ORIGINAL ARTICLE

Allelic imbalance at chromosome 11 in head and neck squamous cell carcinoma in an Indian patient population

G Mondal, A Tripathi, N Bhattacharya, N Sikdar, A Roy, A Sengupta, B Roy, C K Panda, S Roychoudhury

J Clin Pathol 2003;**56**:512–518

See end of article for authors' affiliations

Correspondence to: Dr S Roychoudhury, Human Genetics and Genomics Division, Indian Institute of Chemical Biology, 4, Raja S. C. Mullick Road, Kolkata 700 032, India; susantar@hotmail.com

Accepted for publication 27 February 2003

Background: Genetic instability of chromosome 11 is a frequent event in many solid tumours, including head and neck squamous cell carcinoma (HNSCC).
Aims: To perform allelic imbalance analysis of cytogenetically mapped altered regions of human chromosome 11 in patients with HNSCC from eastern India.
Methods: Genomic alterations were investigated using highly polymorphic microsatellite markers in both HNSCC and leukoplakia tissues.
Results: Microsatellite markers D11S1758 from 11p13–15 and D11S925 from 11q23.3–24 had the highest frequency (38% and 32%, respectively) of loss of heterozygosity among all the markers analysed. Allelic loss at the marker D11S925 was seen in both leukoplakia and in all stages of HNSCC tumour tissues suggesting that it is an early event in HNSCC tumorigenesis. Microsatellite size alteration was also found to be high (> 20%) in several markers. In leukoplakia samples microsatellite instability was seen at a higher frequency than loss of the allele, indicating such alterations might initiate the process of tumorigenesis in HNSCC.
Conclusions: The high rate of chromosomal alterations at 11q21–24 in HNSCC suggests the presence of a putative tumour suppressor gene in this region.

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common human cancer in all parts of the world, and it has been estimated that there are around 400 000 new cases each year worldwide.[1] It accounts for about 30–40% of all cancer types in India and in the subcontinent.[2] Epidemiological studies have linked chronic exposure to tobacco, betel nut leaf quid, alcohol, and some environmental factors to the occurrence of HNSCC,[3] and the characteristic molecular genetic events associated with the initiation and progression of HNSCC are beginning to be understood. The karyotypes of HNSCC tumours are complex, often near triploid, and there are multiple numerical and structural abnormalities, including deletions, balanced and unbalanced translocations, isochromosomes, dicentric chromosomes, and homogeneously staining regions.[4] Classic cytogenetic analysis of HNSCC tumours has identified a gradual increase in chromosomal aberrations from premalignant to malignant lesions.[5] In other studies, consistent chromosomal deletions and amplifications in several chromosome arms have been reported.[6] In addition, centromeric breakage in several chromosomes has been shown to be important for the development of HNSCC.[7]

Chromosome 11 plays a crucial role in several cancers, and two regions in particular, one in the short arm (11p13–15) and the other in the long arm (11q21–24), have been identified as frequent targets for allelic loss. Allelic deletion at 11p15 has been reported to be a common occurrence in oesophageal (53.3%) and gastric adenocarcinomas (61.5%).[8] Similarly, anaplastic thyroid carcinoma is associated with a 33% allelic loss in the 11p13 region.[9] Loss of heterozygosity (LOH) at chromosome 11q23–24 (between loci D11S934 and D11S912) has been reported in 27% of breast cancers in a Chinese population.[10] In nasopharyngeal carcinoma, these two loci showed LOH in 46.7% and 23.3% of the tumours, respectively.[11] Moreover, comparative genomic hybridisation analysis also revealed frequent loss of 11q21–qter regions in nasopharyngeal carcinoma.[12] In addition, 38.8% of cervical carcinomas showed LOH at the 11q23.3 region.[13] Approximately 40% LOH was found at all the markers in the 11q23 region in colorectal carcinoma.[14] Allelotyping studies in patients with HNSCC have revealed frequent LOH affecting many chromosomal loci, including chromosome 11.[15–19] A microsatellite assay covering the region of 11p12–15 showed a variable amount of LOH and microsatellite size alteration (MA) in 19–45% of tumours.[15] In another report, a microsatellite marker flanking the p57KIP2 gene (a cyclin dependent kinase inhibitor) at 11p15.5 showed 33% LOH and loss of imprinting in HNSCC.[17] A genetic progression model of HNSCC has implicated alteration in chromosomal region 11q13 in the transition from dysplasia to in situ carcinoma.[20] In addition, it has been reported that the 11q23–24 region is frequently deleted in nasopharyngeal carcinoma.[19]

> "Chromosome 11 plays a crucial role in several cancers, and two regions in particular, one in the short arm (11p13–15) and the other in the long arm (11q21–24), have been identified as frequent targets for allelic loss"

In our present report, we examine allelic imbalance in HNSCC tumours from the oral cavity, the laryngeal region, and the orofacial region in eastern Indian patient populations at three regions of chromosome 11 (11p15, 11q13, and 11q21–24). We present evidence that the 11q21–24 region may harbour a putative tumour suppressor gene involved in the development of tumours in these three sites of the head and neck. Our result suggests that microsatellite instability is an early event in

Abbreviations: H, heterozygosity; HNSCC, head and neck squamous cell carcinoma; LOH, loss of heterozygosity; MA, microsatellite size alteration; MSI, microsatellite instability; PBL, peripheral blood leucocyte; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism

HNSCC tumorigenesis, and we propose that underlying mechanisms responsible for the generation of microsatellite instability could also be responsible for allelic loss.

