



Multiple regions of chromosome 6q affected by loss of heterozygosity in primary human breast carcinomas

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Summary A total of 80 primary human breast carcinoma DNAs were analysed for loss of heterozygosity (LOH) on the long arm of chromosome 6, using microsatellite markers whose location has been defined physically and by linkage analysis. Loss of heterozygosity was observed in 38 of 80 (48%) tumours that were informative for at least one locus. The analysis revealed partial or interstitial deletions of chromosome 6q. Detailed mapping of chromosome 6q in these tumour DNAs identified two and perhaps three commonly deleted regions. One of these is located between markers *D6S251* and *D6S252* (6q14–q16.2), another between *D6S268* and *D6S261* (6q16.3–q23) and a third between *D6S287* and *D6S270* (6q22.3–q23.1).

Keywords: breast cancer; deletion; chromosome 6q; tumour-suppressor gene

Cytogenetic (Dutrillaux *et al.*, 1990; Lu *et al.*, 1993; Thompson *et al.*, 1993; Trent *et al.*, 1985, 1993) and molecular analyses (reviewed in Callahan *et al.*, 1993) of primary human breast carcinomas have documented frequently occurring genetic alterations that take place during the evolution of tumour development. It is thought that these mutations either inactivate normal growth controls or give the tumour some selective advantage. At the molecular level, loss of heterozygosity (LOH) is the most frequent type of genetic alteration in primary human breast tumours (Callahan *et al.*, 1993). LOH at specific chromosomal loci has been taken as evidence for the presence of putative tumour-suppressor genes within the affected regions (Knudson, 1989). In sporadic primary human breast carcinomas LOH has been detected on at least 12 different chromosome arms (Callahan *et al.*, 1993). However only in the case of chromosome 17p13 has the target gene for LOH (*TP53*) been identified (Hollstein *et al.*, 1991). Generally one allele of the target gene is lost and the remaining allele contains a nonsense or missense mutation. The involvement of chromosome 6q in breast carcinomas has been noted in cytogenetic analysis of primary tumours (Dutrillaux *et al.*, 1990; Lu *et al.*, 1993; Thompson *et al.*, 1993; Trent *et al.*, 1985, 1993). Similarly, molecular analysis of primary breast tumour DNAs has shown that chromosome 6q is frequently affected by LOH (Devilee *et al.*, 1991). In this report, we describe studies aimed at defining the location of putative tumour-suppressor gene(s) on chromosome 6q in primary breast tumour DNAs. We have screened 80 pairs of matched breast tumour and normal DNAs using sequence-tag sites (STSs) whose location has been defined by linkage analysis and have constructed a detailed deletion map of this chromosome arm.

Materials and methods

Thirty invasive ductal carcinomas (IDCs) of the breast and matching peripheral lymphocytes were collected at the Centre Rene Huguenin, Saint Cloud, France (tumour panel 1), and another 50 pairs of IDCs and matched lymphocytes were collected from University of Pisa, Pisa, Italy (tumour panel

2). Patients corresponding to each of the tumour panels had received no prior therapy.

Genomic DNA was extracted and diluted to 100–200 ng μl^{-1} . Polymerase chain reaction (PCR) was performed with 100–200 ng of template DNA, 10 mM Tris-HCl, 1.5 mM magnesium chloride, 50 mM potassium chloride, gelatin 0.1 mg ml^{-1} , 200 μM each dNTP, 0.5 U *Taq* polymerase (Boehringer Mannheim) and 50 pmol of each primer in a total volume of 10 μl or 25 μl . The PCR product was identified by end labelling primers with [γ -³²P]ATP or the PCR product was internally labelled with [α -³²P]dCTP. All PCR reactions were performed on a Perkin Elmer Cetus PCR system with denaturation for 6 min at 94°C followed by 30 cycles of denaturation at 94°C for 1 min, annealing temperature (Table 1) for 1 min, and extension at 72°C for 1 min. The primers for the STS loci that were examined (Gyapa *et al.*, 1994; Volz *et al.*, 1994), their annealing temperatures and the references describing them are shown in Table 1.

The PCR products were diluted with loading buffer (95% formamide, 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol), heat denatured and rapidly cooled. Samples were run in pairs (tumour and lymphocyte PCR product from the same patient) on a denaturing gel (7% acrylamide, 32% formamide, 6 M urea, 1×TBE) at a constant 30–35 W. After electrophoresis the gel was transferred to 3MM Whatman paper and autoradiography performed with Kodak X-Omat AR film at –70°C. When the signal of an allele in tumour DNA was less than 50% of intensity observed in matching normal DNA from a heterozygous patient, LOH was considered to have occurred (Bieche *et al.*, 1993).

