

Allelic Loss on Chromosome Bands 13q11-q13 in Esophageal Squamous Cell Carcinoma

Guang Li,¹ Nan Hu,² Alisa M. Goldstein,² Ze-Zhong Tang,¹ Mark J. Roth,² Quan-Hong Wang,¹ Sanford M. Dawsey,² Xiao-You Han,¹ Ti Ding,¹ Jing Huang,³ Carol Giffen,⁴ Philip R. Taylor,^{2*} and Michael R. Emmert-Buck^{2*}

¹Shanxi Cancer Hospital, Taiyuan, Shanxi, P.R. China

²National Cancer Institute, Bethesda, Maryland, USA

³Cancer Institute & Hospital, Chinese Academy of Medical Sciences, Beijing, P.R. China

⁴Information Management Services, Inc., Silver Spring, Maryland, USA

Allelic loss on chromosome 13 occurs frequently in esophageal squamous cell carcinoma. However, studies of the two known tumor suppressor genes located on 13q, *RBI* and *BRCA2*, have shown few mutations, suggesting that other genes are likely to be involved in the development of this tumor type. To identify a minimal deletion interval, we first analyzed 42 microsatellite markers spanning chromosome bands 13q11-q13 in 56 esophageal squamous cell carcinoma patients, including 34 with a family history of upper gastrointestinal cancer and 22 without a family history of cancer. Lifestyle risk factors and clinical/pathologic characteristics were also collected. Two commonly deleted regions were identified: one was located on band 13q12.11, between markers D13S787 and D13S221; the other was located on bands 13q12.3-q13.1 from markers D13S267 to D13S219. We observed higher allelic loss frequencies for eight of the microsatellite markers in those patients with a family history of upper gastrointestinal cancer compared to patients without such a history. This study suggests that one or more unidentified tumor suppressor genes are located on chromosome arm 13q that play a role in the development of esophageal squamous cell carcinoma. © 2001 Wiley-Liss, Inc.

The molecular events associated with the initiation and progression of esophageal cancer remain poorly understood. Chromosomal regions with frequent allelic loss in these neoplasms may represent inactivation of tumor suppressor genes that play a significant role in the development or progression of these tumors. In addition to helping us better understand esophageal carcinogenesis at a molecular level, identification of such genes likely will serve as the basis for the development of markers for genetic susceptibility testing, screening for early detection, and improved therapies.

We have previously conducted studies of the genetic changes involved in the development of esophageal squamous cell carcinoma (ESCC) (Hu et al., 1999, 2000). An initial genomewide scan in 11 ESCC patients with a family history of upper gastrointestinal (UGI) cancer identified 14 chromosomal regions with a high frequency ($\geq 75\%$) of loss of heterozygosity (LOH) (Hu et al., 2000). In this genomewide scan, nine microsatellite markers were tested on chromosome arm 13q, and all showed a high frequency of allelic loss (86–100%). A second study analyzed 18 of the most frequently lost markers from the genomewide scan in an additional 46 ESCC patients, including 23 with and 23 without a family history of UGI cancer (Hu et al., 1999). In this study, only three markers were

tested on chromosome arm 13q, but all three showed higher frequencies of allelic loss in the patients with a family history of UGI cancer, including one marker (D13S894) for which the difference was statistically significant. These results encouraged us to examine more markers in this region in an effort better to characterize the LOH interval and to confirm the observed difference in LOH by family history status.

Although LOH on chromosome arm 13q is frequently detected in many types of tumors (Kuroki et al., 1995; Montesano et al., 1996; Eiriksdottir et al., 1998; Hyttinen et al., 1999), only two tumor suppressor genes have been identified on this chromosome to date: *RBI* on band 13q14.2 and *BRCA2* on band 13q12.1 (Lee et al., 1987; MacGee et al., 1989; Wooster et al., 1995). LOH at the *RBI* locus has been reported in 54% of esophageal squamous cell carcinomas and 36% of esophageal adenocarci-

Guang Li and Nan Hu contributed equally to this work.