METHODS AND MATERIALS

Tumour samples

Sixty freshly operated primary HNSCC tumours and 13 dysplastic leukoplakia tissue samples, along with their corresponding normal tissues or peripheral blood leucocytes (PBLs), were collected from the patients before treatment. Samples were immediately frozen and stored at −80°C. All tumours were histopathologically diagnosed as squamous cell carcinoma, graded and staged according to the UIC TNM classification. Table 1 shows a detailed history of all 73 patients affected with primary HNSCC tumours and leukoplakia. Among the 60 patients with HNSCC, there were 49 men and 11 women, with a mean age of 49 years. Patients were grouped on the basis of affected primary sites, namely: oral cavity, larynx, and orofacial. Thirty two tumours were from the oral cavity (three alveolus, 16 buccal mucosa, 10 tongue, two tonsil, and one palate), nine tumours were from the larynx, and 14 tumours were from the orofacial region (13 maxilla and one mandible). Histopathologically, the HNSCC tumours were classified as stage I (six tumours), stage II (13 tumours), stage III (17 tumours), or stage IV (19 tumours). Similarly, 13 leukoplakia tissues were classified histologically into three groups, namely: mild (two), moderate (six), and severe (five). Among the 60 tumours, 25 each were lymph node positive and negative. In addition, 34 patients were habitual users of tobacco by different means (bidi, pan, cigarettes, etc) and 17 did not have a tobacco habit. For four patients no history was available (4717b, 4717g, 2917, and 2927).

Microdissection and DNA isolation

The normal cells present as contaminants in the primary tumour tissues were removed by a microdissection procedure.[21] More than 50 to 60 serial tumour specimens (10 to 20 μm) were sectioned and placed on glass slides using a cryostat (model CM 1800; Leica, Heidelberg, Germany). Representative 5 μm tumour specimens from different regions of the tumour were stained with haematoxylin and eosin for diagnosis and for marking the tumour rich regions. The normal cells present in the tumour specimens were removed by microdissection. The adjacent normal tissues of the primary tumour were similarly sectioned to identify the presence of tumour cell infiltration. For those cases in which the "normal" tissues were contaminated with tumour cells, PBLs from the corresponding patient were used as the normal sample. Microdissected tumour samples containing < 60% tumour cells were not further analysed. Genomic DNA was isolated from tissues by standard proteinase K digestion, followed by phenol/chloroform extraction.[22]

Microsatellite markers and PCR analysis

We used 10 highly polymorphic (heterozygosity > 70% except one) CA repeat markers: two markers from 11p13–15 (D11S4177 and D11S1758), one from 11q13 (D11S913), and seven from 11q21–24 (D11S1339, D11S2179, D11S1893, D11S924, D11S925, D11S1345, and D11S1328). These microsatellite loci were amplified by the polymerase chain reaction (PCR) in a 10 μl reaction volume containing 10mM Tris/HCl (pH 8.3), 50mM KCl, 0.2 mM of each dNTP, 2 pmol of each primer, an optimal concentration of $MgCl_2$ (1.5 to 2.0mM; determined separately for each primer pair), and 50–100 ng of template genomic DNA. Reaction mixtures were heated to 94°C for three minutes, cooled to 72°C before the addition of 0.2 units Taq DNA polymerase (Gibco BRL, Gaithersberg, Maryland, USA), and cycled 30 times in a GeneAmp 9700 (Perkin Elmer, Norwalk, Connecticut, USA) PCR machine. Each cycle consisted of 30 seconds at 94°C, 30 seconds at the appropriate annealing temperature (50–58°C), and 30 seconds at 72°C; the final extension was carried at 72°C for seven minutes. Samples without DNA were used as negative controls for

Table 1 Clinical data of patients with leukoplakia and head and neck squamous cell carcinomas used in the study

Tumour	Age (years)/Sex	Site	Stage	Histopathology	Lymph node	Tobacco
L48	59/F	BM	–	MLD	–	+
L49	52M	BM	–	MOD	–	+
L50	64/M	BM	–	MLD	–	+
L51	74/M	BM	–	SD	–	+
L52	45/M	BM	–	MOD	–	+
L53	49/M	BM	–	MOD	–	+
L54	44/M	BM	–	MOD	–	+
L55	44/M	BM	–	SD	–	+
L56	40/M	BM	–	MOD	–	+
L57	40/M	BM	–	MOD	–	+
125	35/F	BM	–	SD	–	–
2024	52/M	BM	–	SD	–	–
3037	55/M	BM	–	SD	–	–
1491	58/M	BM	I	WDSCC	+	+
4119	60/M	BM	I	WDSCC	–	–
4456	45/M	BM	I	WDSCC	+	+
149	38/M	MD	II	WDSCC	–	–
222	60/F	TNG	II	WDSCC	–	–
410	57/F	TNG	II	WDSCC	+	–
558	59/M	AL	II	WDSCC	–	+
825	32/M	P	II	PDSCC	–	–
939	50/M	BM	II	MDSCC	–	–
1047	53/M	BM	II	WDSCC	–	+
1864	45/M	MX	II	MDSCC	+	+
2030	73/M	TNG	II	MDSCC	+	+
2539	48/M	AL	II	WDSCC	U	U
3070	48/M	AL	II	WDSCC	+	+
5959	28/M	TNG	II	MDSCC	–	–
7216	56/M	MX	II	MDSCC	–	+
292	48/M	LX	III	MDSCC	–	+
308	42/M	BM	III	MDSCC	–	+
326	70/M	MX	III	WDSCC	–	–
615	45/M	TNG	III	WDSCC	–	+
693	50/M	LX	III	MDSCC	–	+
4332	54/M	BM	III	WDSCC	+	+
5090	49/M	BM	III	WDSCC	+	+
1295	75/M	LX	III	PDSCC	–	+
1367	60/M	TNS	III	MDSCC	–	–
2323	62/M	MX	III	WDSCC	–	+
2592	70/M	BM	III	MDSCC	+	+
2772	41/M	BM	III	WDSCC	+	+
4248	36/M	BM	III	MDSCC	+	+
5325	46/M	TNG	III	WDSCC	+	+
5364	37/M	MX	III	MDSCC	+	–
7428	40/M	BM	III	WDSCC	+	–
7783	45/F	TNS	III	WDSCC	+	+
311	30/M	BM	IV	WDSCC	+	–
802	50/M	TNG	IV	MDSCC	+	+
1101	58/M	LX	IV	MDSCC	–	+
1445	70/M	LX	IV	WDSCC	–	+
2618	42/M	MX	IV	PDSCC	+	+
4904	60/M	MX	IV	MDSCC	+	+
5184	60/M	BM	IV	MDSCC	–	–
1494	50/M	BM	IV	MDSCC	U	U
4188	65/M	BM	I	MDSCC	+	–
538	50/M	MX	U	WDSCC	U	+
598	45/M	Neck	U	PDSCC	U	U
951	73/M	U	U	WDSCC	–	+
1068	58/M	MX	U	WDSCC	–	–
1128	56/M	MX	U	WDSCC	–	+
1166	28/M	MX	U	WDSCC	–	+
1552	20/F	TNG	IV	MDSCC	+	–
2507	30/F	TNG	U	U	+	–
2579	49/M	MX	I	WDSCC	–	U
2807	52/M	TNG	U	MDSCC	+	+
2888	40/M	MX	I	WDSCC	+	U
3280	60/M	BM	U	U	U	+
5943	U/M	BM	U	U	U	+
7999	70/M	Neck	U	PDSCC	+	+

