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Allelotype analysis of oesophageal adenocarcinoma: loss of heterozygosity occurs at multiple sites

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Summary Deletions of tumour-suppressor genes can be detected by loss of heterozygosity (LOH) studies, which were performed on 23 cases of adenocarcinoma of the oesophagus, using 120 microsatellite primers covering all non-acrocentric autosomal chromosome arms. The chromosomal arms most frequently demonstrating LOH were 3p (64% of tumours), 5q (45%), 9p (52%), 11p (61%), 13q (50%), 17p (96%), 17q (55%) and 18q (70%). LOH on 3p, 9p, 13q, 17p and 18q occurred mainly within the loci of the *VHL*, *CDKN2*, *Rb*, *TP53* and *DCC* tumour-suppressor genes respectively. LOH on 5q occurred at the sites of the *MSH3* mismatch repair gene and the *APC* tumour-suppressor gene. 11p15.5 and 17q25–qter represented areas of greatest LOH on chromosomes 11p and 17q, and are putative sites of novel tumour-suppressor genes. LOH on 9p was significantly associated with LOH on 5q, and tumours demonstrating LOH at both the *CDKN2* (9p21) and *MSH3* (5q11–q12) genes had a significantly higher fractional allele loss than those retaining heterozygosity at these sites. Six of nine carcinomas displaying microsatellite alterations also demonstrated LOH at CDKN2, which may be associated with widespread genomic instability. Overall, there are nine sites of LOH associated with oesophageal adenocarcinoma.

Keywords: oesophageal adenocarcinoma; loss of heterozygosity; fractional allele loss

The incidence of adenocarcinoma of the oesophagus has increased at a greater rate than any other tumour over the last 20 years (Blot et al, 1991), and is now the most common oesophageal malignancy in certain parts of the Western world (Spechler et al, 1994). The reason for this increase is not clear. However, it is known that Barrett's oesophagus, which occurs in approximately 10% of patients with gastro-oesophageal reflux, is associated with a 30–125 times increased risk of developing adenocarcinoma (Spechler et al, 1984; Cameron et al, 1985; Williamson et al, 1991). It is estimated that approximately 1% of patients with Barrett's oesophagus will develop adenocarcinoma each year, and this can occur up to 20 years after the initial diagnosis of Barrett's oesophagus (Spechler, 1987). The histological progression during this period is considered to follow metaplasia–low-grade dysplasia–high-grade dysplasia–carcinoma (Miros et al, 1991). High-grade dysplasia (HGD) has been used as a marker of future cancer development, but there is interobserver variation in the diagnosis of HGD (Reid et al, 1988) and not all patients with HGD will develop cancer (Schnell et al, 1996).

Attention has therefore focused on molecular biomarkers of carcinogenesis. Loss of function of tumour-suppressor genes resulting from genomic insults has been implicated in the development of several different tumours, and these loss of function mutations may be detected by loss of heterozygosity (LOH) studies (Ittman and Wieczorek, 1996; Shimizu and Sakiya, 1996). LOH studies can lead to the identification of tumour-suppressor genes that are inactivated in the metaplasia–dysplasia–carcinoma progression, and may therefore be useful as biomarkers of future carcinogenesis in patients with Barrett's metaplasia and dysplasia undergoing endoscopic surveillance.

Previous alleleotype analyses have detected LOH in more than 40% of oesophageal adenocarcinomas on chromosome arms 1p, 4q, 5q, 9p, 13q, 17p and 18q (Barrett et al, 1996*a* and Hammoud et al, 1996). These allelotype studies were undertaken with 43 and 39 microsatellite primers respectively. We have performed the most comprehensive genomic study of oesophageal adenocarcinoma to date, covering all of the non-acrocentric chromosome arms with 120 microsatellite primers, enabling identification of putative tumour-suppressor gene sites in oesophageal adenocarcinoma.

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MATERIALS AND METHODS

Patients

Twenty-three cases of adenocarcinoma of the oesophagus diagnosed between 1992 and 1996 were studied. Twenty of these patients were male and their mean age was 68 years. At present, six of these patients are alive with no signs of recurrent disease.

DNA extraction

Tissue from the tumour and from normal gastric mucosa were obtained from endoscopic biopsies and from surgical resections, snapped frozen in liquid nitrogen and stored at −70°C. Areas of tumour containing minimal stromal cells were microdissected and DNA extracted from the microdissected tissues using the Nucleon II extraction kit (Scotlab).

