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High frequency allelic loss on chromosome 17p13.3–p11.1 in esophageal squamous cell carcinomas from a high incidence area in northern China

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Allelic loss on chromosome 17p has been reported frequently in esophageal squamous cell carcinoma (ESCC) and generally encompasses the *p53* locus at 17p13.1. However, a good correlation between allelic loss on 17p and mutation of *p53* has not been found. This suggests the possibility that unknown tumor suppressor genes near *p53* may be involved in the development of ESCC. To evaluate this possibility, we analyzed 30 microsatellite markers covering the entire short arm of chromosome 17 in 56 ESCC patients from a high risk population in northern China, including 34 with a family history of upper gastrointestinal (UGI) cancer and 22 without a family history of any cancer. Cancer lifestyle risk factors and clinical/pathological characteristics were also collected. We found frequent allelic loss (≥65%) at 28 of the 30 markers evaluated in these ESCC patients. The highest frequencies of allelic loss (≥80%) were found in three smaller regions: deletion region I located at 17p13.3–p13.2 (between D17S849 and D17S1828); deletion region II located at 17p13.2–p13.1 (between D13S938 and TP53); deletion region III located at 17p13.1–p12 (between D17S804 and D17S799). A number of genes have already been identified in these deleted regions, including: *OVCA1*, *OVCA2* and *HIC-1* in deletion region I; *p53* in deletion region II; *ZNF18*, *ZNF29*, *ALDH3* and *ALDH10* in deletion region III. These results will help us direct future testing of candidate genes and narrow the search region for major new tumor suppressor genes that may play a role in the pathogenesis of ESCC.

Introduction

Esophageal cancer, with a 5 year survival below 10%, is one of the most common fatal cancers world wide. There is great geographical variation in the occurrence of this tumor, including exceptionally high risk areas such as Shanxi Province, a region in north central China with some of the highest esophageal cancer rates in the world (1–4). Epidemiological studies indicate that tobacco and alcohol are the major risk factors for esophageal cancer in the low risk populations of Europe and North America, but the etiology of this tumor in high risk populations remains less clear. Several studies in the high risk regions of China have demonstrated a strong tendency towards familial aggregation or tendency towards clustering of cases within families, suggesting that genetic susceptibility may play an important role in the etiology of esophageal cancer in these populations (5–9).

Previous molecular genetic studies have revealed multiple genetic alterations, including loss of tumor suppressor genes and activation of oncogenes, associated with the development of esophageal cancers (10–17). Chromosomal regions with frequent allelic loss may point to major tumor suppressor genes that can assist our understanding of the molecular events involved in the development of esophageal cancer and may serve as the basis for development of markers for genetic susceptibility testing or screening for early detection of this tumor.

We have previously conducted two studies to better understand the genetic changes involved in the development of esophageal cancer in high risk Shanxi patients. The first study used a genome wide scan with 366 microsatellite markers in 11 esophageal squamous cell cancer (ESCC) patients with a family history of upper gastrointestinal (UGI) cancer and identified 46 markers, representing 14 regions, with very high frequencies (≥75%) of loss of heterozygosity (LOH) (18). In that study, 11 microsatellite markers on chromosome 17 were tested and one marker (D17S1303 at 17p13–p11) showed a very high frequency of LOH (80%) (18). Our second study used 18 of the 46 markers with a very high frequency of allelic loss, including D17S1303, to examine 46 ESCC patients, including 23 with and 23 without a family history of UGI cancer (19). In this study we also found frequent LOH at D17S1303 (in 100 and 89% of the two patient groups, respectively) (19).

Allelic loss on chromosome 17p has been reported frequently in esophageal cancer, including both ESCC and esophageal adenocarcinoma, and generally encompasses the *p53* locus at 17p13.1 (16,20–22). However, a correlation between LOH on 17p and *p53* mutation has not been found. This suggests that unknown tumor suppressor genes near *p53* may be involved in the development of esophageal cancer. To evaluate this possibility, we used 30 microsatellite markers covering the entire short arm of chromosome 17 to look for allelic loss in 56 ESCC patients from Shanxi province, China, that we have previously evaluated (18,19). In addition, we compared the LOH results with the cancer lifestyle risk factors for these patients and the clinical/pathological characteristics of their tumors to look for correlations.

Materials and methods

Patient selection

Patients presenting in 1995 and 1996 to the Shanxi Cancer Hospital in Taiyuan, Shanxi Province, People's Republic of China, who were diagnosed

Abbreviations: ESCC, esophageal squamous cell carcinoma LCM, laser capture microdissection LOH, loss of heterozygosity; UGI, upper gastrointestinal.

*The first two authors contributed equally to this work.