Site: AL, alveoli; BM, buccal mucosa; MD, mandible, MX, maxilla; P, palate; TNG, tongue; TNS, tonsil. Histopathology: MDSCC, moderately differentiated squamous cell carcinoma; MLD, mild dysplasia; MOD, moderate dysplasia; PDSCC, poorly differentiated squamous cell carcinoma; SD, severe dysplasia; WDSCC, well differentiated squamous cell carcinoma. Tobacco: +, consumed at least 10–15 cigarettes/biri or equivalent amount of chewable tobacco each day for at least 10 years. Node: +, lymph node formation with tumour tissue. U, not known. No history is available for the patients 4717b, 4717g, 2917, and 2927.

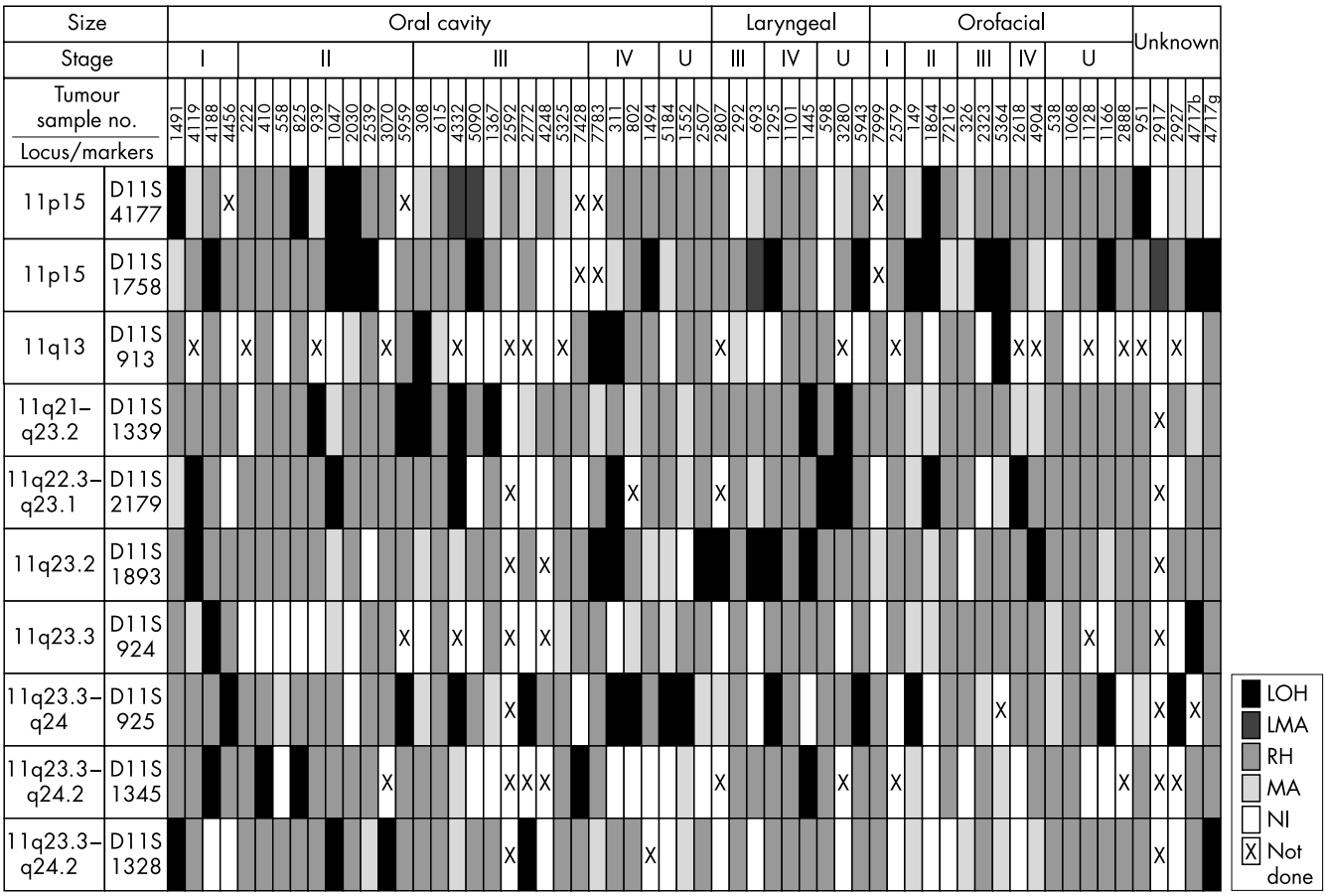

Figure 1 Allelic imbalance data of the chromosome 11 markers used according to tumour type. Patients are arranged according to stage and site of the tumour. Markers with their cytogenetic positions are shown in columns at the left hand side of the figure. LMA, loss of one allele and size alteration of the other; LOH, loss of heterozygosity; MA, microsatellite size alteration; NI, non-informative; RH, retention of heterozygosity; U, unknown.

each primer pair. Before the PCR, one of the paired primers was end labelled with [γ^{32}P] ATP (specific activity, 3000 Ci/mM; Amersham, Buckinghamshire, UK) using T4 polynucleotide kinase (Gibco BRL). The amplified DNA (1 µl) was mixed with formamide dye, denatured, and then electrophoresed on a denaturing 6% polyacrylamide gel at 2300 V for three to four hours. After electrophoresis, gels were dried and exposed to *x* ray films (Kodak X-Omat) for autoradiography.