PCR and LOH analysis

A total of 120 microsatellite primers representing 39 autosomal chromosomal arms were used to study the genome of each tumour (Table 1), the emphasis on chromosome arms that harbour known tumour-suppressor genes or in which a high degree of LOH has been detected in other tumours. However, at least one microsatellite primer was studied for each non-acrocentric chromosome arm. A 25-µl PCR mixture containing 100 ng of extracted DNA, 5 pmol of forward and reverse DNA primers, 200 µM of dNTP, 0.5 units of *Taq* polymerase, and 2.5 µl of standard ammonia buffer containing 1.5 µl of 1.5 mM magnesium chloride (Bioline) was used in the following reaction: 95°C for 5 min, then 30 cycles of 94°C for 30 s, 55–59°C for 30 s (depending on the primer) and 72°C for 1 min, followed by 72°C for 5 min.

An aliquot of 10 µl of the PCR product was electrophoresed overnight on a 10% polyacrylamide gel, and the results visualized by silver staining. There are three possible results for each primer used: heterozygous patients have both alleles present in tumour and normal tissue, homozygous patients have a single corresponding allele in both tumour and normal tissue and LOH is indicated by the absence, or a greater than 50% reduction in intensity, of an allele in the tumour tissue (Figure 1). Homozygous patients are regarded as non-informative at that locus. LOH was taken to indicate the site of a putative tumour-suppressor gene. However, it has been noted that certain PCR techniques cannot distinguish between allelic duplication or low-level amplification leading to LOH (Ah-See et al, 1994). This suggests that LOH may not always be indicative of the presence of a tumour-suppressor gene, and confirmation that a site of LOH contains a tumour-suppressor gene requires mutational analysis.

Microsatellite alterations

The microsatellite primers used to study LOH can also detect microsatellite alterations, which are indicated by a shift in the electrophoretic band of the tumour tissue relative to the band of normal tissue. Seventy-four of the 120 primers used in the LOH analysis were used to study microsatellite alterations.

Fractional allele loss

The fractional allele loss (FAL) was calculated for each tumour as the number of chromosomal arms demonstrating LOH divided by the number of informative chromosomal arms.

Statistical analysis

Comparison of the clinicopathological parameters and FAL values of the tumours was performed by the Fisher exact test, and the Pearson correlation coefficient used to analyse the possible relationships between LOH on different chromosomal arms. Survival was analysed by the Kaplan–Meier method and by log-rank testing.

RESULTS

A total of 120 microsatellite primers (Table 1) were used to study allelic imbalance in 23 cases of oesophageal adenocarcinoma. Each tumour demonstrated LOH with at least three different primers, and one tumour demonstrated LOH with 27 primers. One hundred of the 120 primers used (84%) demonstrated LOH in at least one tumour, and LOH was detected in 36 of the 39 autosomal chromosome arms studied (Figure 2).

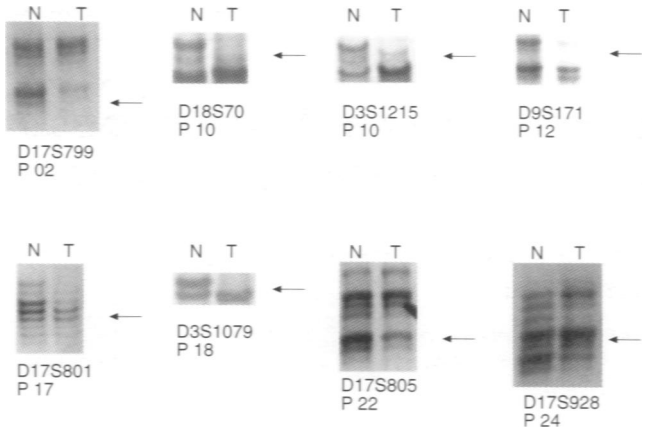

Figure 1 LOH in tumours P02 (D17S799), P10 (D18S70), P10 (D3S1215), P12 (D9S171), P17 (D17S801), P18 (D3S1079), P22 (D17S805) and P24 (D17S928). N, normal gastric tissue; T, tumour tissue

