

The NCAM locus has not been studied before in breast tumours and the finding of a number of allelic rearrangements here is unusual. The obvious explanation is technical problems such as partial restriction digestion of genomic DNA, enzyme star activity or bacterial

contaminants. We cannot exclude this, but the following evidence suggests otherwise. Agarose gel electrophoresis was used to confirm complete digestion of DNA and four other genetic probes subsequently bound to the same nylon membrane showed only the alleles expected. Moreover, all genomic probes were purified from their bacterial vectors before use. A variety of mutations might give rise to the allelic rearrangements observed. These include: partial deletions of the NCAM locus, perhaps extending distally towards D11S29 (see above); point mutations, adding or removing restriction sites; duplications or translocations; or genetic heterogeneity within tumours. It is not clear why the NCAM locus should be so affected.

The results presented provide further evidence for the importance of gene(s) near 11q23 in tumorigenesis. Further work on additional patients may (1) confirm or refute the association between metastasis and allelic loss at D11S29 and investigate the reasons for this observation; (2) enable the allelic rearrangements at NCAM to be proven and characterised; and (3) allow the region(s) of most frequent mutation to be mapped. Candidate loci include the AT group,¹⁶ FA group D⁴ and the progesterone receptor.¹⁷ NCAM itself is a candidate locus and although little is known about its expression in breast tissue, it has been implicated in haematological malignancies,¹⁸ small cell lung cancer¹⁹ and colorectal cancer.²⁰ It must also be determined whether the loci involved are the same or different in each type of cancer that shows mutations at or near 11q23.

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