Definition of LOH and MA

LOH was determined by densitometric scanning (CS-900; Simadju, Osaka, Japan) of the autoradiographs. Because the detection of LOH can be compromised by the presence of normal DNA contaminating the tumour samples, we used genomic DNA from microdissected tumour tissues and calculated LOH using the ratios of the band intensities of paired tumour and normal DNA. For informative cases, allelic loss was scored if there was complete loss of one allele or if the relative band intensity of one allele was decreased by at least 50% in the tumours, compared with the same allele in the normal control. Values were calculated as the ratio of band intensities of the larger to the smaller alleles in the tumour DNA divided by the same ratio in the corresponding normal DNA sample. LOH indices of > 1.5 and < 0.67 were considered to be loss of smaller and larger alleles, respectively.[23] MA was scored if one (MA1) or both (MA2) alleles at a given locus showed size variation—either expansion or contraction—in comparison with the same alleles in normal control DNA.[24] The determination of MA can be erroneous if the samples are not properly paired, so that in our study this was ensured by the genotyping of tumour and normal DNA with four restriction fragment length polymorphism (RFLP) markers (see results). To calculate the frequency of LOH, samples showing homozygosity (H) and MA only were not considered; that is, LOH = (LOH + LMA)/(total − MA − H), whereas for the MA frequency calculation the samples showing LOH only were not considered; that is, MA = (MA + LMA)/(total − LOH).[25] The samples showing both LOH and MA at the same locus was considered for calculating both LOH and MA. MA can sometimes falsely be designated as LOH, and this can be resolved by scanning the autoradiogram. All samples showing LOH and/or MA were subjected to repeat analysis after a second independent amplification for confirmation.

RESULTS

Analysis of LOH and MA in the HNSCC samples

We genotyped 60 primary HNSCC tumours at different stages of development for LOH and MA at 10 different loci on chromosome 11 using highly polymorphic microsatellite markers; two from 11p13–15, one from 11q13, and seven from 11q21–24 (table 1; fig 1). All markers used in our study were informative in more than 70% of the tumours analysed. Each tumour was genotyped with at least eight markers. Broadly, two types of allelic alterations were seen in the matched tumour tissues compared with normal tissues of the same individuals. These were loss of an allele and an increase or decrease in the size of the allele (fig 2). In a few cases, biallelic alterations in the tumour tissues were also seen (data not shown). In those samples, proper matching between the normal and tumour sample was confirmed by genotyping with four RFLP markers, namely: Alu FXIII3B (H = 0.43), Alu D1 (H = 0.49), Alu TPA25 (H = 0.48), and Alu ACE (H = 0.44), respectively (data not shown). It was found that 51 of the 60