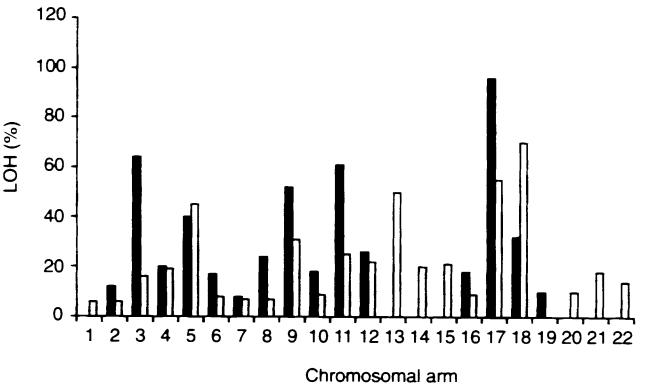

Figure 2 Individual allelotypes of 23 cases of oesophageal adenocarcinoma. ■, LOH: □, retention of heterozygosity; and the remaining blank areas are non-informative at that locus. FAL is displayed for each tumour. ■, p; □, q

Percentage LOH on each chromosomal arm

The percentage of tumours displaying LOH was calculated for each chromosomal arm (Table 1 and Figure 3). Eight chromosomal arms displayed LOH in at least 45% of the tumours: 3p (64%), 5q (45%), 9p (52%), 11p (61%), 13q (50%), 17p (96%), 17q (55%), and 18q (70%). LOH on these chromosomal arms was significantly greater than LOH detected on other chromosomal arms ($P < 0.02$, Fisher's exact test). Nineteen of 23 tumours demonstrated LOH in at least half of these chromosomal arms. Excluding the eight chromosomal arms with LOH in more than 45% of tumours, the background LOH was 15%, which is similar to that previously reported in oesophageal carcinoma (Wagata et al, 1991; Huang et al, 1992; Boynton et al, 1992). Allelic loss was detected in only one of the p or q chromosomal arms in 78% of chromosomes, indicating that the majority of deletions represented subchromosomal events.

Table 1 LOH analysis of 23 oesophageal adenocarcinomas using 120 microsatellite primers

Chromosome arm	Primer	Site	LOH/informative cases (%)	Total LOH on each arm (%)
1p	D1S159	1p22.3–p21	0/10 (0)	0/10 (0)
1q	D1S53	1q31–q32	1/16 (6)	1/16 (6)
2p	D2S207	2p25–p23	0/10 (0)	2/17 (12)
	TPO	2p25–p24	2/13 (15)	
2p	D2S104	2q33–q37	1/17 (6)	1/17 (6)
3p	D3S1079	3p13	3/9 (33)	**14/22 (64)**
	D3S659	3p13	2/12 (17)	
	D3S1235	3p21.3–p21.2	0/6 (0)	
	D3S1211	3p22	3/10 (30)	
	THRBS	3p24	0/2 (0)	
	D3S1293	3p25–p24.2	0/12 (0)	
	D3S656	3p25.1	3/15 (20)	
	D3S587	3p26–p24	9/16 (56)	
3q	D3S1215	3q12	3/19 (16)	3/19 (16)
4p	HOX7	4p16.3–p16.1	2/10 (20)	2/10 (20)
4q	D4S392	4q12–q13	3/16 (19)	3/16 (19)
5p	D5S117	5p15.3–p15.1	3/11 (27)	8/20 (40)
	D5S392	5p15.3–pter	5/17 (29)	
5q	D5S118	5qcen–q11.2	2/13 (15)	**10/22 (45)**
	D5S107	5q11.2–q13.3	8/17 (47)	
	D5S346	5q21–q22	6/18 (33)	
	D5S404	5q23.3	2/15 (13)	
	D5S421	5q23.3	3/15 (20)	
	IL9	5q22.9–q32.1	4/12 (33)	
	D5S209	5q31.3–q33.3	2/10 (20)	
6p	D6S470	6p25	3/18 (17)	3/18 (17)
6p, 6q	TRM1	6p23–q12	1/6 (17)	
6q	D6S305	6q	1/12 (8)	1/12 (8)
7p	D7S531	7p22–pter	1/13 (8)	1/13 (8)
7q	D7S473	7q	0/13 (0)	1/15 (7)
	D7S550	7q31–qter	1/11 (9)	
8p	D8S57	8p12	1/12 (8)	5/21 (24)
	ANK1	8p21.1–p11.2	2/11 (18)	
	D8S261	8p23–p11	2/18 (11)	
8q	D8S164	8q13–q22.1	1/15 (7)	1/15 (7)
9p	D9S200	9p21–p12	1/11 (9)	**12/23 (52)**
	D9S104	9p21	1/10 (10)	
	D9S161	9p21.1–p21.3	2/11 (18)	
	D9S171	9p21.3–p21.1	8/16 (50)	
	D9S162	9p23–p22	1/13 (8)	
	D9S157	9p23–p22.1	4/19 (21)	
	D9S156	9p23.3–p22.1	3/9 (33)	
	D9S199	9p23.3–p23.1	0/6 (0)	
	D9S51	9p22.3–p33	0/13 (0)	
9q	D9S103	9q33–qter	3/10 (30)	5/16 (31)
	D9S67	9q34–qter	3/11 (27)	
10p	D10S249	10p	2/11 (18)	2/11 (18)
10q	D10S212	10qter	1/11 (9)	1/11 (9)
11p	D11S554	11p12–p11	2/13 (15)	**14/23 (61)**
	WT1	11p13	0/13 (0)	
	D11S865	11p13–p14	2/16 (13)	
	D11S419	11p15.4–p13	3/7 (43)	
	D11S875	11p15.4–p13	3/13 (23)	
	TH	11p15.5	3/12 (25)	
	HRAS	11p15.5	6/16 (38)	
11q	DRD2	11q23.1	4/16 (25)	4/16 (25)
12p	D12S61	12p	1/15 (7)	5/19 (26)
	D12S94	12pter–p13.2	4/12 (33)	
12q	D12S63	12qter	1/6 (17)	2/9 (22)
	D12S43	12q12–q24.1	1/8 (43)	
13q	D13S217	13q12	3/13 (23)	**11/22 (50)**
	D13S157	13q13	1/9 (11)	
	D13S220	13q13	6/17 (35)	
	D13S175	13q11–q13	3/12 (25)	
	D13S168	13q11–q22	4/13 (30)	
	Rb	13q14.1–q14.2	5/16 (31)	
	D13S166	13q21	0/11 (0)	
	D13S71	13q32	0/12 (0)	
14q	D14S47	14q11.2–q22	3/13 (23)	3/15 (20)