Table I. Demographics, clinical/pathological characteristics, cancer lifestyle risk factors[a] and allelic loss of ESCC patients

Patient no.	Patient ID	Age/sex	Location of tumor	Grade	Stage	Lymph node metastasis	Smoker	Drinks alcohol	Eats pickled vegetables	Eats scalding hot food	Family history of upper gastrointestinal cancer[b]	Loss/no. informative (%)
1	SHE021	57/F	Middle	2	III	N	N	Weekly	Daily	Daily	2 EC (father, paternal grandfather)	17/18 (94)
2	SHE027	59/M	Middle	2	III	N	Y	Daily	Seldom	Seldom	CC (brother)	1/16 (6)
3	SHE057	50/F	Middle	2	III	Y	N	Weekly	Never	Never	EC (sister)	17/20 (85)
4	SHE066	51/M	Middle	3	III	Y	Y	Never	Monthly	Daily	CC (father), EC (mother)	13/18 (72)
5	SHE080	39/F	Middle	2	III	N	N	Never	Daily	Seldom	EC(father)	14/14 (100)
6	SHE081	58/M	Lower	2	III	N	N	Weekly	Daily	Daily	EC (mother)	14/16 (88)
7	SHE083	64/M	Lower	2	III	N	Y	Weekly	Never	Never	EC(father)	12/12 (100)
8	SHE093	55/M	Middle	2	III	N	N	Never	Seldom	Daily	EC(father)	14/15 (93)
9	SHE098	53/M	Upper	2	III	N	Y	Weekly	Daily	Never	EC (paternal grandmother)	11/15 (73)
10	SHE108	47/M	Middle	2	III	Y	Y	Never	Daily	Never	EC (paternal uncle), CC (paternal uncle)	14/14 (100)
11	SHE109	59/F	Middle	2	III	Y	N	Daily	Never	Daily	EC (paternal uncle)	2/13 (15)
12	SHE113	43/F	Middle	2	III	N	N	Never	Seldom	Seldom	EC (maternal uncle)	17/17 (100)
13	SHE123	56/M	Middle	2	III	NA	Y	Never	Daily	Seldom	2 EC (paternal uncle, paternal grandfather)	10/11 (91)
14	SHE138	55/F	Middle	2	III	N	N	Never	Daily	Never	EC (mother)	1/15 (7)
15	SHE150	57/M	Middle	1	III	N	Y	Weekly	Daily	Daily	EC (mother)	18/20 (90)
16	SHE152	44/F	Lower	2	III	N	N	Never	Weekly	Daily	CC (mother)	12/13 (92)
17	SHE186	43/M	Middle	2	III	N	N	Weekly	Daily	Never	EC (paternal uncle), CC (brother)	17/17 (100)
18	SHE235	59/F	Middle	2	III	N	Y	Weekly	Monthly	Monthly	EC (father)	14/19 (74)
19	SHE252	57/F	Middle	2	III	Y	N	Never	Monthly	Monthly	5 EC (father, mother, sister, sister, paternal grandmother)	13/14 (93)
20	SHE263	50/M	Middle	2	III	NA[c]	Y	Weekly	Weekly	Daily	3 EC (maternal uncle, maternal grandfather, maternal grandmother)	17/18 (94)
21	SHE265	49/F	Middle	1	III	N	N	Never	Monthly	Daily	EC (father), BC (mother)	15/16 (94)
22	SHE328	50/F	Middle	2	III	N	N	Never	Monthly	Monthly	2 EC (maternal uncle, maternal cousin)	13/14 (93)
23	SHE340	50/M	Lower	2	III	Y	Y	Weekly	Monthly	Daily	CC (father), EC (paternal uncle)	11/14 (79)
24	SHE360	55/M	Middle	3	III	Y	N	Never	Monthly	Daily	EC (father), BC (brother)	19/20 (95)
25	SHE384	53/F	Middle	2	III	N	N	Never	Monthly	Daily	2 EC (father, sister), BC (brother)	21/22 (95)
26	SHE391	55/M	Middle	2	III	N	N	Weekly	Weekly	Monthly	EC (father)	15/15 (100)
27	SHE408	65/M	Middle	1	III	N	Y	Daily	Weekly	Daily	EC (paternal uncle's son)	10/13 (77)
28	SHE409	42/M	Middle	2	III	N	Y	Weekly	Monthly	Monthly	EC (father)	6/18 (33)
29	SHE437	47/F	Middle	2	III	N	N	Never	Monthly	Monthly	2 EC (father, mother)	3/18 (17)
30	SHE444	65/M	Lower	2	III	N	Y	Never	Monthly	Monthly	EC (father)	14/15 (93)
31	SHE459	43/F	Lower	2	III	N	N	Never	Monthly	Daily	EC (paternal aunt)	14/16 (88)
32	SHE480	65/M	Lower	1	III	Y	Y	Daily	Monthly	Daily	EC (paternal uncle)	14/14 (100)
33	SHE495	54/M	Middle	2	III	Y	Y	Weekly	Daily	Daily	EC (father)	14/17 (82)
34	SHE516	58/M	Middle	2	III	N	N	Daily	Monthly	Monthly	2 EC (mother, brother)	9/12 (75)
35	SHE034	63/M	Lower	2	III	Y	Y	Daily	Monthly	Daily	N	7/15 (47)
36	SHE052	57/M	Middle	2	III	N	N	Weekly	Daily	Never	N	14/15 (93)
37	SHE069	48/M	Middle	3	III	N	Y	Never	Daily	Never	N	11/12 (92)
38	SHE095	58/M	Middle	1	III	N	Y	Never	Daily	Daily	N	15/17 (88)
39	SHE096	57/M	Middle	1	III	N	Y	Weekly	Monthly	Daily	N	15/18 (83)

