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Allelotype of squamous cell carcinoma of the head and neck: fractional allele loss correlates with survival

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Summary Allelic imbalance or loss of heterozygosity (LOH) studies have been used extensively to identify regions on chromosomes that may contain putative tumour-suppressor genes. We have undertaken an extensive allelotype of 80 specimens of squamous cell carcinoma of the head and neck (SCCHN) using 145 polymorphic microsatellite markers on 39 chromosome arms. Allelic imbalances were found most frequently on chromosome arms 3p, 9p, 17p and 18q with over 45% LOH and imbalances on 1p, 1q, 2p, 5q, 6p, 6q, 8p, 8q, 9q, 11q, 13q, 17q and 19q were found in more than 20% of SCCHN. These LOH data were analysed against a range of clinicopathological parameters which included previously untreated and previously treated tumours; correlations were found between LOH on 9q and nodes at pathology ($P = 0.02$) and between histopathological grade and LOH on 12q ($P = 0.02$) and 13q ($P = 0.01$). In the group of previously untreated tumours, a correlation was found between site of tumour and LOH on 3p ($P = 0.019$), and 8p ($P = 0.029$), while TNM staging correlated with LOH on 3p ($P = 0.019$) and 17p ($P = 0.016$). Fractional allele loss (FAL) was calculated for 52 tumours with LOH data on nine or more chromosomal arms and found to have a median value of 0.22 (range 0.0-0.80). Correlations were found between FAL > median value and nodes at pathology ($P = 0.01$) and tumour grade ($P = 0.06$), demonstrating that advanced tumours with lymph node metastasis often had LOH at multiple sites. FAL > median value was found to correlate with a poor survival ($P < 0.03$) and, furthermore, FAL > median value correlated with poor survival in the previously untreated patients ($P < 0.019$). These results indicate that assessment of the accumulation of genetic damage, as provided by allelotype data, provides a useful molecular indicator of the tumour behaviour and clinical outcome.

Keywords: head and neck cancer; oral cancer; allelotype; microsatellites; loss of heterozygosity; fractional allele loss

The chromosomal locations of many different putative human tumour-suppressor genes have been identified by loss of heterozygosity (LOH) studies (Rees *et al.*, 1989; Lasko *et al.*, 1991; Latif *et al.*, 1992; Cunningham *et al.*, 1993; Adamson *et al.*, 1994). A number of oncogenic and tumour-suppressor gene functions have been demonstrated in squamous cell carcinoma of the head and neck (SCCHN) (Field *et al.*, 1989, 1991; Field, 1992), and the results of LOH analysis currently point to several novel tumour-suppressor gene sites in this disease (Maestro *et al.*, 1993; Adamson *et al.*, 1994; Ah-See *et al.*, 1994; Field *et al.*, 1994; Kiaris *et al.*, 1994; Li *et al.*, 1994; Loughran *et al.*, 1994; Merlo *et al.*, 1994; Nawroz *et al.*, 1994; van der Reit *et al.*, 1994).

To date only two global analyses of the whole genome have been undertaken with the view to determine the fractional allele loss (FAL) of specific tumours and thus provide information concerning the 'genetic burden' of the disease during its progression as measured by clinicopathological parameters and survival data. This type of analysis has been undertaken in colorectal (Vogelstein *et al.*, 1989) and bladder cancers (Knowles *et al.*, 1994), and provides an indication of interacting genetic mechanisms in the development of these diseases. In addition, the results of such detailed allelotypes may aid the interpretation of carcinogenesis and the development of molecular progression models for specific tumours.

We have undertaken a very comprehensive allelotype of SCCHN using 145 microsatellite markers in order to identify common regions of allelic imbalance and to analyse the interactions of these regions by calculating the fractional allele loss (FAL) in these tumours.

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Materials and methods

Specimens

Eighty SCCHN tumour specimens were collected at the Royal Liverpool University Hospital, Department of Otorhinolaryngology and at the Walton Hospital Liverpool, Maxillofacial Unit. Tumour samples obtained from surgical specimens were frozen in liquid nitrogen and stored at −70°C. The clinicopathological data on the 52 SCCHN used in fractional allele loss analysis is given in Table I. This group of patients had LOH information on nine or greater (9-39) chromosome arms.

DNA extraction

All the tumour specimens used for LOH analysis were microdissected to yield at least 60% tumour cells before DNA preparation. Genomic DNA was extracted from tumour specimens using the Nucleon II DNA extraction kit (Scotlab) following the manufacturer's instructions. Genomic DNA samples were stored at 4°C.