Table 1 cont.

Chromosome arm	Primer	Site	LOH/informative cases (%)	Total LOH on each arm (%)
	D14S51	14q32.1–q32.2	0/9 (0)	
15q	CYP19	15q21.1	1/13 (8)	4/19 (21)
	D15S87	15q25–qter	3/13 (23)	
16p	HBAP1	16p13.3	3/17 (18)	3/17 (18)
16q	D16S303	16p24.3	1/11 (9)	1/11 (9)
17p	D17S935	17p11.1	3/5 (60)	**22/23 (96)**
	D17S959	17p11.1	5/12 (42)	
	TCF2	17p11.1–p12	10/17 (59)	
	D17S261	17p11.1–p12	6/14 (43)	
	D17S842	17p11.2	5/10 (50)	
	D17S58	17p11.2	5/6 (83)	
	CHRNB1	17p12–11.1	5/11 (45)	
	D17S953	17p12–11.2	1/6 (17)	
	D17S122	17p12–p11.2	3/12 (25)	
	D17S805	17p12	8/16 (50)	
	D17S520	17p13–p12	9/19 (47)	
	TP53	17p13.1	8/17 (47)	
	D17S740	17p13.1	2/7 (29)	
	D17S799	17p13.1–p12	7/12 (59)	
	D17S922	17p13.1–p12	3/8 (38)	
	D17S955	17p13.1–p12	1/6 (16)	
	D17S839	17p13.1–p12	1/11 (9)	
	D17S921	17p13.1–p12	1/10 (10)	
	D17S578	17p13.3–q11.2	2/8 (25)	
17q	D17S783	17q11.2	0/9 (0)	**12/22 (55)**
	D17S798	17q11.2	3/18 (17)	
	D17S841	17q11.2	0/7 (0)	
	D17S250	17q11.2–q12	3/16 (19)	
	THRA1	17q11.1–q12	2/18 (11)	
	MPO	17q21.3–q22	1/8 (13)	
	GP3A	17q21.32	3/12 (25)	
	D17S940	17q23	0/4 (0)	
	D17S515	17q23–q25	0/9 (0)	
	AFMc008wel	17q24	2/7 (17)	
	D17S801	17q25	1/4 (25)	
	D17S928	17q25–qter	5/20 (25)	
18p	D18S52	18pter–p11.2	2/11 (18)	6/19 (32)
	D18S59	18pter–p11.2	4/15 (27)	
18q	D18S43	18q	0/8 (0)	**16/23 (70)**
	D18S34	18q12.2–q12.3	5/17 (29)	
	DCC	18q21.1	4/13 (30)	
	D18S35	18q21.1–q21.3	5/16 (31)	
	D1838	18q21.31	3/10 (30)	
	D18S42	18q22.1	0/8 (0)	
	D18S70	18q23–qter	6/17 (35)	
19p	D19S20	19p13.3	1/10 (10)	1/10 (10)
19q	D19S180	19q13.4	0/17 (0)	0/17 (0)
20p	D20S57	20p13	0/18 (0)	0/18 (0)
20q	D20S120	20q13.3	2/20 (10)	2/20 (10)
21q	D21S156	21q22.3	2/11 (18)	2/11 (18)
22q	IL-2RB	22q13	1/7 (14)	1/7 (14)