Table I. *Continued*

Patient no.	Patient ID	Age/sex	Location of tumor	Grade	Stage	Lymph node metastasis	Smoker	Drinks alcohol	Eats pickled vegetables	Eats scalding hot food	Family history of upper gastrointestinal cancer[b]	Loss/no. informative (%)
40	SHE118	56/F	Middle	3	III	Y	N	Never	Daily	Never	N	21/21 (100)
41	SHE170	52/F	Middle	2	III	N	Y	Weekly	Daily	Monthly	N	1/16 (6)
42	SHE198	53/M	Middle	2	III	Y	Y	Weekly	Daily	Weekly	N	15/17 (88)
43	SHE200	59/F	Middle	2	III	Y	N	Weekly	Monthly	Daily	N	16/17 (94)
44	SHE208	56/F	Middle	2	III	N	Y	Never	Daily	Daily	N	1/14 (7)
45	SHE216	43/F	Middle	2	II	N	N	Weekly	Weekly	Daily	N	18/19 (95)
46	SHE240	51/M	Middle	2	III	Y	Y	Daily	Weekly	Never	N	17/21 (81)
47	SHE247	45/M	Middle	2	III	Y	Y	Never	Monthly	Daily	N	20/23 (87)
48	SHE261	57/M	distal	2	III	N	N	Weekly	Weekly	Monthly	N	0/17 (0)
49	SHE273	62/M	Middle	2	III	Y	N	Weekly	Monthly	Monthly	N	14/15 (93)
50	SHE297	50/M	Middle	2	III	Y	Y	Weekly	Daily	Monthly	N	15/15 (100)
51	SHE308	58/F	Middle	3	III	Y	Y	Never	Never	Daily	N	11/16 (69)
52	SHE322	50/F	Middle	2	III	N	N	Never	Daily	Daily	N	11/12 (92)
53	SHE488	48/F	Lower	2	III	N	N	Never	Weekly	Daily	N	5/13 (38)
54	SHE497	60/M	Middle	2	II	Y	Y	Weekly	Daily	Daily	N	7/18 (39)
55	SHE507	47/M	Middle	2	III	Y	Y	Weekly	Monthly	Monthly	N	22/22 (100)
56	SHE510	59/M	Middle	2	III	Y	Y	Daily	Monthly	Monthly	N	7/12 (58)

[a]For definitions see Materials and methods.
[b]Includes esophageal cancer (EC), cardia cancer (CC) and body of stomach cancer (BC).
[c]NA, not applicable.

with ESCC and considered candidates for curative surgical resection were identified and recruited to participate in this study. The study was approved by the Institutional Review Boards of the Shanxi Cancer Hospital and the US National Cancer Institute. For this study a total of 56 patients with ESCC were selected who had a histological diagnosis of esophageal squamous cell cancer confirmed by pathologists at both the Shanxi Cancer Hospital and the National Cancer Institute. None of the patients had prior therapy and Shanxi was the ancestral home for all. Of the 56 ESCC patients studied, 34 had a family history of UGI cancer (i.e. a first, second or third degree relative with cancer of the esophagus, gastric cardia or body of the stomach) and 22 had no a family history of any cancer (Table I).

After obtaining informed consent, patients were interviewed to obtain information on demographic and cancer lifestyle risk factors, including tobacco use, frequencies of alcohol, pickled vegetable and scalding hot food consumption and a detailed family history of cancer (including all cancers in the first, second and third degree relatives). Data were also recorded concerning the clinical/pathological characteristics of the patients' tumors, including location (upper, middle or lower third), pathological grade (G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated), pathological TNM stage (I–IV) and lymph node metastasis (yes or no).

Biological specimen collection and processing

Ten milliliters of venous blood were taken from each patient prior to surgery and genomic DNA was extracted and purified. Tumor tissue obtained during surgery was fixed in ethanol and embedded in paraffin.