PCR and LOH analysis

Microsatellite repeat primers were obtained from Isogen (The Netherlands). PCR reactions were performed in a 25 µl reaction volume and contained 200 ng of genomic DNA, 200 µM each dNTP, 5 pmol each of forward and reverse primers,

Table I Clinicopathological characteristics of the 52 squamous cell carinomas investigated on nine or more chromosomal arms

Patient number	*History*	*TNM*	*Histology*	*Nodes at pathology*	*Survival (months)*	*Fate*	*FAL[a] ≤ Median*
0128	PT	TI	Moderate	−ve	8	Died of disease	0.00
0041	PU	TII	Well	−ve	35	Alive	0.00
1052	PU	TIV	Moderate	−ve	29	Alive	0.00
0353	PU	TIV	Moderate	−ve	12	Died of disease	0.05
1091	PU	TIII	Well	−ve	21	Alive	0.07
0302	PU	TIII	Moderate	−ve	32	Alive	0.10
0310	PT	TIV	Moderate	−ve	5	Alive	0.10
0223	PT	TI	Poor	−ve	44	Alive	0.11
0325	PU	TIV	Moderate	+ve	27	Alive	0.11
1086	PU	TIV	Poor	+ve	17	Alive	0.11
0342	PU	TIII	Moderate	+ve	18	Alive	0.11
0339	PT	TI	Moderate	−ve	68	Alive	0.13
0359	PT	TIII	Moderate	−ve	12	Alive	0.13
0204	PU	TIII	Moderate	−ve	21	Died of disease	0.14
1087	PU	TIV	Moderate	−ve	22	Alive	0.15
1101	PU	TII	Well	−ve	17	Alive	0.15
0350	PT	TIII	Moderate	+ve	11	Alive	0.15
1075	PU	TII	Moderate	−ve	23	Alive	0.17
0358	PU	TIV	Moderate	+ve	12	Alive	0.17
0336	PU	TIII	Well	−ve	2	Died of disease	0.19
0318	PT	TIII	Moderate	−ve	34	Alive	0.20
0340	PT	TIII	Moderate	−ve	10	Alive	0.20
0361	PU	TII	Moderate	+ve	8	Alive	0.20
0315	PU	TIV	Moderate	+ve	99	Alive	0.21
1062	PU	TI	Moderate	No data	7	Alive	0.21
							> Median
0215	PU	TI	Well	−ve	36	Alive	0.22
0347	PT	TIII	Moderate	−ve	48	Alive	0.22
1164	PT	TIV	Poor	−ve	21	Died of disease	0.22
0305	PU	TIV	Moderate	+ve	13	Died of disease	0.23
0225	PT	TIII	Moderate	−ve	87	Died of disease	0.23
0228	PT	TIV	Poor	No data	56	Alive	0.24
0202	PT		Moderate	+ve	74	Alive	0.28
0224	PU	TIII	Moderate	+ve	20	Alive	0.30
0327	PU	TIV	Moderate	+ve	25	Alive	0.30
0313	PU	TIV	Moderate	+ve	8	Alive	0.31
0346	PU	TIV	Moderate	+ve	17	Died of disease	0.33
0360	PU	TIV	Moderate	+ve	9	DOC	0.33
1084	PU	TII	Well	−ve	9	Died of disease	0.33
0352	PU	TIV	Moderate	+ve	11	Alive	0.33
0192	PU	TIV	Poor	+ve	14	Died of disease	0.35
0351	PU	TIV	Moderate	−ve	11	Alive	0.35
0348	PT	TIII	Not defined	+ve	15	Alive	0.35
0314	PU	TIV	Moderate	+ve	8	Died of disease	0.36
0343	PU	TIV	Moderate	+ve	19	Alive	0.38
0218	PU	TIV	Poor	+ve	5	Died of disease	0.41
0335	PT	TI	Moderate	+ve	18	Alive	0.44
0341	PU	TIII	Moderate	+ve	0	Died of disease	0.45
0329	PU	TIV	Moderate	+ve	7	Alive	0.46
0338	PU	TIV	Moderate	−ve	20	Alive	0.47
0184	PU	TIII	Poor	+ve	16	Died of disease	0.50
0324	PU	TIV	Moderate	+ve	12	Alive	0.56
0078	PT	TIII	Poor	−ve	22	Died of disease	0.80

PU, previously untreated; PT, previously treated; DOC, died of other causes. [a]FAL, fractional allele loss (number of chromosome arms lost divided by the number of chromosome arms examined). Median value of FAL in this group of SCCHN was 0.22.

0.5 units of *Taq* polymerase (Advanced Biotechnologies) and 2.5 µl 10 × buffer [670 mM Tris-HCl, pH 8.5; 166 mM ammonium sulphate; 67 mM magnesium chloride; 1.7 mg ml^{-1} bovine serum albumin (BSA); 100 µM β-mercaptoethanol; 1% (w/v) Triton X-100]. The reactions were denatured for 5 min at 95°C then the DNA was amplified for 30 cycles of 95°C for 30 s, 57°C for 30 s and 72°C for 30 s. Aliquots of 10 µl of the PCR product were electrophoresed for 10 h on a 10% non-denaturing polyacrylamide gel at 250 V and visualised by silver staining.

LOH or allelic imbalance was scored by direct visual comparison of the allelic ratios of the normal and tumour specimens. Examples of heterozygous, homozygous and LOH in tumour/normal SCCHN specimens are given in Figure 1. Complete loss or reduced intensity of one allele in the tumour was considered as LOH. In the cases where there was only a reduced intensity of one allele this was considered to be due to contamination of tumour tissue by normal stroma. It has been previously noted by Ah-See *et al.* (1994) that the PCR techniques used by a number of authors in similar studies cannot readily distinguish between allelic duplication or low-level amplification leading to loss of heterozygosity. This caveat has to be taken into consideration when interpreting these results and thus LOH may not necessarily be indicative of the presence of a tumour-suppressor gene.