Results

Preliminary results obtained at 6 loci (*D6S254*, *D6S251*, *D6S252*, *D6S249*, *ARG1* and *D6S255*) on chromosome 6q in 30 breast tumour DNAs (tumour panel 1) suggested that LOH on this arm of chromosome 6 was a frequent event (Table II). LOH was detected in nine tumour DNAs. In three tumour DNA samples all informative loci were affected by LOH. Six other tumour DNAs had loss of one allele at *D6S251* or *D6S252* or *D6S249*. However, the data set was too small to determine with any precision the location of the target region(s). Therefore, this study was extended to include another 50 primary breast carcinoma DNAs (tumour panel 2) with eight additional microsatellite markers whose localisa-

Table I STS loci and primers on chromosome 6q

Locus/STS	Annealing temperature (°C)	Primers (5'-3')	References
D6S254	55	AGAGAGGCTGAAGACCAATC TCCCATAGCTACAAGCCACT	Wilkie et al. (1993)
D6S286	55	GGCCCAGCATCACCCCTAAT CCAATCGTGCATCCCAAAGA	Gyapa et al. (1994)
D6S284	53	CATGGCTGTCTATCAAACCC AAGCATTTGTGTGGCTCTTG	Gyapa et al. (1994)
D6S251	55	TTCCTAACCAGGTTTCAATG ATATTTTTAAAGTAAAGTTGCVAC	Wilkie et al. (1993)
D6S252	55	TGAAAGGAAAGTCCTGCTTC ATGGCTCAGGATTCACATTG	Wilkie et al. (1993)
D6S249	55	TTCTATTTCTGAAGGTGAACTA ATAGTTACCATCAGTCACTG	Wilkie et al. (1993)
D6S268	50	CTAGGTGGCAGAGCAACATA AAAAGGAGGTCATTTAATCG	Gyapa et al. (1994)
D6S302	50	TTCCACAATGACAAGTCCAATACACG TTCTTTAGGATAAGCCAATACACG	Gyapa et al. (1994)
D6S261	50	GTGAAACCCTGTCTCACTGC GGATTTATAGTGACCATGCCA	Gyapa et al. (1994)
D6S287	50	ATATTAGTGCCTTATGCTTCTG AAATTGGATATTCATGCTTG	Gyapa et al. (1994)
D6S262	50	ATTCTTACTGCTGGAAAACCAT GGAGCATAGTTACCCTTAAAATC	Gyapa et al. (1994)
D6S270	55	GTGTAACCTGATCTGAATGGTTCC GTAGTGAAGCCTGGATGTGG	Gyapa et al. (1994)
ARG1	55	CTACATATTTCTAAATACATGC ACTTAGTAGTTTTAAGCAGGA	Wilkie et al. (1993)
D6S255	55	TCAGCATCAAGGTAAGTCTGAG TTAGTGCCCTATGCAAGGCA	Wilkie et al. (1993)

Table II LOH on chromosome 6q in breast tumour panel 1

Regional assignment	Locus	n	LOH/inf. (%)	Tumour DNA No.									
				14	21	22	25	48	51	54	55	66	
6q13	D6S254	30	0/5 (0)	NI	NI	NI	H	NI	NI	NI	NI	NI	
6q14-q16.2	D6S251	30	6/28 (21)	D	H	D	H	D	D	D	H	D	
	D6S252	30	4/16 (25)	NI	D	NI	NI	H	D	NI	D	D	
6q16.3-q21	D6S249	30	5/14 (36)	D	NI	H	D	H	D	D	NI	D	
6q22.3-q23.1	ARG	30	2/12 (17)	NI	D	NI	NI	H	NI	NI	H	D	
6q25.2	D6S255	30	3/9 (33)	D	D	NI	NI	NI	NI	NI	H	NI	

The genotypes of nine tumour DNA samples from tumour panel 1 at STS markers between D6S254 and D6S255 that were tested and their regional locations are listed. The genetic order of the STS loci is according to published linkage studies (Gyapa et al., 1994; Volz et al., 1994). n, total number of tumour DNA samples examined for each marker; LOH/inf., fraction of tumours from informative patients that showed LOH at each marker; the number in parenthesis is the percentage of tumours having LOH at each locus; D, LOH; H, STS loci that were informative but unaffected; NI, STS loci that were not informative.

tion and order were determined by linkage analysis (Gyapa et al., 1994; Volz et al., 1994).