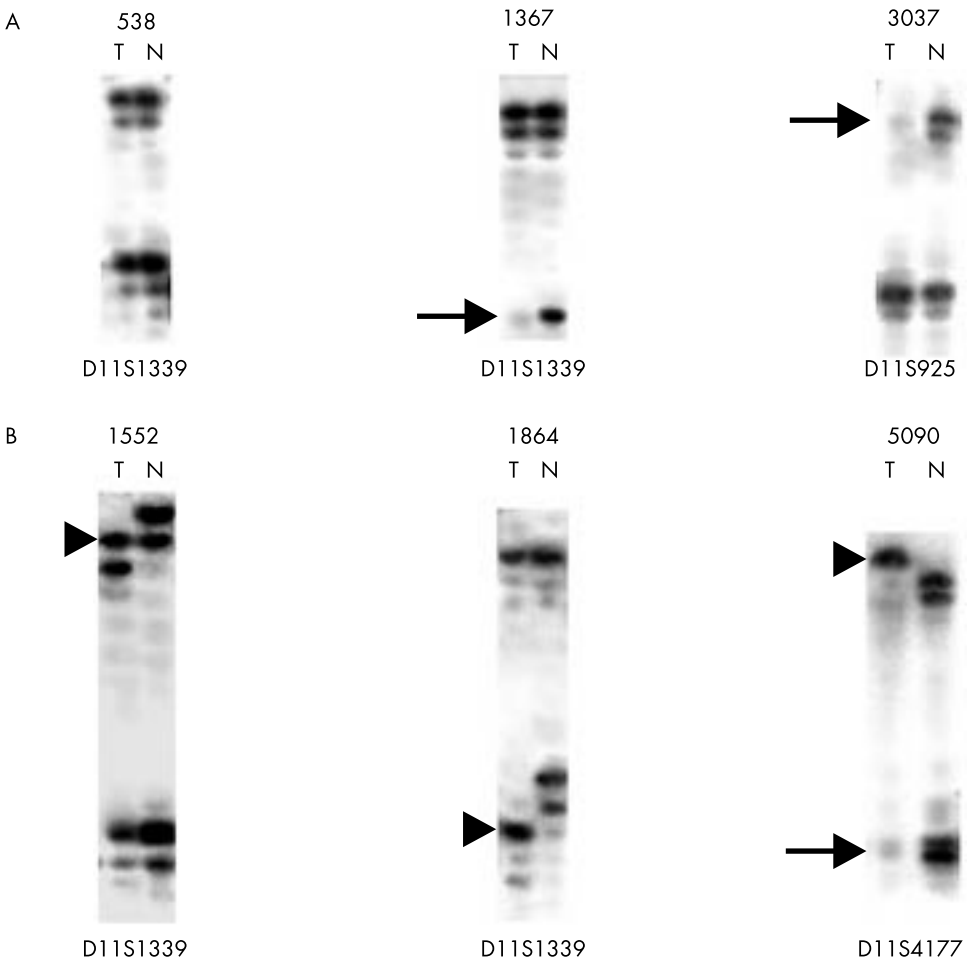

Figure 2 Representative autoradiographs showing loss of heterozygosity and microsatellite size alteration at different markers on chromosome 11. The markers (shown at the bottom of each panel) were amplified from paired tumour (T) and normal (N) tissues taken from selected patients (shown at the top of each panel), analysed by denaturing 6% polyacrylamide gel electrophoresis, and autoradiographed. (A) The samples from patient 538 show the retention of both alleles; the samples from patients 1367 and 3037 show loss of the lower and upper alleles, respectively. (B) The samples from patient 5090 show loss of the lower allele and a shift in the upper allele in the tumour tissue. The samples from patients 1552 and 1864 show a shift in the upper and lower allele, respectively. The arrows indicate the loss of an allele and the arrowheads indicate a shift of the allele.

HNSCC samples exhibited LOH and/or MA on chromosome 11 for at least one marker (fig 1).

Figure 3 depicts the frequency of LOH and MA in the HNSCC tumours analysed in relation to the markers used. All markers exhibited LOH and MA at a moderate to high frequency. LOH was found in more than 20% of the tumours for three markers (D11S1758, D11S913, and D11S925). Similarly, MA was found in more than 20% of the tumours for four markers (D11S4177, D11S1758, D11S924, and D11S925). Among the markers analysed on the long arm, D11S925 at 11q23.3 displayed the highest frequency of LOH (32%). Interestingly, both D11S925 and another adjacent marker D11S924 within the 11q23.3 region also exhibited a high frequency of MA (22%). Thus, it appears that the 11q23.3 region is prone to genetic instability in HNSCC tumours. It should be noted that two markers from the 11p15 region also exhibited a high frequency of instability (fig 3). Another interesting observation is the moderate degree of instability (16% LOH and 12% MA) of D11S2179, which is intragenic to the ATM gene (fig 3).

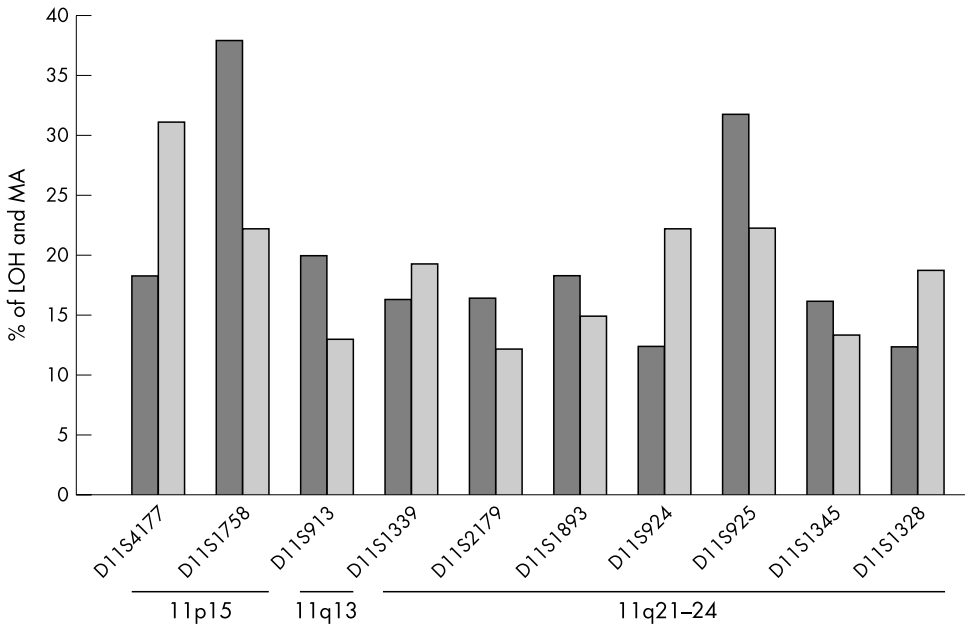

Figure 3 Frequency of loss of heterozygosity (LOH) and microsatellite size alteration (MA) at different markers on chromosome 11 in primary head and neck squamous cell carcinomas. The dark bars indicate LOH and the lighter bars indicate MA. The names of the markers and their cytogenetic positions are shown at the bottom of each histogram.

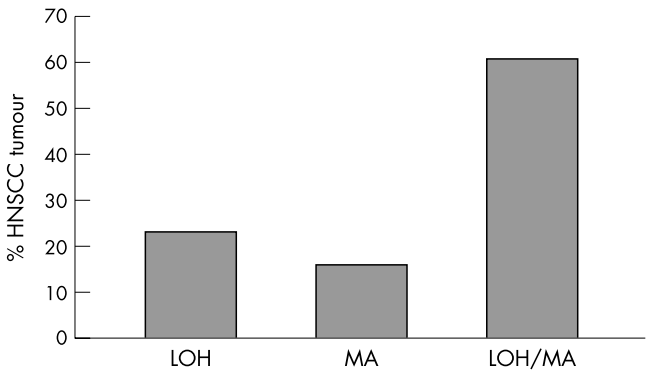

Figure 4 The frequency of type of allelic imbalance in head and neck squamous cell carcinomas. Bars represent the percentage of head and neck squamous cell carcinoma tumours (HNSCC) exhibiting only loss of heterozygosity (LOH), only microsatellite size alteration (MA), or both LOH and MA in at least one marker. Each tumour was genotyped with eight to 10 markers.