Statistical analysis

Quantitative data were analysed by χ^2 or Fisher's exact test where appropriate. Survival curves were drawn using the Kaplan–Meier (1958) product limit estimate. Differences between survival times were analysed by the log-rank method (Peto *et al.*, 1976).

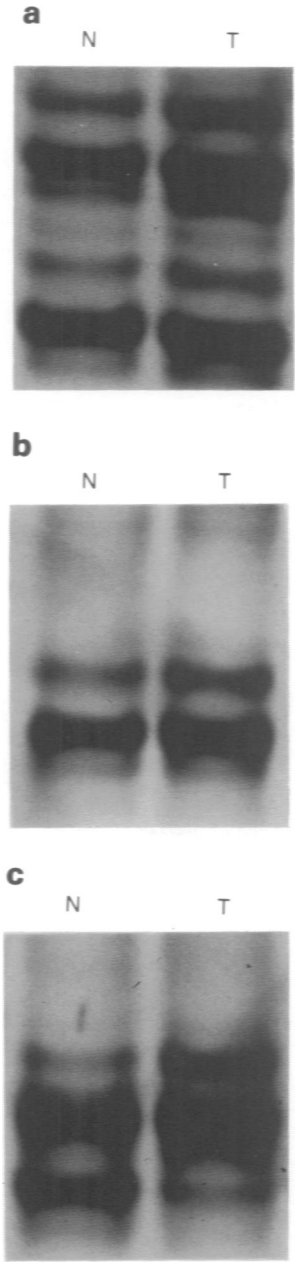

Figure 1 Representative figure of allelic imbalance (loss of heterozygosity). (**a**) Retention of heterozygosity. (**b**) Homozygosity. (**c**) Allelic imbalance (loss of heterozygosity), loss of the upper band in the tumour specimen. N, normal; T, tumour DNA. 'Stutter' or 'shadow bands' may be seen in both the normal and tumour lanes.

Results

A total of 80 SCCHN tumours were investigated for LOH using 145 microsatellite markers and loss on individual chromosome arms was calculated using the total data set (Table II). A total of 1092 chromosomal arms were analysed, of which 956 (88%) were informative. The most frequent losses were found on chromosome arms 3p, 9p, 17p and 18q, with over 45% LOH; losses on 1p, 1q, 2p, 5q, 6p, 6q, 8p, 8q, 9q, 11q, 13q, 17q and 19q were found in more than 20% of cancers (Figure 2).

Loss of heterozygosity at specific loci

The highest incidence of LOH was found on chromosome 9p (24/39) with a 62% loss and this allelic imbalance was especially concentrated between the *D9S161* (9p21) and *D9S156* (9p23–9p22) informative markers in this region (JK Field *et al.*, in preparation), which agrees with the observations of van der Riet *et al.* (1994).

The second highest percentage of allelic imbalance (52%) was found on chromosome arm 3p from 61 informative tumours. Using 18 markers we found the greatest loss in the 3p24–p25 and 3p13 regions and a very small incidence of LOH at the 3p21 site, including the *D3S1217* marker (3p21) which had a LOH of only 10% (Field *et al.*, 1994; JK Field *et al.*, unpublished).

Chromosome 17p revealed an LOH of 50% with the highest loss at the *CHRNB1* locus (17p12–p11.1). Furthermore, as previously reported by Adamson *et al.* (1994), LOH at this locus was found in 77% of the hypopharyngeal carcinomas studied.

Markers on 18q showed an overall LOH of 49% (20/41), however the main area of loss was not associated with the *DCC* marker at 18q21.1 but was found at *D18S35* (18q21.1–q21.3) (33%) (Rowley *et al.*, 1995).

Significant losses, 29% (13/45), were also found on chromosome arm 5q, eight markers were used, including *D5S346* (5q21–q22) in the *APC/MCC* region which showed 35% LOH (9/26). Six patients in this group of tumours showed loss only in this region, which was bounded in each case by informative heterozygous markers centromeric and telomeric of *D5S346*, thereby indicating that this region plays an important role in some SCCHN.

LOH on 8p has been demonstrated in a range of tumours with the 8p21.3–p11.22 region being of most interest. Our investigations using five microsatellite markers on 8p have shown 35% (14/40) LOH, which appears to be concentrated particularly at the *D8S87* (8p12) and *ANK1* (8p21.1–p11.2) loci (29% and 20% LOH respectively). However, when the results of these two markers were combined, 37% (13/35) LOH was demonstrated (Kiaris *et al.*, 1994).

LOH has also been observed on chromosome arm 1p in a range of tumours, with the 1p31.2–p21.3 region indicating that this may be a further target region in *SCCHN*. The cumulative loss of two markers in this region, *D1S159* (1p22.1–p21) and *D1S167* (1p22–p21), was 24% (14/46).

Chromosome 11 contains an amplicon region at 11q13 which includes the *int-2*, *cyclin D* and *EMS-1* genes. We have found 23% LOH (9/39) on the 11q arm and LOH at the *int-2* locus was 17% (3/18).