Laser microdissection and extraction of DNA

Tumor cells were obtained by laser capture microdissection (LCM) (Pixcell 100; Arcturus Engineering, Mountain View, CA) using methods previously described (23,24). Briefly, unstained, ethanol-fixed, paraffin-embedded 5 µm histological tissue sections were prepared on glass slides, deparaffinized twice with xylene, rinsed twice with 95% ethanol, stained with eosin and air dried. Specific cells of interest were selected from the eosin-stained slides and microdissected by LCM. The cells obtained were immediately resuspended in an 80 µl solution containing 0.01 M Tris–HCl, 1 mM EDTA, 1% Tween-20 and 0.1 mg/ml proteinase K (pH 8.0) and incubated for two nights at 37°C. The mixture was then boiled for 5 min to inactive the proteinase K. Two microliters of this solution were used for each PCR reaction.

Markers, PCR and LOH reading and interpretation

Thirty polymorphic microsatellite markers on chromosome 17p, with heterozygosity ranging from 50 to 93%, were used for this study (Human MapPairs; Research Genetics, Huntsville, AL) (Table II). Of the 30 markers, 13 are found on the physical map (http://www.ncbi.nlm.nih.gov/) and 17 are on the genetic map located at 17p13–p11.

DNA extracted from tumor cells microdissected from the resection specimen and genomic DNA extracted from venous blood were used for each patient. PCR reactions were carried out using a 10 µl final volume containing 1.0 µl of 10× PCR buffer I (100 mM Tris–HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$), 1.0 µl of 1.25 mM dNTP, 2 µl of DNA extraction buffer, 0.6 µl of each primer, 0.09 µl of AmpliTaq DNA polymerase (Perkin Elmer) and 1 µCi [α-^{32}P]dCTP. Typical PCR conditions were as follows: 10 min denaturation at 94°C, then 30 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1 min. An elongation step at 72°C for 10 min was added to the final cycle. The PCR products were mixed with 5 µl of formamide loading dye (95% formamide, 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol), denatured for 6 min at 95°C and chilled on ice until being loaded onto a 6% polyacrylamide gel. Samples were electrophoresed at 60 W for 1–3 h and radiographed for 1–2 days using Kodak BioMax MR film.

LOH was defined as either complete or nearly complete loss of a band in the tumor sample relative to the corresponding normal DNA (Figure 1). There was no convincing evidence of a homozygous deletion in any tumor sample at any of the 30 markers employed. The results were reviewed independently by three investigators (J.Huang, N.Hu and A.M.Goldstein). Discrepant cases were re-evaluated, repeated if necessary and the data were accepted and included in the analysis only if all three reviewers agreed on the results.

Calculation of the frequency of allelic loss

The frequency of allelic loss at each chromosome locus was calculated as the number of tumors with allelic loss at that locus divided by the number of informative tumors at that locus. The frequency of allelic loss at each chromosome locus was classified as low (0–24%), medium (25–49%), high (50–74%) or very high (≥75%).

Statistical analysis

All statistical analyses were performed using Statistical Analysis Systems (SAS Corp., NC). The *t*-test (for continuous variables) and χ^2 or Mantel–Haenszel χ^2 or Fisher's exact test (for nominal variables) were used for statistical analysis of the relationship between LOH and lifestyle risk factors

Table II. Summary of frequency of allelic loss in ESCC patients with/without a family history of UGI cancer