Percentage LOH at specific sites

The chromosomal arms previously identified as demonstrating high LOH in other tumours were examined using at least seven primers (range 7–19).

Fifty-four per cent of Barrett's adenocarcinomas demonstrated LOH at 3p26–p24 (D3S587), which spans the site of the von Hippel–Lindau (*VHL*) tumour-suppressor gene. LOH on chromosome 5q mainly occurred at two sites: half of oesophageal adenocarcinomas demonstrated LOH at 5q11.2–q13.3 (D5S107), which encompasses the site of *MSH3*, a DNA mismatch repair gene, and one-third were found to have LOH at 5q21–q22 (D5S346), the site of the adenomatous polyposis coli (*APC*) tumour-suppressor gene. The D9S171 (9p21.3–p21.1) primer identified LOH in 8 of 16 informative cases. 9p21.3–p21.1 spans the site of the cyclin-dependent kinase inhibitor 2A and 2B (*CDKN2*) tumour-suppressor genes. LOH on chromosome arm 9p was significantly correlated with LOH on 5q ($P = 0.008$, Pearson correlation coefficient) and LOH on 18q ($P = 0.015$, Pearson correlation coefficient). Fourteen of 23 cases of oesophageal adenocarcinoma had LOH detected on chromosome arm 11p, with half of these cases demonstrating LOH at 11p15.5, the site of the *H-ras* oncogene. LOH on 13q occurred in 8 of 16 tumours and the site of greatest LOH on 13q was at the retinoblastoma (*Rb*) locus (30%). The most common site of LOH

Table 2 Comparison of three allelotype analyses of adenocarcinoma of the oesophagus

	No. of primers used			LOH (%)		
Chromosome	Dolan	Barrett	Hammoud	Dolan	Barrett	Hammoud
1p	1	1	1	0	41	20
3p	8	1	1	64	33	26
4q	1	1	1	19	16	54
5q	7	1	1	45	80	18
9p	9	1	1	52	64	27
11p	7	1	1	61	17	5
13q	8	2	1	50	43	15
17p	19	2	1	96	100	63
17q	13	1	1	55	24	25
18q	7	2	1	67	43	40

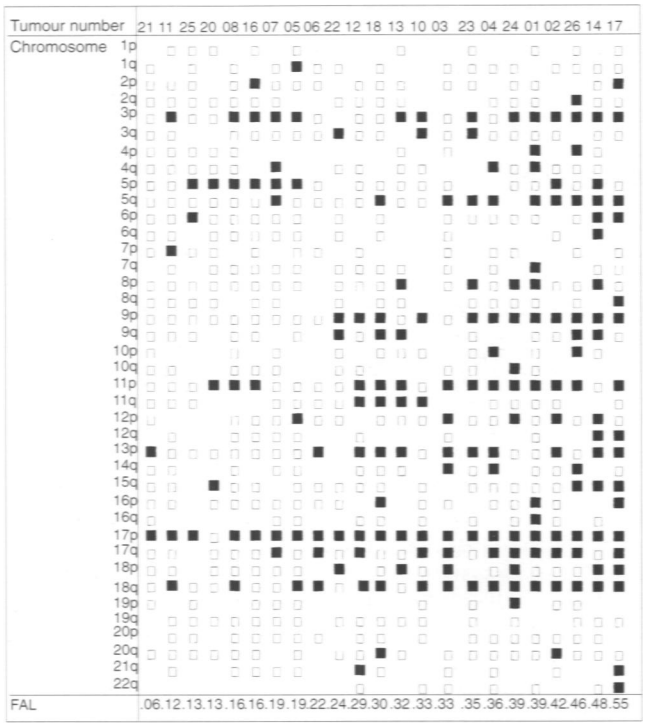

Figure 3 Percentage of tumours demonstrating loss of heterozygosity in each chromosomal arm.