In this data set, whole chromosome loss was seen only on chromosome 17 and in four tumours: 78, 192, 225 and 335 (11% of cases). All of these chromosome arms showing LOH at greater than 20% (3p, 17p, 9p, 18q, 5q, 8p, 1p and 11q) have been previously shown to contain either known or putative tumour-suppressor genes. However, there are other arms in this study with greater than 20% LOH (1q, 2p, 6p, 6q, 8q, 13q, 17q and 19q) and these may also be target regions involved in the development of SCCHN.

LOH data analysed against a range of clinicopathological parameters

LOH data for each chromosomal arm were analysed against a range of clinicopathological parameters, including site of the tumour, histology, TNM staging, nodes at pathology and survival (Table I). These calculations were undertaken on the whole data set of 80 tumours (previously untreated and previously treated) and on the two subgroups separately. In the whole data set (80 SCCHN), correlations were found between nodes at pathology and LOH on 9q ($P = 0.020$) and between histopathological grading and LOH on 12q ($P = 0.022$) and on 13q ($P = 0.012$). In the group of previously untreated tumours, a correlation was found between site and LOH on 3p ($P = 0.032$) and 8p ($P = 0.029$), while TNM staging correlated with LOH on 3p ($P = 0.019$) and 17p ($P = 0.016$). Only one association was found in the group of previously treated patients, between nodes at pathology and LOH on 11p ($P = 0.045$) (Table III).

Fractional allele loss

LOH data from all 39 chromosome arms were assessed separately for the 52 SCCHN tumours which had inform-

Table II Loss of heterozygosity analysis of 145 microsatellite markers in SCCHN on 39 chromosomal arms

Chromosome arm	Locus	Localisation	Allele loss/ informative cases	Percentage LOH on each arm
1p	*D1S171*	1p36.3	10/37	27
	D1S186	1p35–p32		
	D1S162	1p31–p22		
	D1S159	1p22.3–p21		
	D1S167	1p22–p21		
	AMY2B	1p21		
1q	*D1S187*	1q12	5/18	28
	D1S176	1q12–q21		
	CRP	1q21–q23		
	D1S104	1q21.1–q23		
	D1S179	1q31–q42		
	ACTN2	1q42–q43		
2p	*TP*	2p25–p24	7/31	23
	D2S207	2p25–p23		
	D2S162	2p25–p22		
	D2S126	2p22–p12		
	MHC/CD8A	2p12		
2q	*IL1A*	2q13	2/16	13
	D2S103	2q23–q33		
	D2S104	2q33–q37		
3p	*D3S1435*	3pter–p24.2	32/61	52
	D3S587	3p26–p24		
	D3S1038	3p26.1–p25.2		
	D3S1304	3p25.1–p24.2		
	D3S656	3p25.1		
	D3S1252	3p25–p24.2		
	D3S1293	3p25–p24.2		
	THRB	3p24		
	D3S1266	3p25–p24		
	D3S1235	3p21.3–p21.2		
	D3S966	3p21.3–p21.2		
	D3S1289	3p21.2–p21.1		
	D3S1067	3p21.1–p14.3		
	D3S1217	3p21		
	D3S1261	3p14–p12		
	D3S1079	3p13		
	D3S659	3p13		
	D3S1284	3p13–p12		
3q	*D3S1269*	3q21	4/32	13
	RHO	3q21.3–q24		
4p	*D4S43*	4p16.3	1/13	8
	HOX7	4p16.3–p16.1		
	GABRB1	4p13–p12		
4q	*D4S392*	4q12–q13	2/15	13
	D4S194	4q25–q34		
	D4S243	4q31–q32		
5p	*D5S111*	5p14.1–p13.1	2/12	17
5q	*D5S118*	5cen–q11.2	13/45	29
	D5S357	5q11–q13.3		
	D5S107	5q11.2–q13.3		
	D5S346	5q21–q22		
	IL9	5q22.3–q31.3		
	D5S210	5q31.3–q33.3		
	D5S209	5q31.3–q33.3		
	D5S211	5q33.3–qter		
6p	*D6S344*	6p24	3/14	21
	TRM1	6p23–q12		
	D6S271	6p21.2–p21.1		
6q	*D6S286*	6q16.3–q21	6/24	25
	D6S262	6q22.3–q23.1		
	D6S281	6q27		
7p	*D7S531*	7p	1/13	8
7q	*D7S473*	7q	1/14	7
	D7S550	7q31–qter		
8p	*D8S201*	8pter–p22	14/40	35
	D8S265	8p23–p11		
	D8S261	8p23–p11		
	D8S87	8p12		
	ANK1	8p21.1–p11.2		
8q	*D8S166*	8q11.23–q12	7/33	21
	D8S164	8q13–q22.1		
	D8S88	8q22.1–q22.3		
	D8S85	8q23–qter		
	D8S198	8q23.1–qter		
	MYC	8q24.12–q24		
9p	*D9S54*	9pter–p22	24/39	62
	D9S199	9p23		
	D9S156	9p23.3–p22		
	D9S157	9p23–p22		
	D9S162	9p23–p22		
	IFNA	9p21		
	D9S171	9p21		
	D9S161	9p21		
	D9S200	9p21–p12		
	D9S104	9p21		
	D9S50	9p21–pter		
9q	*D9S51*	9q22.3–q33	6/30	20
	D9S103	9q33–qter		
	D9S67	9q34–qter		
10p	*D10S249*	10p	1/11	9
10q	*D10S109*	10q11.2–qter	2/16	13
	D10S212	10qter		
11p	*HRMS*	11p15.5	5/39	13
	TH	11p15.5		
	D11S875	11p15.4–p13		
	D11S419	11p15.4–p13		
11q	*INT2*	11q13.3	9/39	23
	DRD2	11q23.1		
	D11S439	11q23.3		
	D11S874	11q24–qter		
12p	*D12S94*	12pter–p13.2	1/7	14
12q	*D12S43*	12q12–q24.1	2/13	15
	D12S63	12q22–q24.33		
13q	*D13S115*	13q11–q12.1	8/30	27
	D13S175	13q11–q13		
	D13S168	13q11–q22		
	D13S165	13q		
	D13S155	13q		
	D13S71	13q32		
14q	*TCRD*	14q11.2	2/19	11
	D14S47	14q11.2–q22		
	D14S51	14q32.1–q32		
15q	*GABRB3*	15q11.2–q12	2/17	12
	CYP19	15q21.1		
	D15S87	15q32.1–qter		
16p	*HBAP1*	16p13.3	2/15	13
16q	*D16S303*	16q24.3	0/17	0
17p	*D17S578*	17p13.3–p11.2	18/36	50
	TP53	17p13.3		
	D17S520	17p13–p12		
	D17S799	17p13.1–p12		
	CHRNB1	17p12–p11.1		
	D17S122	17p12–p11.2		
17q	*TCF2*	17q11.2–q12	12/40	30
	GP3A	17q21.32		
	MPO	17q21.3–q23		
	D17S515	17q23–q25		
18p	*D18S59*	18pter–p11.22	5/31	16
	D18S52	18pter–p11.22		
	D18S40	18p11.21		