Marker no.	Locus	Marker location on chromosome 17	LOH (%) (no. of cases with allelic loss/no. of informative cases/total no. cases)			*P* (two-sided Fisher's exact test)
			All patients (n = 56)	Patients with a family history of UGI cancer (n = 34)	Patients without a family history of any cancer (n = 22)	
1	D17S1798	17p13.3	87 (20/23/54)	80 (8/10/32)	92 (12/13/22)	0.560
2	D17S849	17p13.3	83 (25/30/52)	80 (16/20/34)	90 (9/10/18)	0.640
3	D17S926	17p13.3	86 (19/22/51)	100 (10/10/29)	75 (9/12/22)	0.221
4	D17S1832	17p13.3	84 (31/37/54)	91 (21/23/32)	71 (10/14/22)	0.173
5	D17S379	17p13.3	89 (17/19/46)	91 (10/11/28)	88 (7/8/18)	1.000
6	D17S1566	17p13.3	82 (18/22/51)	80 (8/10/31)	83 (10/12/20)	1.000
7	D17S1828	17p13.2	81 (26/32/50)	86 (19/22/31)	70 (7/10/19)	0.346
8	D17S1810	17p13.2	84 (37/44/54)	90 (26/29/34)	73 (11/15/20)	0.207
9	CHRNB1	17p13.2	78 (18/23/54)	85 (11/13/33)	70 (7/10/21)	0.618
10	D17S578	17p13.2	80 (24/30/51)	88 (15/17/31)	69 (9/13/20)	0.360
11	D17S938	17p13.2	84 (31//37/52)	83 (20/24/32)	85 (11/13/20)	1.000
12	D17S1854	17p13.2	71 (15/21/55)	92 (11/12/33)	44 (4/9/22)	0.046
13	D17S570	17p13.2	67 (22/33/56)	74 (14/19/34)	57 (8/14/22)	0.459
14	TP53	17p13.1	80 (33/41/53)	88 (23/26/33)	67 (10/15/20)	0.117
15	D17S786	17p13.1	70 (30/43/56)	64 (16/25/34)	78 (14/18/22)	0.503
16	D17S804	17p13.1	88 (30/34/53)	95 (19/20/31)	79 (11/14/22)	0.283
17	D17S945	17p13.1	84 (21/25/49)	82 (14/17/32)	88 (7/8/17)	1.000
18	D17S520	17p13.1	65 (29/44/51)	67 (16/24/31)	65 (13/20/20)	1.000
19	D17S1176	17p13.1	72 (33/46/51)	70 (21/30/30)	75 (12/16/21)	1.000
20	D17S559	17p13	88 (28/32/49)	86 (18/21/31)	91 (10/11/18)	1.000
21	D17S525	17p13	85 (11/13/54)	71 (5/7/32)	100 (6/6/22)	0.462
22	D17S513	17p13	76 (34/45/55)	84 (21/25/34)	65 (13/20/21)	0.176
23	D17S799	17p12	90 (26/29/51)	93 (14/15/30)	86 (12/14/21)	0.598
24	D17S922	17p12	76 (22/29/54)	82 (14/17/32)	67 (8/12/22)	0.403
25	D17S261	17p11.2	74 (17/23/56)	92 (12/13/34)	50 (5/10/22)	0.052
26	D17S122	17p11.2	72 (18/25/55)	67 (10/15/33)	80 (8/10/22)	0.659
27	D17S1857	17p11.2	75 (9/12/56)	86 (6/7/34)	60 (3/5/22)	0.523
28	D17S1358	17p11.2	74 (20/27/56)	69 (11/16/34)	82 (9/11/22)	0.662
29	D17S1288	17p11.1	33 (10/30/50)	38 (6/16/28)	29 (4/14/22)	0.709
30	D17S783	17p11.1	47 (16/34/54)	48 (12/252/33)	44 (4/9/21)	1.000

as well as clinical/pathological characteristics. All *P* values were two-sided and considered statistically significant at $P < 0.05$.

Results

Patient characteristics

A total of 56 ESCC patients, including 34 males and 22 females, were evaluated (Table I). Patients with a family history of UGI cancer included 24 patients with cancer in a first degree relative, eight with cancer in a second degree relative and two with cancer in a third degree relative. Patients without a family history of UGI cancer included 22 patients without a family history of any cancer. There were no significant differences between patients with and without a family history of UGI cancer for age (53 versus 54 years, $P = 0.31$), gender (59 versus 64% male; $P = 0.78$), tumor location (lower, 21 versus 14%; middle, 71 versus 86%; upper, 8 versus 0%; $P = 0.26$), tumor grade (G1, 12 versus 9%; G2, 82 versus 77%; G3, 6 versus 14%; $P = 0.60$), tumor stage (100 versus 91%, stage III; $P = 0.15$), lymph node metastasis (yes, 28 versus 55%; $P = 0.09$), tobacco use (44 versus 64%; $P = 0.15$), alcohol consumption (53 versus 64%; $P = 0.43$), any pickled vegetable consumption (82 versus 95%; $P = 0.15$) or very hot food consumption (71 versus 82%; $P = 0.34$).

Allelic loss in ESCC patients

Allelic loss was detected in 55 of 56 patients (98%) at one or more loci on 17p. Eighteen percent of patients ($n = 10$) had LOH at 100% of informative markers, half the patients showed loss for ⩾90% of markers (including 14 with retention of just a single informative marker) and four-fifths of the patients had allelic loss at >50% of the informative markers (Table I).

Deletion regions

Of the 30 markers covering the entire short arm of chromosome 17, 19 had a very high frequency of LOH (⩾75%), nine had a high frequency of LOH (50–74%) and two markers located at 17p11.1 showed medium frequency of allelic loss (25–49%) (Table II). Indeed, all 28 markers on 17p13.3–p11.2 showed allelic loss in ⩾65% of informative tumors, implying a very high degree of genetic instability throughout this entire area.