was on chromosome arm 17p, where 22 of 23 adenocarcinomas demonstrated LOH. LOH was detected in 10 of 17 informative tumours (59%) with the TCF2 primer (17p11.1–p12), and in 8 of 17 tumours (47%) with the TP53 primer (17p13.1), the site of the *TP53* tumour-suppressor gene. LOH on 17q occurred in 55% of tumours (12 of 22 cases), and was always associated with LOH on 17p. Five of 20 tumours demonstrated LOH on 17q25–qter (D17S928). The deleted in colon cancer (*DCC*) tumour-suppressor gene is located at 18q21.1, and LOH using the *DCC* microsatellite primer was detected in 4 of 13 (31%) oesophageal adenocarcinomas. LOH was also detected in 5 of 16 cases (31%) at 18q21.1–q21.3 (D18S35).

Microsatellite alterations

Nine of 23 cases (39%) of oesophageal adenocarcinoma demonstrated microsatellite alterations with 13 different microsatellite primers from a total of 70 primers studied. However, only two cases displayed microsatellite alterations with more than two primers. Seven of nine cases with microsatellite alterations also demonstrated LOH on chromosome arm 9p (P = 0.048, Fisher exact test), and six cases demonstrated LOH at 9p21, the site of the *CDKN* tumour-suppressor genes. Interestingly, only two cases displaying microsatellite alterations also demonstrated LOH at 5q11.1–q13.3 (D5S107), the site of the mismatch repair gene *MSH3*.

FAL

FAL was calculated for each tumour as the number of chromosomal arms displaying LOH divided by the number of informative chromosomal arms, and it reflects the quantity of genetic abnormality in each tumour. The median FAL was 0.30 and the mean FAL was 0.29, indicating that on average each tumour demonstrated LOH on 29% of its chromosomal arms. FAL was not significantly related to survival, TNM classification or grade of tumour. However, it is of note that tumours displaying LOH at the sites of the *CDKN2* and *MSH3* genes had significantly higher FAL values than tumours retaining heterozygosity at these sites (P = 0.003 and P = 0.015 respectively, Fisher's exact test).

Survival

Survival was not significantly affected by the FAL of each tumour, nor by LOH on individual chromosomes.

DISCUSSION

This represents the most in-depth study to date of allelic imbalance in oesophageal adenocarcinoma. In 23 cases of oesophageal adenocarcinoma, LOH on chromosomes 3p, 5q, 9p, 11p, 13q, 17p, 17q and 18q was significantly greater than LOH on other chromosomes. The majority of LOH (78%) was detected in only one of the p or q arms for each chromosome, suggesting that subchromosomal events are mainly responsible for LOH.

A previous allelotype analysis of adenocarcinoma of the oesophagus found significant LOH on chromosome arms 5q, 9p, 13q and 17p (Barrett et al, 1996*a*), which is in agreement with this study (Table 2). Although a previous study has documented LOH on chromosome 4q in more than 50% of oesophageal adenocarcinomas (Hammoud et al, 1996), both our own and Barrett's allelotype study reported that fever than 20% of adenocarcinomas demonstrated LOH on 4q. We also detected significant LOH on chromosome arms 3p, 11p, 17q and 18q in our study, most probably due to the use of a greater number of microsatellite primers for each chromosomal arm in our study. In our study, microdissection was used to minimize stromal cell contamination of the tumour DNA, and this may also have contributed to our high LOH findings. Other investigators have used flow cytometry to separate aneuploid cells for use in LOH studies (Barrett et al, 1996*a*), although not all oesophageal carcinomas exhibit aneuploidy on DNA analysis (Dorman et al, 1992; Porschen et al, 1993; Blount et al, 1994) and the sensitivity and specificity of the detection of aneuploidy by flow cytometry is only 79% and 60% respectively

(Walsh et al. 1992). Hence, not all oesophageal carcinomas will be amenable to this method of tumour cell procurement.

Chromosome 3

LOH on 3p has been detected in a variety of tumours, e.g. pulmonary (Sozzi et al. 1996), gastrointestinal (Ohta et al. 1996) and ovarian (Chuaqui et al. 1996), and in our study of oesophageal adenocarcinoma 64% of tumours displayed LOH. The region of greatest LOH on 3p was at 3p26–p24 (primer D3S587), which contains the *VHL* tumour-suppressor gene locus. A previous study failed to show any involvement of the *VHL* tumour-suppressor gene in squamous cell carcinoma of the upper aerodigestive tract (Waber et al. 1996), but allelic loss of the *VHL* gene has been described in sporadic colon cancer (Zhuang et al. 1996) and sporadic renal cell carcinomas (Van den Berg et al. 1996). LOH at 3p25 has been shown to be associated with lymph node metastases in squamous cell carcinoma of the oesophagus (Ogasawara et al. 1995): 7 of 13 Barrett's adenocarcinomas displayed LOH at 3p26–p24 in this study, and six of these seven tumours had positive nodes. It is likely that 3p26–p24 contains a tumour-suppressor gene involved in oesophageal carcinogenesis, but whether it is the *VHL* tumour-suppressor gene or a nearby novel tumour-suppressor gene remains to be determined.