In this second set of 50 breast tumour DNA samples, all were informative for at least one locus and of these 29 (58%) had LOH at one or more loci. The frequency of LOH at the different STSs loci on chromosome 6q for tumour panel 2 is shown in Table III. Autoradiographs of two tumour DNA samples from tumour panel 2, each at three different STS loci, is shown in Figure 1. Sample 281 had LOH at D6S286 and D6S251 but was informative and unaffected at D6S252. These results taken together with those summarised in Table III for tumour DNA samples 127, 28, 91, 304, 263 and 49 are consistent with the presence of a tumour-suppressor gene located in the 10 cM interval between D6S284/D6S286 and D6S252 on chromosome 6q14-q16.2 (region 1). If tumour DNA 82 is also considered the size of region 1 may be further reduced to the 1.1 cM interval between D6S284/D6S286 and D6S251.

Shown in Figure 1 are autoradiographs of tumour DNA sample 82, which indicate that a second region of chromosome 6q is independently affected by LOH. In this tumour DNA, D6S268 and D6S261 are both informative and unaffected whereas LOH was detected at D6S302. The results

summarised in Table III for tumour DNA samples 63, 224 and 204 are also consistent with the presence of a tumour-suppressor gene located in the 5.2 cM interval between D6S268 and D6S261 (region 2). Evidence for a third region affected by LOH was found in tumours DNAs 204, 99, 208, 49, 114 and 83 (Table III). In these tumour DNA samples D6S262 was affected by LOH, whereas the more centromeric locus D6S287 was informative and unaffected. The telomeric boundary of this region could be D6S270 since in tumour DNAs 204 and 99 it was unaffected by LOH.

Several of the tumour DNAs were remarkable in that more than one region of chromosome 6q was affected by LOH. For instance tumour DNA samples 82, 263 and 281 exhibited independent LOH of regions 1 and 2 whereas in tumour DNA 204 regions 2 and 3 were independently affected by LOH (Table III). Similarly in tumour DNA sample 208 regions 1 and 3 were affected by LOH and in sample 49, each of the three regions were independently affected by LOH. Cytogenetic analysis of metastatic breast carcinomas have also identified tumours with multiple chromosome 6 alterations (Dutrillaux et al., 1990; Lu et al., 1993; Thompson et al., 1993; Trent et al., 1993). In several cases it was not possible to unambiguously determine which

Table III LOH on chromosome 6q in breast tumour panel 2

Regional assignment	Locus	Recombination frequency (cM)	n	LOH/inf. (%)	127	28	91	304	281	263	82	63	224	204	99	208	49	114	83	551	86	50	178	394	125	272	14	376	321	62	396	376	267			
					Patient no.																															
					D	NI	H	NI	H	NI	H	NI	H	NI	H	NI	H	NI	H	NI	H	NI	H	NI	H	NI	H	NI	H	NI	H	NI	H			
6q14-q15	D6S284	0	9	1/5 (20)	D	NI	H	NI	H	NI	H	NI	H	NI	H	NI	H	NI	H	NI	H	NI	H	NI	H	NI	H	NI	H	NI	H	NI	H	NI	H	
6q14-q16.2	D6S286	1.1	21	4/11 (36)	D	NI	NI	NI	D	NI	D	NI	D	NI	H	H	D	D	D	H	H	NI	NI	NI	D	H	H	NI	D	NI	NI	D	NI	D	NI	
	D6S251	8.9	50	10/39 (26)	D	D	D	NI	D	D	H	H	H	H	H	D	D	D	D	H	H	NI	NI	NI	D	H	H	NI	D	NI	NI	D	NI	D	NI	
	D6S252	3.3	50	7/32 (22)	H	H	H	D	H	NI	H	H	H	H	NI	D	NI	NI	D	NI	D	NI	D	D	D	NI	H	H	NI	H	H	H	D	NI	NI	
6q16.3-q21	D6S249	10.3	26	4/14 (29)	H	H	NI	NI	NI	NI	H	H	NI	NI	H	NI	H	D	NI	NI	NI	NI	H	H	NI	NI	NI	NI	H	D	H	NI	D	D	D	
	D6S268	2.6	28	4/14 (29)	NI	H	H	D	H	NI	H	NI	H	D	NI	H	D	NI	H	D	NI	D	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	
6q21-q23	D6S302	2.6	50	13/30 (40)	NI	NI	NI	H	NI	NI	D	D	D	D	NI	NI	D	D	D	NI	D	H	D	H	D	D	D	D	D	D	D	H	NI	H	NI	
	D6S261	1	40	5/25 (20)	NI	NI	NI	H	H	D	H	H	H	NI	H	NI	NI	NI	NI	H	H	H	H	NI	NI	NI	D	D	D	D	NI	NI	D	NI	D	
6q22.3-q23.1	D6S287	9	50	4/33 (12)	NI	H	H	H	NI	H	H	NI	H	H	H	H	H	H	H	H	H	NI	NI	H	D	D	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	D6S262	4	50	10/28 (36)	NI	NI	NI	NI	H	H	NI	H	NI	NI	D	D	D	D	D	D	D	D	NI	NI	H	D	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	D6S270		5	1/5 (20)																																