Table II–continued

Chromosome arm	Locus	Localisation	Allele loss/ informative cases	Percentage LOH on each arm
18q	*D18S34*	18q12.2–q12.3	20/41	49
	D18S35	18q21.1–q21.3		
	DCC	18q21.1		
	D18S38	18q21.31		
	D18S42	18q22.1		
	D18S43	18q22.3–q23		
	MBP	18q23		
	D18S70	18q23–qter		
19p	*D19S20*	19p13.3	0/9	0
19q	*D19S49*	19q12–q13.1	5/17	29
	D19S180	19q13.4		
20p	*D20S57*	20p13	0/14	0
20q	*D20S120*	20q	1/11	9
21q	*D21S156*	21q22.3	1/12	8
22q	*IL2RB*	22q11.2–q12	0/15	0

ation on nine or greater (range 9–39) chromosome arms. This subgroup of 52 tumours was composed of 36 previously untreated tumours and 16 previously treated tumours. Allelotypes derived from 145 microsatellite markers are presented diagrammatically in Figure 3. The fractional allele loss (FAL) in a tumour is defined as the number of chromosomal arms on which allelic imbalance was observed divided by the number of chromosomal arms for which markers were informative in the patient's normal cells (Vogelstein *et al.*, 1989). The FAL values for this group of 52 SCCHN showed a median value of 0.22 and a mean of 0.25 (range 0.0–0.80).

FAL values were assessed against the clinicopathological data (tumour site, tumour grade, TNM staging, nodes at pathology) by dividing the tumours into those with FAL > median value and those with FAL < median value. A positive correlation was found between FAL and nodes at pathology ($P = 0.01$) and between FAL and tumour grade ($P = 0.06$) (Table IV). This demonstrates that advanced tumours with lymph nodal metastasis often had LOH at multiple sites. No correlation was found between FAL and the patient's history of smoking or drinking (Table V).

The FAL data was also investigated for a possible association with clinical outcome using the log-rank analysis. It was found that a FAL > median value correlated with poor survival ($P < 0.032$), and furthermore that a FAL > median value also correlated with a poor survival in the previously untreated patients ($P < 0.019$) when analysed separately. In order to analyse a homogeneous group of patients for FAL with clinical outcome, we calculated the log rank on the subset of 40 advanced tumours (TNM III and IV) and this also demonstrated a correlation between FAL and prognosis ($P < 0.05$).

Discussion

In this detailed allelotype of SCCHN we have demonstrated a complex set of genetic alterations, a finding that has also been described in a range of human cancers (Vogelstein *et al.*, 1989; Sato *et al.*, 1990, 1991; Fujimori *et al.*, 1991; Morita *et al.*, 1991; Tsuchiya *et al.*, 1992; Yamaguchi *et al.*, 1992; Aoki *et al.*, 1994; Fujino *et al.*, 1994; Knowles *et al.*, 1994). The highest LOH was found on the chromosome arms 3p, 9p, 17p and 18q which is in general agreement with previous studies on SCCHN (Ah-See *et al.*, 1994; Nawroz *et al.*, 1994). Table VI provides an overview of the results