Chromosome 5

LOH on chromosome arm 5q was detected in 9 of 20 cases (45%) of oesophageal adenocarcinoma, and this LOH was concentrated in two sites. One-third of tumours demonstrated LOH at 5q21–q22 (D5S346), the site of the *APC* tumour-suppressor gene. LOH at the *APC* tumour-suppressor gene has previously been described in 23 of 61 cases (38%) of squamous cell carcinoma of the oesophagus (Ogasawara et al. 1996). However, single-strand conformation polymorphism analysis found only one *APC* mutation in 35 cases of oesophageal squamous cell carcinoma, and one *APC* mutation in 18 cases of oesophageal adenocarcinoma (Powell et al. 1994). Hence the role, if any, of the *APC* tumour-suppressor gene in oesophageal adenocarcinoma is yet to be determined. 5q11.2–q13.3 (D5S107) represented the site of greatest LOH on chromosome 5q, with 8 of 17 oesophageal adenocarcinomas demonstrating LOH. *MSH3*, a mismatch repair gene, has been mapped to 5q11–q12, and may be the target of LOH detected by D5S107 in oesophageal adenocarcinoma. Of the eight tumours demonstrating LOH at this site, two also displayed microsatellite alterations with other primers.

Chromosome 9

Eight of 16 tumours displayed LOH at 9p21.3–p21.1 (D9S171), and LOH at this site has previously been detected in 24 of 32 adenocarcinomas of the oesophagus (Barrett et al. 1996*b*). DNA sequencing has detected mutations in the *CDKN2* tumour-suppressor genes in both adenocarcinoma and squamous cell carcinoma of the oesophagus (Zhou et al. 1994; Barrett et al. 1996*b*). The target of LOH at 9p21.3–21.1 is most likely to be the *CDKN2* tumour-suppressor genes, but confirmatory mutational analysis is required. Three of 23 cases of oesophageal adenocarcinoma in our study were classified as T1 N0 M0, and two of these tumours demonstrated LOH at the site of the *CDKN2* tumour-suppressor genes. Hence, allelic loss at the site of the *CDKN2* tumour-suppressor genes is a frequent and perhaps early event in oesophageal carcinogenesis, and deserves further study as a potential marker of carcinogenesis in patients with Barrett's oesophagus.

Chromosome 11

The *HRAS1* primer was used to detect 38% LOH at 11p15.5 in oesophageal adenocarcinoma, and has previously been used to demonstrate LOH in 40% of squamous cell carcinomas of the oesophagus (Shibagaki et al. 1994). LOH at 11p15.5 has also been demonstrated in adenocarcinoma of the stomach (Baffa et al. 1996), and candidate tumour-suppressor genes in this region include *WT2* and *H19*, loss of which have been described in Wilms' tumours (Besnard-Guerin et al. 1996) and in cervical cancer (Douc-Rasy et al. 1996) respectively. This area on 11p obviously requires further study in oesophageal and other malignancies.

Chromosome 13

LOH at the *Rb* locus was detected in 5 of 16 cases (31%) of oesophageal adenocarcinoma, which is similar to the 36% LOH detected in a previous study of 14 cases of oesophageal adenocarcinoma (Boynton et al. 1991).

Chromosome 17

Twenty-two of 23 oesophageal adenocarcinomas had LOH detected on chromosome 17p, and 8 of 17 tumours demonstrated LOH at the site of the *TP53* tumour-suppressor gene (*TP53* primer). Previous studies have detected LOH on chromosome 17p in 14 of 14 (Neshat et al. 1994), 30 of 31 (Blount et al. 1994) and 11 of 16 (Gleeson et al. 1995) oesophageal adenocarcinomas. Two of three intramucosal adenocarcinomas (T1 N0 M0) in our study demonstrated LOH at the site of the *TP53* tumour-suppressor gene, suggesting that LOH at this site is an early event in oesophageal carcinogenesis. Similarly, *TP53* mutations have been detected in HGD adjacent to adenocarcinomas (Hamelin et al. 1994; Gleeson et al. 1995). The *TP53* gene merits further study as a marker of carcinogenesis in patients with Barrett's oesophagus. LOH was detected in 59% of informative tumours with the TCF-2 primer and in 43% of tumours with D17S261, markers at 17p11.1–p12, indicating the presence of a putative tumour-suppressor gene, originally reported by Swift et al (1995).