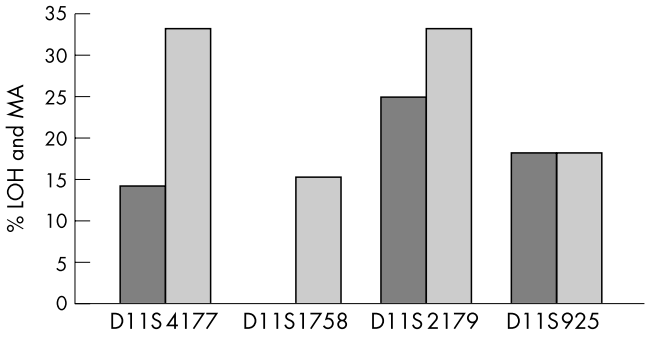

Figure 5 Frequency of loss of heterozygosity (LOH) and microsatellite size alteration (MA) at different markers on chromosome 11 in leukoplakia. The darker bars show the percentage of LOH and the lighter bars show the percentage of MA.

Figure 1 shows the allelic imbalance data for the different markers used in relation to the tumour location and stage. Primary site and tumour stage information were available for 55 and 45 tumours, respectively. For five tumours, neither of these pieces information were available. LOH was seen most frequently for markers D11S925 (13 of 41) and D11S1758 (17 of 45). D11S925 was deleted in eight of 24 (33%) oral cavity tumours and four of 15 (27%) laryngeal/orofacial tumours, whereas D11S1758 was deleted in eight of 17 (47%) laryngeal/orofacial tumours and six of 23 (26%) oral cavity tumours. However, the numbers do not suggest a site specific LOH pattern. Both markers exhibited LOH in all stages of the oral cavity tumours. The group of laryngeal/orofacial tumours was too small to determine the stage specific LOH pattern. Six tumours (numbers 2030, 2539, 5090, 1494, 2323, and 951) exhibited LOH on the short arm of chromosome 11 and none on the long arm. Recently, such chromosome arm specific loss has been documented in colorectal cancer.[26] Further analysis with more markers on the short arm is required to substantiate these data. Four tumours (numbers 4332, 5090, 693, and 2917) suffered biallelic alterations in markers from 11p15, where one allele was lost and the other allele exhibited size alteration (figs 1 and 2). However, biallelic alterations were not seen for the 11q markers.

Furthermore, among the tumours showing changes, 12 of 51 (23%) exhibited only LOH and eight of 51 (16%) displayed MA exclusively (fig 4). Thus, 31 of 51 (61%) tumours exhibited both LOH and MA (fig 4). Interestingly, 19 of 60 (32%) tumours exhibited MA at two or more loci, indicating a microsatellite instability (MSI) phenotype (fig 1).

Analysis of LOH and MA in leukoplakia

Genotyping of 13 leukoplakia tissues of various stages using a subset (D11S4177, D11S1758, D11S2179, and D11S925) of the above 10 markers revealed several intriguing features (figs 5 and 6). Analysis of the allelic imbalance in leukoplakia samples revealed that dysplastic tissues of mild and moderate stages exhibited more MA and infrequent LOH, whereas severe dysplastic tissues exhibited both types of alteration at equal frequencies (fig 6). Moreover, it was found that three markers had a higher degree of MA than LOH, and one marker (D11S925) exhibited an equal frequency of LOH and MA in these samples (fig 5). These results suggest that MA could be an early event in HNSCC tumorigenesis. Furthermore, the presence of LOH in D11S925 in the leukoplakia tissues suggests that alterations in the 11q23.3–24 region were an early event in the development of HNSCC. In contrast, the absence of LOH in D11S1758 suggests that it is a late event and associated with tumour progression.

DISCUSSION

In our present study, we examined allelic imbalance mainly in two regions (11p15 and 11q21–24) of chromosome 11 in

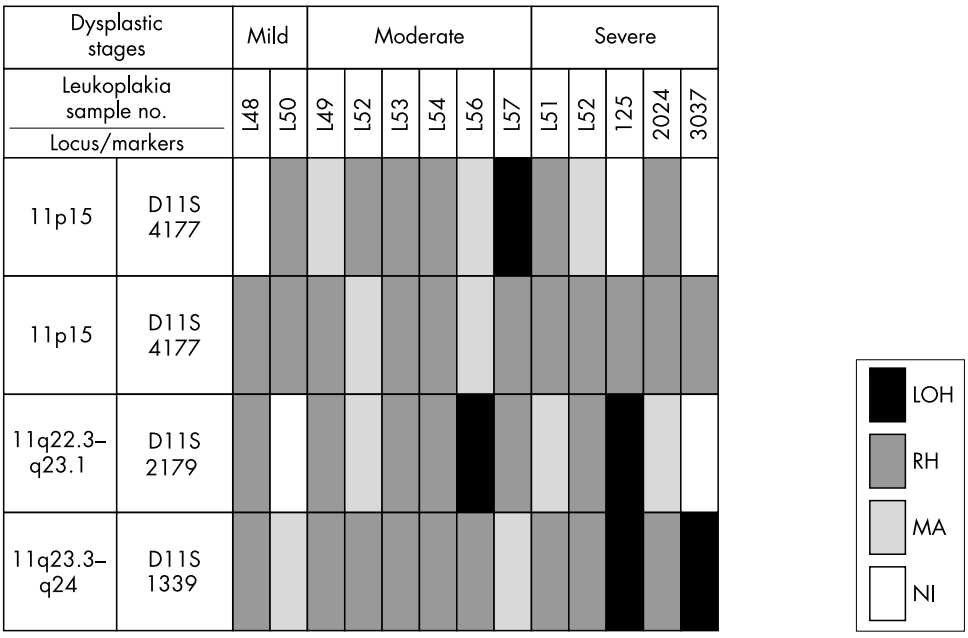

Figure 6 Allelic imbalance data of the chromosome 11 markers in leukoplakia samples. Leukoplakia samples are arranged according to the stage of dysplasia. Markers with their cytogenetic positions are shown in columns at the left hand side of the figure. LOH, loss of heterozygosity; MA, microsatellite size alteration; NI, non-informative; RH, retention of heterozygosity.