Table III LOH data analysed against a range of clinicopathological parameters

	Chromosome arm	Site	Nodes	TNM	Pathology
Total data ($n = 80$) previously treated/untreated	9q	NS	0.020	NS	NS
	12q	NS	NS	NS	0.022
	13q	NS	NS	NS	0.012
Previously untreated	3p	0.032	NS	0.019	NS
	8p	0.029	NS	NS	NS
	17p	NS	NS	0.016	NS
Previously treated	11p	NS	0.045	NS	NS

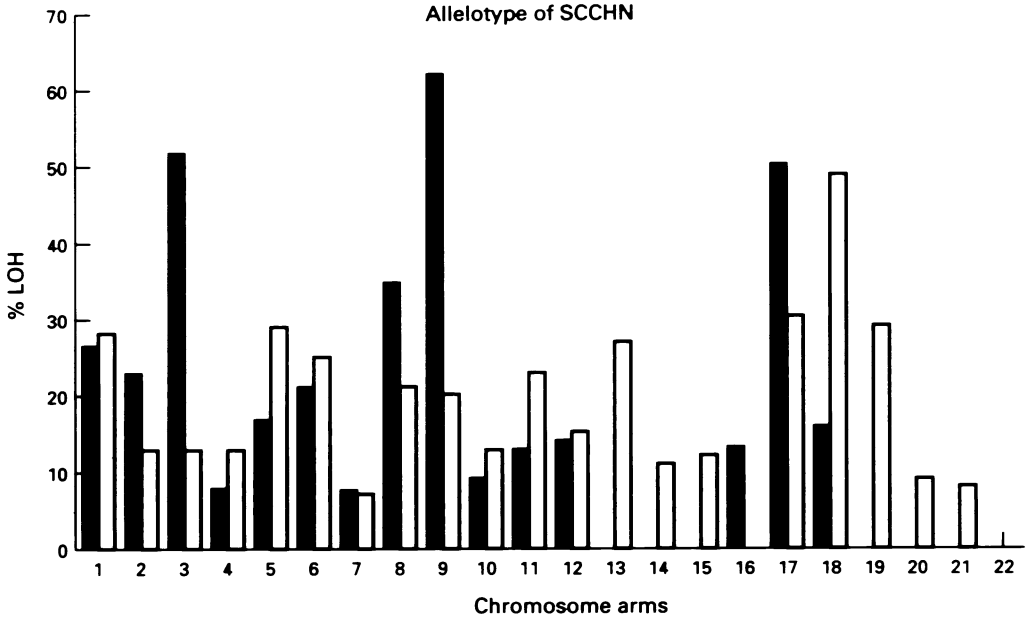

Figure 2 Frequency of allele loss on each chromosome arm in 80 squamous cell carcinomas of the head and neck. ■, p arm; □, q arm.