Within this area of high genome instability, however, smaller regions with especially high frequency LOH could be identified. Using the 13 markers located on the physical map, there were three regions where adjacent markers were deleted in ⩾80% of the informative tumors (Figure 2). Deletion region I displayed allelic loss frequencies of 83, 86 and 81% at markers D17S849, D17S926 and D17S1828, respectively, a distance of at least 9 cM or 3.2 Mb on 17p13.3–p13.2. (As D17S849 was our most telomeric marker, the telomeric boundary was not identified.) Deletion region II spanned only 1.67 cM or 0.313 Mb on 17p13.2–p13.1 and included markers D17S938 and TP53, which showed frequencies of 84 and 80%, respectively. Deletion region III included markers D17S804, D17S945 and D17S799, with LOH frequencies of 88, 84 and 90% and covered 11 cM or 1.868 Mb on 17p13.1–p12.

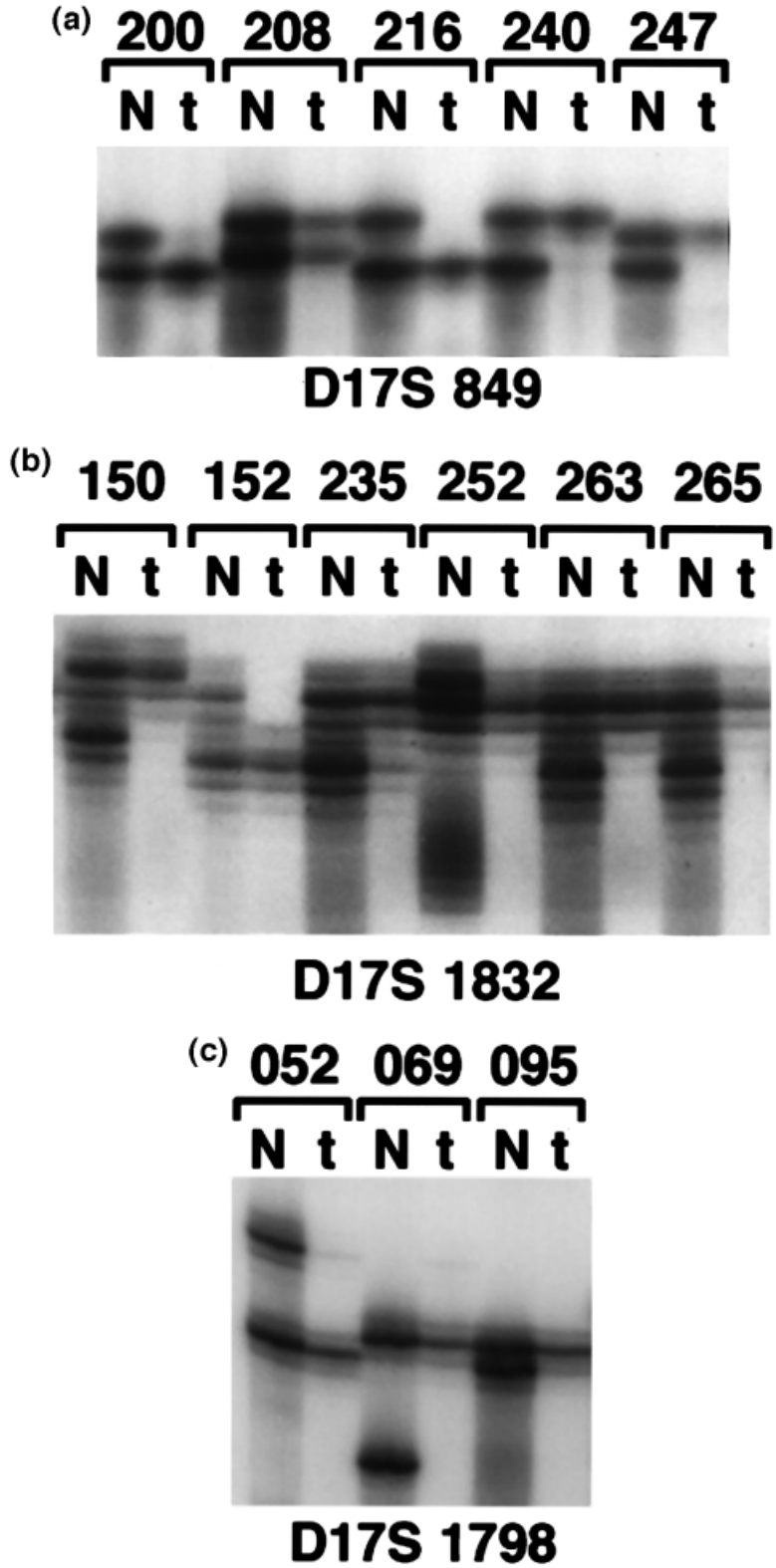

Fig. 1. Allelic loss on markers D17S849 (**a**), D17S1832 (**b**) and D17S1176 (**c**). N, normal DNA from blood; t, DNA from tumor cells.