The genotypes of 29 tumour DNAs from tumour panel 2 that had LOH at one or more informative STS loci are shown. The regional location of the STS loci and the recombination frequency between them in centimorgans (cM) is according to published studies (Gyapa et al., 1994; Volz et al., 1994). n, total number of tumours DNA samples examined for each marker; LOH/inf, fraction of tumours from informative patients that showed LOH at each marker; the number in parenthesis is the percentage of tumours having LOH at each locus; D, LOH; H, STS loci that were informative but unaffected; NI, STS loci that were not informative; vertical bars highlight the smallest regions affected by LOH. Tumours not tested at a particular locus are blank.

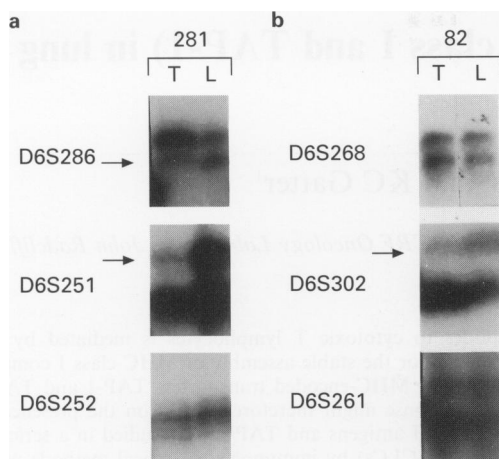


Figure 1 Autoradiographs of three tumour DNA samples having LOH at STS markers on chromosome 6q. (a) The STS markers were *D6S286*, *D6S251* and *D6S252*. Arrows mark the deleted allele at *D6S286* and *D6S251* in tumour DNA 281. (b) The STS markers were *D6S268*, *D6S302*, *D6S261*. The arrow marks the deleted allele of *D6S302* in tumour DNA 82. The autoradiograph for tumour and lymphocyte DNA at *D6S268* represent different exposures of the same gel.

region was the target for LOH. Thus in tumour DNAs 351 and 86 either region 1 or region 2 could have been the target for LOH. Similarly, in samples 178, 394, 125, 272, 14, 150, and 62 either region 2 or region 3 could contain the target tumour-suppressor gene.

Discussion

Our results confirm the findings of Devilee *et al.* (1991) that LOH on chromosome 6q is a frequent event in primary human breast carcinomas. They detected LOH at *MYB* and/or *D6S37* in 50% of the tumour DNA samples. The *MYB* locus is 5 cM telomeric of *D6S270* (Gyapa *et al.*, 1994; Volz *et al.*, 1994) and *D6S37* is at the distal end of chromosome 6q. Our study extends their results by defining three regions

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of chromosome 6q that are independently affected by LOH. During the course of our work Orphanos *et al.* (1995) reported two regions on chromosome 6q that are affected by LOH in human breast tumour DNAs. One of these spans 6q13–q21 and probably corresponds to regions 1 and 2 in our data set. The second region in their study was located at 6q21–q27 and is distal to region 3 in our study. LOH on chromosome 6q is not unique to breast carcinomas, it has been detected in 40% of melanomas in the region of 6q16–q23 (Millikin *et al.*, 1991). Similarly, Saito *et al.* (1992) found that 51% of ovarian carcinomas had LOH at one or more of nine loci on chromosome 6q24–q27. In this study the commonly deleted region was 6q26–q27.

At the present time there are few, if any, candidate target genes for LOH on chromosome 6q. However, Negrini *et al.* (1994) have shown that microcell-mediated transfer of chromosome 6 into the human breast tumour cell line MDA-MB-231 inhibits its tumorigenicity in BALB/c-*nu/nu* mice as well as causing the cells to age in culture. An analysis of polymorphic loci which identifies the portions of the transferred chromosome 6 that were retained in the cell line, suggested that at least two functional regions of 6q are important for tumour suppression. One functional region was at the distal end of 6q near *D6S48*. Based on current linkage maps of chromosome 6q (Gyapa *et al.*, 1994; Volz *et al.*, 1994) the second functional region is located between the *CNR* locus (6q14–q15) and *D6S310* (9 cM telomeric of *D6S270*, see Table III). This second functional region is consistent with regions 1, 2 and possibly 3 presented in our study. Clearly the development of a physical map of the polymorphic STSs loci on chromosome 6q should lead to further definition of the regions affected by LOH and to the target gene(s) in primary breast tumours.

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