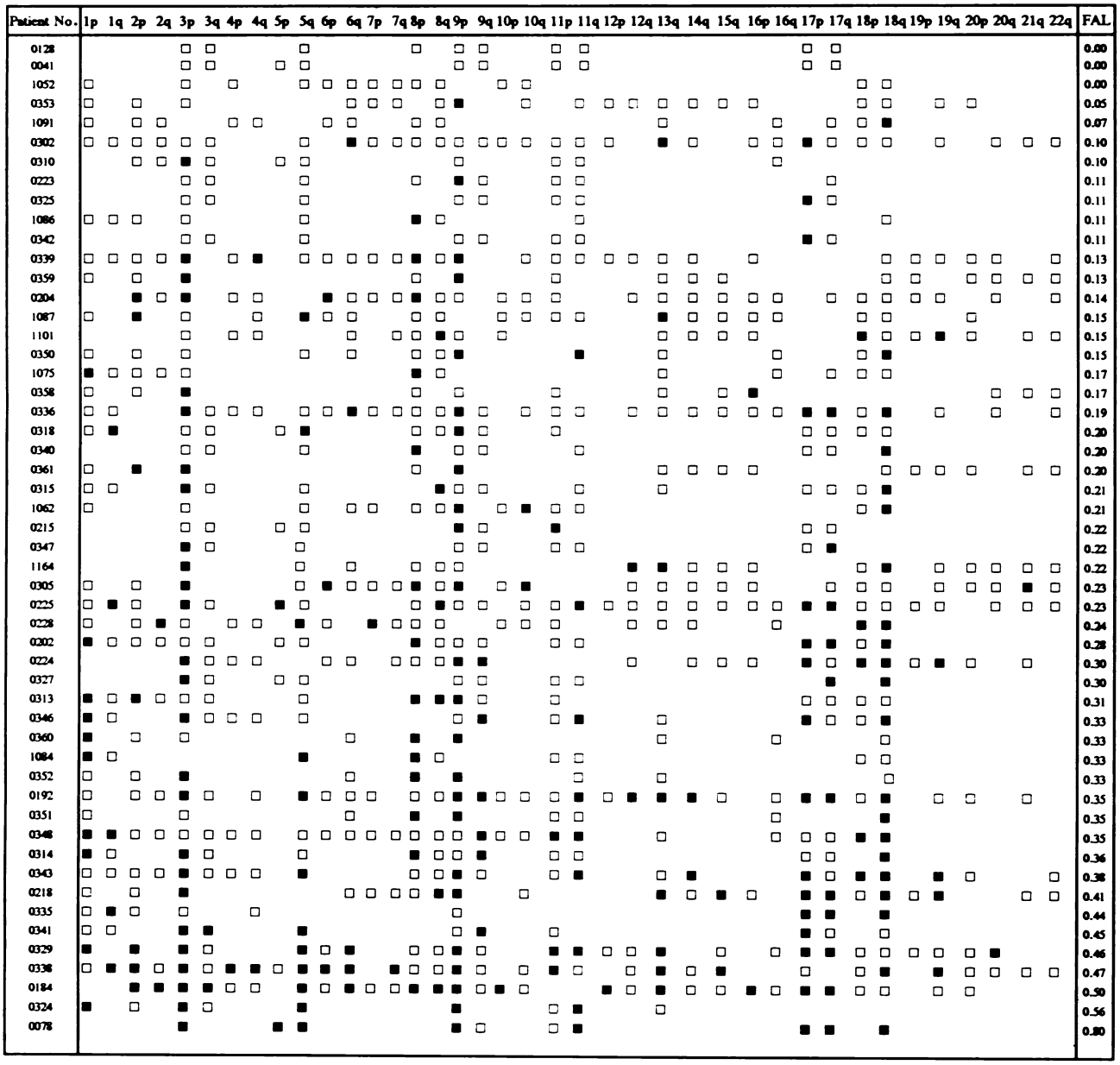

Figure 3 Individual allelotypes for 52 squamous cell carcinomas of the head and neck. These SCCHN tumours were investigated on nine or more informative chromosomal arms (range 9–39). The FAL (fractional allele loss) data has been given for each tumour specimen (range 0.0–0.80). ■, LOH; □, retention of heterozygosity; chromosome arms which were uninformative or not done are not shown. Each square represents the summation of the LOH results on a single chromosome arm using all of the informative markers, i.e. if there was allelic imbalance for any one of the markers tested for that specific chromosomal arm, then it is indicated as a filled square.

undertaken in this study in comparison with those in the two other allelotypes undertaken on SCCHN tumours. Both of the previous studies used about one-third of the number of markers used in this analysis: 58 markers (Nawroz *et al.*, 1994) and 52 markers (Ah-See *et al.*, 1994) respectively. Similar LOH values have been found at 3p, 5q, and 9q between Ah-See *et al.* (1994) and these data (± 15%), in the cases where the results may be compared. Also, similar LOH findings may be seen between Nawroz *et al.* (1994) and this data set for chromosome arms, 1p, 1q, 3p, 5q, 8p, 8q, 9p, 9q, 11p, 17p, 17q and 19q (± 15%). However, a number of differences (> ± 20%) do exist between the previous reports and our results on 9p, 11q, 13q and 18q. The percentage LOH for 11q varies from 23%, 45% to 61% in the three studies (Ah-See *et al.*, 1994; Nawroz *et al.*, 1994; these data respectively), however the two previous studies only used two markers on this chromosome arm. The data on 18q from Nawroz *et al.* (1994) based on one marker give an LOH of 29%, whereas eight markers have been used in this study, demonstrating an LOH of 49%. This analysis demonstrates

Table IV Association between FAL and nodes at pathology and tumour grade

	FAL		
Clinical parameter	*≤ Median value*	*> Median value*	P[a]
Nodes at pathology			
No nodes	17	8	
Positive nodes	7	18	0.01
TNM status			
TNM I and II	8	3	
TNM III and IV	16	23	0.06

[a]Fisher's exact *t*-test.

that the results of the three allelotypes on SCCHN agree in general, however when only a limited number of markers are chosen per arm there is a very high probability that a target region may be missed.

The results from this study provide further confirmation of

Table V Association between FAL and a history of smoking and drinking in patients with SCCHN

	Non-smoker	*Moderate smoker <20 per day*	*Heavy smoker >20 per day*	*Stopped smoking*	P[a]	*Non-drinker*	*Moderate <21 units per week*	*Heavy >21 units per week*	*Stopped drinking*	P[a]
FAL ≤ median	5	5	8	2		6	4	8	1	
FAL > median	5	3	9	4	0.7	4	6	10	1	0.9

[a]Fisher's exact *t*-test.

Table VI Comparison of LOH from three allelotypes of SCCHN

Chromosome arm	*Ah-See* et al. *(1994)* *No. of markers*	*LOH (%)*	*Nawroz* et al. *(1994)* *No. of markers*	*LOH (%)*	*This study* *No. of markers*	*LOH (%)*
1p	1	14[a]	2	30	6	27
1q	2	0[a]	1	23	6	28
3p	3	44	2	67	18	52
5q	4	43	1	25	8	29
8p	1	10[a]	1	40	5	35
8q	1	7[a]	2	38	6	21
9p	1	24[a]	3	72	11	62
9q	2	35	2	18	3	20
11p	1	5[a]	2	17	4	13
11q	2	45	2	61	4	23
13q	1	0[a]	2	54	6	27
17p	2	31	3	52	6	50
17q	1	19[a]	2	31	4	30
18q	1	0[a]	2	29	2	49
19q	1	0[a]	2	40	2	29

[a]Data taken from Figure 2, Ah-See *et al.* (1994).