Cancer lifestyle risk factors, clinical/pathological characteristics and LOH

A higher frequency of LOH was observed in patients with a family history of UGI cancer at 19 markers, but for only one of these markers (D17S1854) was this difference statistically significant (92 versus 44%; $P = 0.046$) (Table II). A higher LOH frequency at D17S926 (in deletion region I) was seen in male (73%) compared with female patients (27%) ($P = 0.046$). Pickled vegetable consumption was associated with a significantly higher LOH frequency at D17S849 (in deletion

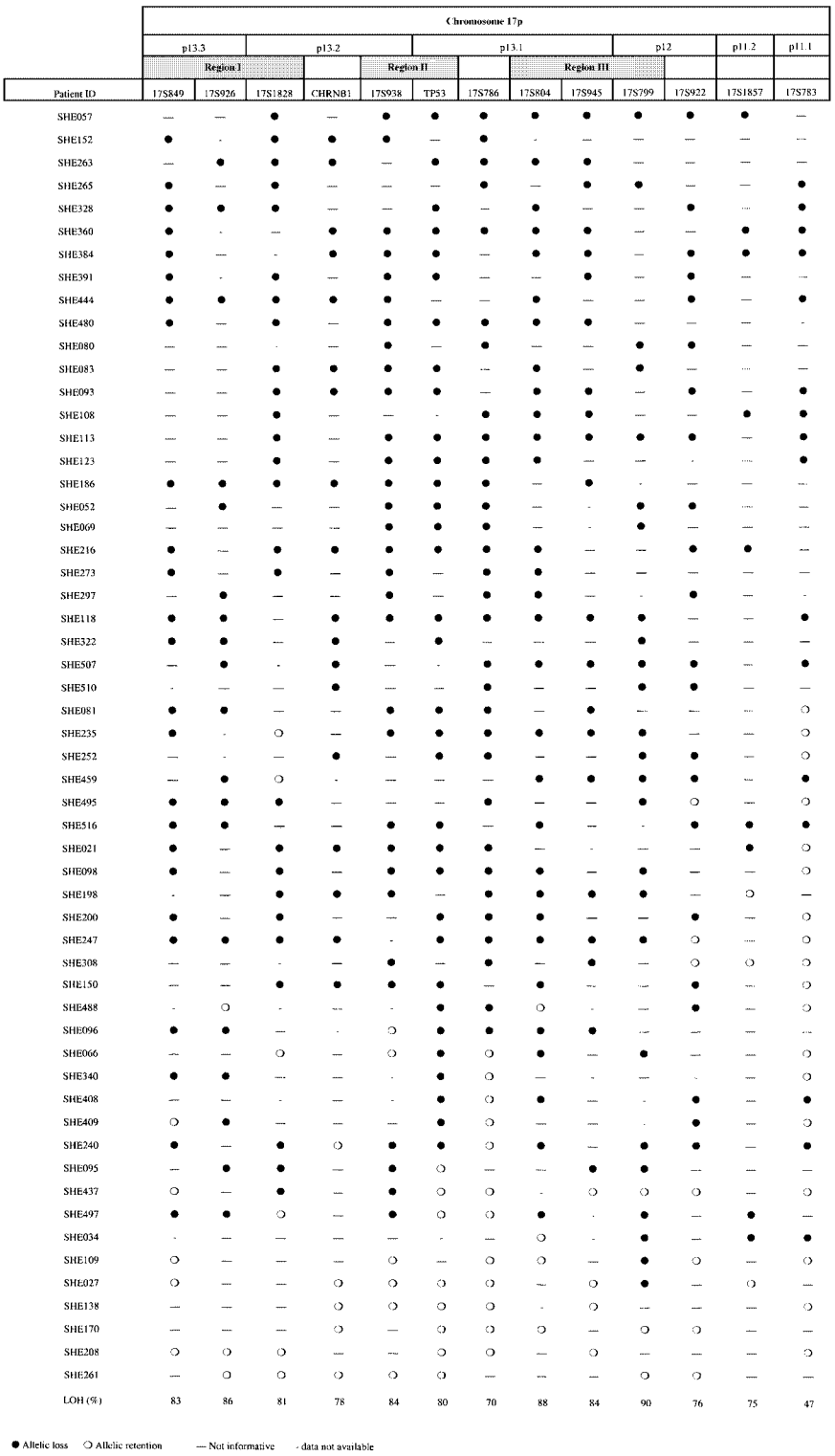

Fig. 2. Deletion map for chromosome 17p13.3–p11.1 in esophageal squamous cell carcinoma patients.

region I) (89 versus 0%; $P = 0.023$). Also, scalding hot food consumption was associated with a significantly lower frequency of allelic loss at D17S1288 (18 versus 75%; $P = 0.007$). There were no significant associations between tobacco use or alcohol consumption and higher LOH frequencies at any of the 30 markers tested. In addition, none of the four clinical/pathological characteristics examined was associated with a higher frequency of allelic loss at any of the 30 markers.