*Correspondence to: Philip R. Taylor, Cancer Prevention Studies Branch, NCI, 6006 Executive Plaza, Rm 321, Bethesda, MD 20892-7058. E-mail: phil_taylor@nih.gov; or Michael R. Emmert-Buck, Pathogenetics Unit, Laboratory of Pathology, NCI, Rm. 2A33, Bldg. 10, 9000 Rockville Pike, Bethesda, MD 20892. E-mail: mb184z@nih.gov

Received 24 December 2000; Accepted 24 January 2001

TABLE I. Summary of Frequency of Allelic Loss in Esophageal Squamous Cell Carcinoma Patients With and Without a Family History of Upper Gastrointestinal Cancer

| Marker no. | Locus | Location on chromosome 13 | LOH (%) (no. of cases with allelic loss/no. of informative cases/total no. of cases) | | | P value |
|------------|-----------------------|---------------------------|--|--|--|----------------|
| | | | All patients (n = 56) | Patients with a family history of UGI cancer ^a (n = 34) | Patients without a family history of any cancer (n = 22) | |
| 1 | D13S141 | 13q11 | 29 (2/7/55) | 67 (2/3/34) | 0 (0/4/21) | — ^b |
| 2 | D13S175 ^c | 13q11 | 65 (17/26/48) | 62 (8/13/27) | 69 (9/13/21) | 1.000 |
| 3 | D13S1236 ^c | 13q11 | 70 (23/33/55) | 88 (14/16/34) | 53 (9/17/21) | 0.031 |
| 4 | D13S115 ^c | 13q11 | 25 (7/28/56) | 25 (5/20/34) | 25 (2/8/22) | 1.000 |
| 5 | D13S145 | 13q12.1 | 19 (9/48/55) | 18 (6/33/34) | 20 (3/15/21) | 1.000 |
| 6 | D13S308E | 13q12.1 | 66 (25/38/56) | 70 (16/23/34) | 60 (9/15/22) | 0.728 |
| 7 | D13S246 | 13q12.1 | 40 (16/40/56) | 29 (8/28/34) | 67 (8/12/22) | 0.037 |
| 8 | D13S310 | 13q12.1 | 55 (12/22/48) | 69 (11/16/31) | 17 (1/6/17) | 0.029 |
| 9 | D13S232 | 13q12.11 | 67 (26/39/53) | 63 (17/27/32) | 75 (9/12/21) | 0.714 |
| 10 | D13S292 ^c | 13q12.11 | 62 (16/26/41) | 71 (10/14/20) | 50 (6/12/21) | 0.422 |
| 11 | D13S787 ^c | 13q12.11 | 79 (23/29/49) | 83 (15/18/29) | 73 (8/11/20) | 0.646 |
| 12 | D13S1243 ^c | 13q12.11 | 79 (23/29/55) | 86 (12/14/34) | 73 (11/15/21) | 0.651 |
| 13 | D13S283 ^c | 13q12.11 | 76 (29/38/54) | 86 (18/21/34) | 65 (11/17/20) | 0.249 |
| 14 | D13S221 ^c | 13q12.11 | 89 (32/36/52) | 94 (16/17/30) | 84 (16/19/22) | 0.605 |
| 15 | D13S1294 ^c | 13q12.11 | 57 (12/21/56) | 58 (7/12/34) | 56 (5/9/22) | 1.000 |
| 16 | D13S1285 | 13q12.12 | 52 (14/27/49) | 57 (13/23/31) | 25 (1/4/18) | 0.326 |
| 17 | D13S1304 | 13q12.12 | 40 (16/40/55) | 52 (12/23/33) | 24 (4/17/22) | 0.104 |
| 18 | D13S1254 | 13q12.12 | 56 (15/27/52) | 71 (10/14/31) | 38 (5/13/21) | 0.128 |
| 19 | FLT1 ^c | 13q12.12 | 0 (0/0/53) | 0 (0/0/31) | 0 (0/0/22) | — ^b |
| 20 | D13S1244 | 13q12.12 | 61 (33/54/55) | 76 (25/33/33) | 38 (8/21/22) | 0.001 |