patients with HNSCC from thee Indian populations. Overall, we found a very high frequency (85%) of allelic alterations in these two regions of chromosome 11 compared with other cancers in different populations. Forty three of the 60 HNSCC tumours (72%) exhibited LOH in at least one marker. The analysis showed that markers D11S925 at 11q23.3–24 and D11S1758 at 11p15 exhibited the highest frequency of LOH (32% and 38%, respectively). A high frequency of LOH in the 11p15.5 region (D11S988) has also been reported in HNSCC patient populations from the USA.[14] Although frequent allelic loss in the 11q21–24 regions has been reported in many cancers,[8 10 13] such alterations in oral cavity, laryngeal, and orofacial carcinomas of the head and neck region have not been reported so far. The maximum single locus incidence of LOH in the 11q21–24 region varied from 27% in breast cancer[10] to 47% in nasopharyngeal carcinoma,[11] which is comparable to our present data (32% at D11S925) in HNSCC tumours. Several genes involved in the DNA damage response (ATM, MRE11A, and CHEK) and apoptosis (CASP1, CASP4, CASP5, and P53AIP1) are located at the 11q21–24 region. Similarly, a few functionally uncharacterised genes (ANC, BRCA3, MLL, and ST3), implicated in various cancers, are also located in this region. Another putative tumour suppressor gene (PPP2R1B) found in this region has been excluded from involvement in cervical cancer,[27] but has been predicted to be a possible target in colorectal cancer.[28] It is notable that the two markers flanking D11S925 (D11S924 and D11S1345) had a lower frequency of LOH, so that in our study we have identified a minimal region of 7 cM between D11S924 and D11S1345 that may harbour putative tumour suppressor genes involved in the development of HNSCC. The marker D11S2179, which is intragenic to the tumour suppressor gene ATM, exhibited 16% LOH in HNSCC tumours, indicating that the ATM gene might be involved in the development of HNSCC, and this possibility needs to be investigated in greater detail.

> "In our study we have identified a minimal region of 7 cM between D11S924 and D11S1345 that may harbour putative tumour suppressor genes involved in the development of head and neck squamous cell carcinoma"

We found that MA was very frequent (39 of 60; 65%) in HNSCC tumours from Indian populations. Similar results were also reported in a HNSCC patient population from the USA, where 51% of the tumours showed MA in one or more loci.[15] All 10 markers used in our present study exhibited MA in between 12% and 31% of the HNSCC tumours. The markers D11S4177 from 11p15 and D11S924 and D11S925 from the 11q21–24 region exhibited the highest frequency of MA. Interestingly, both markers D11S4177 and D11S924 are adjacent to the markers that exhibited the highest LOH, indicating that these regions are very susceptible to genetic alterations during the development of HNSCC. Recently, it has been suggested that MSI and chromosomal instability phenotypes can coexist in tumours.[29] Previously, we have reported that the MSI phenotype exhibited by HNSCC tumours differs distinctly from that of colorectal tumours.[30] Based on these results, we argue that the underlying mechanisms for MA could also be responsible for LOH.

Take home messages

- The high rate of chromosomal alterations at 11q21–24 in head and neck squamous cell carcinoma (HNSCC) suggests the presence of a putative tumour suppressor gene in this region
- Allelic loss at the marker D11S925 was seen in leukoplakia and in all stages of HNSCC, suggesting that it is an early event in the tumorigenesis of HNSCC
- Microsatellite size alteration was also found to be high (> 20%) in several markers
- In leukoplakia samples, microsatellite instability was seen at a higher frequency than loss of the allele, indicating such alterations might initiate the process of tumorigenesis in HNSCC

ACKNOWLEDGEMENTS

We thank Dr S Gupta (Director, Cancer Centre and Welfare Home, Calcutta) for providing access to the patients, and Dr K Choudhury and Dr K Ray for critical reading of the manuscript. We gratefully acknowledge the technical support of Mr S Mitra. We are grateful to all members of Human Genetics and Genomics Division of IICB, and the Department of Oncogene Regulation of CNCI for their kind cooperation and encouragement during the study. GM is supported by a predoctoral fellowship from the Department of Biotechnology, Government of India. This work was supported by research grants from the Department of Biotechnology (grant no. BT/MB/05/002/94), and the Department of Science and Technology (grant no. SP/SO/D-75/96), Government of India.

Authors' affiliations
G Mondal, S Roychoudhury, Human Genetics and Genomics Division, Indian Institute of Chemical Biology, Kolkata 700 032, India
A Tripathi, N Bhattacharya, C K Panda, Department of Oncogene Regulation, Chittaranjan National Cancer Institute, Kolkata 700 026, India
A Roy, Department of Pathology, Medical College and Hospital, Kolkata 700 073, India
A Sengupta, Cancer Centre and Welfare Home, Kolkata 700 063, India
N Sikdar, B Roy, Anthropology and Human Genetics Unit, Indian Statistical Institute, Kolkata 700 035, India