Discussion

This study reports the pattern of allelic loss found at 30 microsatellite markers on chromosome 17p13–p11 in 56 eso-

phageal squamous cell carcinomas from a high risk population in north central China. Our results indicate that LOH is widespread in this chromosomal region in most patients. Median LOH in our patients was 89%, compared with 43–65% reported in other studies (16). Potential explanations for our higher LOH rates include: the use of LCM tumor samples, which contain purer tumor DNA in which LOH is more easily seen; the possibility that tumors in our study were more advanced than in other studies; the possibility that there truly is greater genetic instability in ESCCs from this high incidence population. Although the number of studies and markers evaluated for LOH on 17p to date is small, the most likely explanation for our higher LOH rate would seem to be our use of LCM samples. Stage differences seem less likely since all reported ESCC LOH studies have analyzed specimens from patients who had been operated on and in the very few studies that examined ESCC from US patients without LCM, LOH has been comparable with studies from Asia (in the range of 60%).

Several tumor suppressor genes and candidate tumor suppressor genes are located on 17p and genetic alterations of these genes have been detected in many human tumors (16,22,25,27–29). Our results showed that all markers on 17p13.3–p11.2 had a high frequency of LOH, indicating that almost the entire short arm of chromosome 17 was very unstable in these ESCC patients. Within this broad area of genetic instability, however, we identified three smaller regions at 17p13.3–p13.2, 17p13.2–p13.1 and 17p13.1–p12 with especially high frequencies of LOH.

Several tumor suppressor genes have been described in our deletion region I (primarily 17p13.3). Phillips *et al.* reported that 80% of 57 early ovarian cancers showed LOH at 17p13.3 (25) and Schultz *et al.* identified two candidate tumor suppressor genes, *OVCA1* and *OVCA2*, at 17p13.3 (26). *HIC-1* (hypermethylated in cancer 1), another putative tumor suppressor gene at 17p13.3, has been shown to be hypermethylated in a number of cancers (27,28). Fujii *et al.* found LOH in the telomeric portion of 17p in 67% of the breast cancers they examined and determined that this correlated strongly with methylation of *HIC-1*, suggesting that loss of an unmethylated *HIC-1* allele may contribute to the inactivation of HIC-1 protein in cells with a pre-existing methylated allele (29). Recently, Dunn *et al.* reported allelic loss at D17S849 (on 17p13.3) in Barrett's esophageal adenocarcinoma (22). The very high frequencies of LOH that we found in both markers (D17S849 and D17S926) tested at 17p13.3 raises the possibility that *OVCA1*, *OVCA2* and/or *HIC-1* may be important in the development of ESCC in our patients. In addition, we also found a high frequency of LOH in the next adjacent marker (D17S1828), suggesting that an unknown tumor suppressor gene downstream from these previously described genes may also be important.

The principal known tumor suppressor gene in our deleted region II (17p13.2–p13.1) is *p53*. Dunn *et al.* also found a high frequency of LOH at the *p53* locus in esophageal adenocarcinomas (22) and suggested three possible interpretations for the observed loss: that *p53* is the sole target, a shared target or not a target at all. Further analyses of our samples will be required before we can more fully address these potential interpretations.

Our deletion region III at 17p13.1–p12, centromeric to *p53*, contains several interesting genes: *ZNF18* (zinc finger protein 18), *ZNF29* (zinc finger protein 29), *ALDH3* (aldehyde dehydrogenase 3) and *ALDH10* (aldehyde dehydrogenase 10). Allelic loss in this region may affect or reflect the activation or inactivation of these genes or it may be somehow related to *p53*. To the best of our knowledge, high frequency allelic loss in this region has not previously been reported in esophageal cancer.

There was no strong association between patterns of allelic loss on chromosome 17p and cancer lifestyle risk factors of our patients or the clinical/pathological characteristics of their tumors. Only one of 30 markers was significantly associated with a family history of UGI cancer, male gender, pickled vegetables consumption and eating scalding hot food, and no markers were significantly associated with smoking, alcohol consumption, tumor location, pathological grade, clinical stage or lymph node metastasis. Given the large number of evaluations, the significant associations may have occurred by chance alone.

In summary, we found high frequencies of LOH in nearly all of the 30 microsatellite markers we evaluated on chromosome 17p13.3–p11.2 in 56 ESCCs from a high risk population in northern China. The highest frequencies of allelic loss were found in three smaller regions, which may help us narrow the search for major tumor suppressor genes involved in the development of ESCC.

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