target regions in SCCHN containing putative tumour-suppressor genes on 3p and 9p as well as a high LOH associated with the p53 gene. In addition, this analysis provides evidence for regions of minimal loss in SCCHN on 1p, 8p, 17p and 18q (Adamson *et al.*, 1994; Kiaris *et al.*, 1994; Rowley *et al.*, 1995; K Taylor *et al.*, in preparation). The 1p minimal area of loss has been located at 1p31.2–p21.3, a region previously shown by karyotype analysis to contain cytogenetic abnormalities (Jin *et al.*, 1990, 1993; Owens *et al.*, 1992). A minimal area of loss has also been identified on 8p between 8p12 and 8p21.2–p11 in this series of tumours, a region considered to contain a candidate tumour-suppressor gene in colonic and hepatocellular carcinomas (Fujiwara *et al.*, 1993; Cunningham *et al.*, 1994). We have recently described a novel region on 17p distinct from *TP53*, at *CHRNB1* (17q12–p11.1), in SCCHN which has a particularly high loss in hypopharyngeal carcinomas (77%) (Adamson *et al.*, 1994). Furthermore the detailed analysis of 18q has allowed us to identify a region at 18q21.1–q21.3 as a target region in SCCHN, which does not appear to be the *DCC* (deleted in colon cancer) gene as we found a very low LOH at the *DCC* locus. Thus it may be argued that there is a second tumour-suppressor gene in this region on 18q that is involved in SCCHN.

Two further chromosomal regions considered to contain tumour-suppressor genes in other neoplasms have not been shown to play an important role in SCCHN. Even though there is frequent LOH on 13q (Yoo *et al.*, 1994) there does not appear to be inactivation of the retinoblastoma gene, and it has been argued by these authors that there may be another tumour-suppressor locus at 13q14. Also, the *APC/MCC* locus on 5q, which has been demonstrated to be involved in colorectal carcinomas (Kinzler *et al.*, 1991) and has previously been shown to have a high LOH in SCCHN (Ah-See *et al.*, 1994), may not in fact be the target locus, as mutations in the *APC* gene have rarely been found in oral cancers (Uzawa *et al.*, 1994).

Analysis of LOH for each chromosomal arm was assessed against a range of clinicopathological parameters (Table III). In particular, a correlation was found between site and LOH on 3p and 8p, while TNM staging correlated with LOH on 3p and 17p in previously untreated tumours. Also, in the group of previously untreated and previously treated tumours a correlation was found between LOH on 9q and positive nodes at pathology, and histological grading correlated with LOH on 12q and 13q. In a detailed study undertaken by Lee *et al.* (1994), on chromosome 13 (using 13 markers), a correlation was found between LOH on 13q and lymph node metastasis. Moreover, these authors reported that they found similar LOH in a subset of the tumours in the adjacent non-malignant mucosa. However, no correlation between LOH at 13q and lymph node metastasis was observed in the study described here.

The phenomenon of microsatellite instability (MI) (Mao *et al.*, 1994; Field *et al.*, 1995) has been demonstrated in some of these SCCHN tumours, but no correlation was found between MI and LOH on any chromosome arm in this study. MI is therefore considered to be a separate pathogenic mechanism in the development of SCCHN.

The fractional allele loss (FAL) data were assessed for 52 tumours on which there was LOH information on nine or more chromosome arms. In this group we found a median FAL value of 0.22, mean of 0.25 (range 0.0–0.80). This demonstrates that alleles were lost on average from 25% of the chromosome arms in these tumours; a figure that is comparable with that obtained in non-small-cell carcinoma and colorectal carcinomas (0.2), bladder and breast carcinomas (0.11) and osteosarcarcinomas (0.32) (Sato *et al.*, 1990, 1991; Morita *et al.*, 1991; Tsuchiya *et al.*, 1992; Knowles *et al.*, 1994). The FAL values were compared with the clinicopathological data based on FAL ≤ median value and FAL > median value. A correlation was found between FAL and positive nodes at pathology ($P = 0.01$), a clinical parameter considered to be the most useful prognostic indicator in head and neck cancer. No statistical correlation was found between FAL and site, TNM staging or histological differentiation. A history of smoking and drinking has been correlated with overexpression and mutations in the p53 gene (Field *et al.*, 1991, 1994; Field, 1992; Brennan *et al.*, 1995), but no correlation has been found between these carcinogens and FAL in this analysis. In colorectal carcinomas, Vogelstein *et al.* (1989) found no correlation between FAL and Dukes' classification or tumour size, whereas in the allotype on bladder carcinomas, a correlation

was found between FAL and tumour grade but not with the stage of the disease (Knowles *et al.*, 1994). Thus, all three analyses demonstrate no correlation between FAL and tumour stage.

We have demonstrated that a FAL > median value correlated with a poor prognosis in all 52 patients analysed ($P < 0.032$) and also in the subset of previously untreated patients ($P < 0.019$) calculated by the log-rank method. Vogelstein *et al.* (1989) also showed a relationship between FAL and prognosis for colon cancers with a similar number and distribution of patients ($P < 0.01$) using Fisher's exact test. Thus the argument originally proposed by Vogelstein *et al.* (1989) that recognition of accumulated genetic damage, as provided by the allelotype, provides a useful molecular indicator of the tumour behaviour is supported by the findings of this study.

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