REFERENCES

1 **Parkin DM**, Pisani P, Ferlay J, *et al*. Estimates of the worldwide incidence of eighteen major cancers in 1985. *Int J Cancer* 1993;**54**:594–600.
2 **Saranath D**, Bhoite LT, Deo MG, *et al*. Molecular lesion in human oral cancer: the Indian scene, oral oncology. *Eur J Cancer* 1993;**29**:107–12.
3 **Wu CL**, Solan P, Thakker N, *et al*. Deletion mapping on the short arm of chromosome 3 in squamous cell carcinoma of the oral cavity. *Cancer Res* 1994;**54**:6484–8.
4 **Sauders WS**, Shuster S, Huang X, *et al*. Chromosomal instability and cytoskeletal defects in oral cancer cells. *Proc Natl Acad Sci U S A* 2000;**97**:303–8.
5 **Van Dyke D**, Worsham MJ, Beninger MS, *et al*. Recurrent cytogenetic abnormalities in squamous cell carcinomas of the head and neck region. *Genes Chromosomes Cancer* 1994;**9**:192–206.
6 **Gollin SM**. Chromosomal alterations in squamous cell carcinomas of the head and neck; window to the biology of disease. *Head Neck* 2001;**23**:238–53.
7 **Jin Y**, Mertens F, Jin C, *et al*. Nonrandom chromosome abnormalities in short-term cultured primary squamous cell carcinomas of the head and neck. *Cancer Res* 1995;**55**:3204–10.
8 **Moskaluk CA**, Rumpel CA. Allelic deletion in 11p15 is a common occurrence in esophageal and gastric adenocarcinoma. *Cancer* 1998;**83**:232–9.
9 **Kitamura Y**, Shimizu K, Tanaka S, *et al*. Allelotyping of anaplastic thyroid carcinoma: frequent allelic losses on 1q, 9p, 11, 17, 19p, and 22q. *Genes Chromosomes Cancer* 2000;**27**:244–51.
10 **Shen KL**, Yang LS, Harn HJ, *et al*. Microsatellite alterations on human chromosome 11 in in situ and invasive breast cancer: a microdissection microsatellite analysis and correlation with p53, ER, and PR protein immunoreactivity. *J Surg Oncol* 2000;**2**:100–7.
11 **Harn HJ**, Fan HC, Chen CJ, *et al*. Microsatellite alteration at chromosome 11 in primary human nasopharyngeal carcinoma in Taiwan. *Oral Oncol* 2002;**38**:23–9.
12 **Fang Y**, Guan X, Guo Y, *et al*. Analysis of genetic alterations in primary nasopharyngeal carcinoma by comparative genomic hybridization. *Genes Chromosomes Cancer* 2001;**30**:254–60.
13 **O'Sullivan MJ**, Rader JS, Gerhard DS, *et al*. Loss of heterozygosity at 11q23.3 in vasculoinvasive and metastatic squamous cell carcinoma of the cervix. *Hum Pathol* 2001;**32**:475–8.
14 **Lee AS**, Seo YC, Chang A, *et al*. Detailed deletion mapping at chromosome 11q23 in colorectal carcinoma. *Br J Cancer* 2000;**83**:750–5.
15 **El-Nagger AK**, Hurr K, Huff V, *et al*. Allelic loss and replication errors at microsatellite loci on chromosome 11p in head and neck squamous carcinoma: association with aggressive biological features. *Clin Cancer Res* 1996;**2**:903–7.

16 **Jiang WW**, Fujii H, Shirai T, *et al*. Accumulative increase of loss of heterozygosity from leukoplakia to foci of early cancerization in leukoplakia of the oral cavity. *Cancer* 2001;**92**:2349–56.
17 **Lai S**, Goefert H, El-Nagger AK, *et al*. Loss of imprinting and genetic alterations of the cyclin-dependent kinase inhibitor pKIP2 gene in head and neck squamous cell carcinoma. *Clin Cancer Res* 2000;**6**:3172–6.
18 **Nawroz H**, van der Riet P, Hurban RH, *et al*. Allelotype of head and neck carcinoma. *Cancer Res* 1994;**4**:1152–5.
19 **Lo KW**, Teo PM, Hui AB, *et al*. High resolution allelotype of microdissected primary nasopharyngeal carcinoma. *Cancer Res* 2000;**60**:3348–53.
20 **Califano J**, Riet PV, Westra W, *et al*. Genetic progression model for head and neck cancer: implications for field cancerization. *Cancer Res* 1996;**56**:2488–92.
21 **Dasgupta S**, Mukherjee N, Roy S, *et al*. Mapping of the candidate tumour suppressor genes loci on human chromosome 3 in head and neck squamous cell carcinoma of an Indian patient population. *Oral Oncol* 2002;**38**:6–15.
22 **Sambrook J**, Fritsch EF, Maniatish T. *Molecular cloning: a laboratory manual*, 2nd ed. Cold Spring Harbour, NY: Cold Spring Harbour Laboratory, 1989.
23 **MacGrogan D**, Levy A, Bostwick D, *et al*. Loss of chromosome arm 8p loci in prostate cancer: mapping by quantitative allelic imbalance. *Genes Chromosomes Cancer* 1994;**10**:151–9.
24 **Wisruba II**, Behrens C, Milchgrub S, *et al*. Sequential molecular abnormalities are involved in the multistage development of squamous cell lung carcinoma. *Oncogene* 1999;**18**:643–50.
25 **Dillon EK**, Boer WB, De Papadimitriou JM, *et al*. Microsatellite instability and loss of heterozygosity in mammary carcinoma and its probable precursors. *Br J Cancer* 1997;**76**:156–62.
26 **Thiagalingam S**, Vogelstein B, Lengauer C, *et al*. Mechanism underlying losses of heterozygosity in human colorectal cancers. *Proc Natl Acad Sci U S A* 2001;**98**:2698–702.
27 **Pulido HA**, Fakruddin MJ, Chatterjee A, *et al*. Identification of a 6-cM minimal deletion at 11q23.1–23.2 and exclusion of PPP2R1B gene as a deletion target in cervical cancer. *Cancer Res* 2000;**60**:6677–82.
28 **Takagi Y**, Futamura M, Yamaguchi K, *et al*. Alterations of the PPP2R1B gene located at 11q23 in human colorectal cancers. *Gut* 2000;**47**:268–71.
29 **Abdel-Rahman WM**, Katsura K, Rens W, *et al*. Spectral karyotyping suggests additional subsets of colorectal cancers characterized by pattern of chromosome rearrangement. *Proc Natl Acad Sci U S A* 2001;**98**:2538–43.
30 **Chakraborty S**, Roychoudhury S, Panda CK, *et al*. Microsatellite instability in squamous cell carcinoma of head and neck from the Indian patient population. *Int J Cancer* 2001;**92**:555–61.