BRCA1 is a tumour-suppressor gene located at 17q21, and 3 of 12 oesophageal adenocarcinomas demonstrate LOH at this site. Five of 20 cases displayed LOH at 17q25–qter (D17S928), but LOH was not detected in the intervening region 17q23 (D17S940). LOH at 17q25 has been described in breast and ovarian carcinomas (Kalikin et al. 1997).

Chromosome 18

The *DCC* tumour-suppressor gene is most commonly inactivated in carcinoma of the colon (Fearon et al. 1990). In our study, LOH at the *DCC* locus was detected in 4 of 13 (31%) oesophageal adenocarcinomas, which is similar to the 29% detected previously (Huang et al. 1992).

FAL and genomic instability

The median FAL for oesophageal adenocarcinoma was 0.30 (0.06–0.55). This is significantly higher than the FAL of 0.20

detected for colorectal carcinoma (Vogelstein et al, 1989), head and neck (FAL of 0.22) (Field et al, 1995) and non-small-cell lung cancer (FAL of 0.09) (Neville et al, 1996), but is similar to a FAL of 0.28 calculated for 20 cases of oesophageal adenocarcinoma (Barrett et al, 1996*a*). This higher FAL suggests that a greater degree of genetic abnormality occurs in oesophageal adenocarcinoma than occurs in colorectal carcinoma. FAL was not significantly related to survival, grade or TNM classification of the tumours. This is in agreement with studies of squamous cell carcinoma of the oesophagus (Shibagaki et al, 1994) and of osteosarcomas (Yamaguchi et al, 1992), in which the FAL was not related to the clinicopathological parameters of the tumours. It is probable that, with respect to the stage of the tumour and its prognosis, the quantity of the genetic abnormalities is less important than the actual site of the mutations. In fact, tumours demonstrating LOH at 9p21.3–p21.1 (which span the sites of the *CDKN2* tumour-suppressor genes) had a significantly greater FAL than those retaining heterozygosity at this site. It is also of note that six of nine patients displaying microsatellite alterations also demonstrated LOH at the site of the *CDKN2* tumour-suppressor genes. Hence, allelic inactivation at 9p21.3–p21.1 increases the probability of mutations at other sites, and may be associated with widespread genomic instability. Similarly, LOH at 5q11.2–q13.3 (*MSH3* mismatch repair gene) was associated with a high FAL, and there was a significant correlation between LOH on 5q and 9p. LOH at the sites of the *CDKN2* and *MSH3* genes tend to occur together, and are associated with LOH at multiple sites, with allelic loss at *CDKN2* also being correlated with microsatellite alterations. Overall, however, the level of microsatellite alterations detected in oesophageal adenocarcinoma was low, with 39% of tumours demonstrating alterations and only two tumours demonstrating alterations with more than two microsatellite primers. Other studies have also found low levels of microsatellite alterations in adenocarcinoma of the oesophagus (Keller et al, 1995; Gleeson et al, 1996) and of the stomach (Dos Santos et al, 1996). These low levels of microsatellite alterations in adenocarcinoma of the upper gastrointestinal tract may reflect that the mutator phenotype is acquired late in the carcinogenesis sequence.

In conclusion, there are eight chromosomal arms demonstrating a significantly high level of LOH in adenocarcinoma of the oesophagus: 3p, 5q, 9p, 11p, 13q, 17p, 17q and 18q. Significantly high LOH occurred at the sites of the *VHL*, *CDKN2* and *TP53* tumour-suppressor genes, and the site of the *MSH3* mismatch repair gene. A lesser degree of LOH also occurred at the sites of the *APC*, *Rb* and *DCC* tumour-suppressor genes. LOH was detected at 11p15.5 and 17q25–qter, and these areas represent putative sites of novel tumour-suppressor genes. LOH at the sites of the *CDKN2* and *TP53* tumour-suppressor genes occurred in two of three intramucosal carcinomas studied, and may be useful as biomarkers of early carcinogenesis in patients with Barrett's oesophagus.

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