| 21 | D13S243 | 13q12.12 | 69 (20/29/52) | 79 (15/19/33) | 50 (5/10/19) | 0.205 |
| 22 | D13S625 | 13q12.12 | 75 (9/12/55) | 100 (7/7/31) | 40 (2/5/14) | 0.045 |
| 23 | D13S1242 ^c | 13q12.12 | 46 (18/39/52) | 68 (15/22/32) | 18 (3/17/20) | 0.003 |
| 24 | D13S217 ^c | 13q12.12 | 55 (12/22/50) | 67 (8/12/28) | 40 (4/10/22) | 0.391 |
| 25 | D13S1250 | 13q12.12 | 42 (11/26/55) | 58 (11/19/33) | 0 (0/7/22) | 0.010 |
| 26 | D13S120 | 13q12.12 | 59 (16/27/54) | 65 (11/17/32) | 50 (5/10/22) | 0.687 |
| 27 | D13S802 | 13q12.12 | 79 (31/39/55) | 81 (21/26/33) | 77 (10/13/22) | 1.000 |
| 28 | D13S629 | 13q12.13 | 46 (17/37/50) | 57 (16/28/33) | 11 (1/9/17) | 0.023 |
| 29 | D13S1299 | 13q12.13 | 54 (13/24/56) | 64 (9/14/34) | 40 (4/10/22) | 0.408 |
| 30 | D13S1246 | 13q12.13 | 66 (19/29/54) | 61 (11/18/32) | 73 (8/11/22) | 0.694 |
| 31 | D13S1287 | 13q12.13 | 48 (13/27/48) | 59 (10/17/31) | 30 (3/10/17) | 0.236 |
| 32 | D13S289 ^c | 13q12.13 | 60 (18/30/53) | 82 (14/17/33) | 31 (4/13/20) | 0.008 |
| 33 | D13S1229 | 13q12.13 | 55 (21/38/56) | 64 (16/25/34) | 38 (5/13/22) | 0.178 |
| 34 | D13S1238 | 13q12.13 | 61 (20/33/56) | 63 (12/19/34) | 57 (8/14/22) | 1.000 |
| 35 | D13S290 ^c | 13q12.2 | 71 (10/14/51) | 75 (6/8/29) | 67 (4/6/22) | 1.000 |
| 36 | D13S893 ^c | 13q12.2 | 0 (0/4/33) | 0 (0/1/16) | 0 (0/3/17) | — ^b |
| 37 | D13S1226 | 13q12.2 | 0 (0/2/56) | 0 (0/2/34) | 0 (0/0/22) | — ^b |
| 38 | D13S260 ^c | 13q12.2 | 57 (17/30/54) | 60 (12/20/32) | 45 (5/11/22) | 0.477 |
| 39 | D13S1293 | 13q12.3 | 71 (29/41/55) | 77 (20/26/34) | 60 (9/15/21) | 0.300 |
| 40 | D13S267 ^c | 13q12.3 | 83 (33/40/56) | 83 (19/22/34) | 82 (14/17/22) | 1.000 |
| 41 | D13S220 ^c | 13q12.3 | 71 (10/14/55) | 75 (6/8/34) | 67 (4/6/21) | 1.000 |
| 42 | D13S219 ^c | 13q13.1 | 70 (28/40/55) | 77 (20/26/34) | 57 (8/14/21) | 0.281 |

^aUpper gastrointestinal cancer.^bMarker dropped as the number of informative cases was fewer than 10.^cMarker is on the physical map.

nomas (Boynton et al., 1991; Huang et al., 1992), but few mutations have been detected (Maesawa et al., 1994). *BRCA2* has not been found to be altered frequently in primary ESCC (Montesano et al., 1996). Recently, Harada et al. (1999) reported that LOH at D13S171, a marker flanking *BRCA2*,

showed a significant correlation with lymph node metastasis in ESCC. However, no specific tumor mutation was observed in the *BRCA2* gene itself. Whereas *RBI* and *BRCA2* could still be the target genes that are affected by 13q LOH through inactivation by mechanisms other than mutation (e.g.,

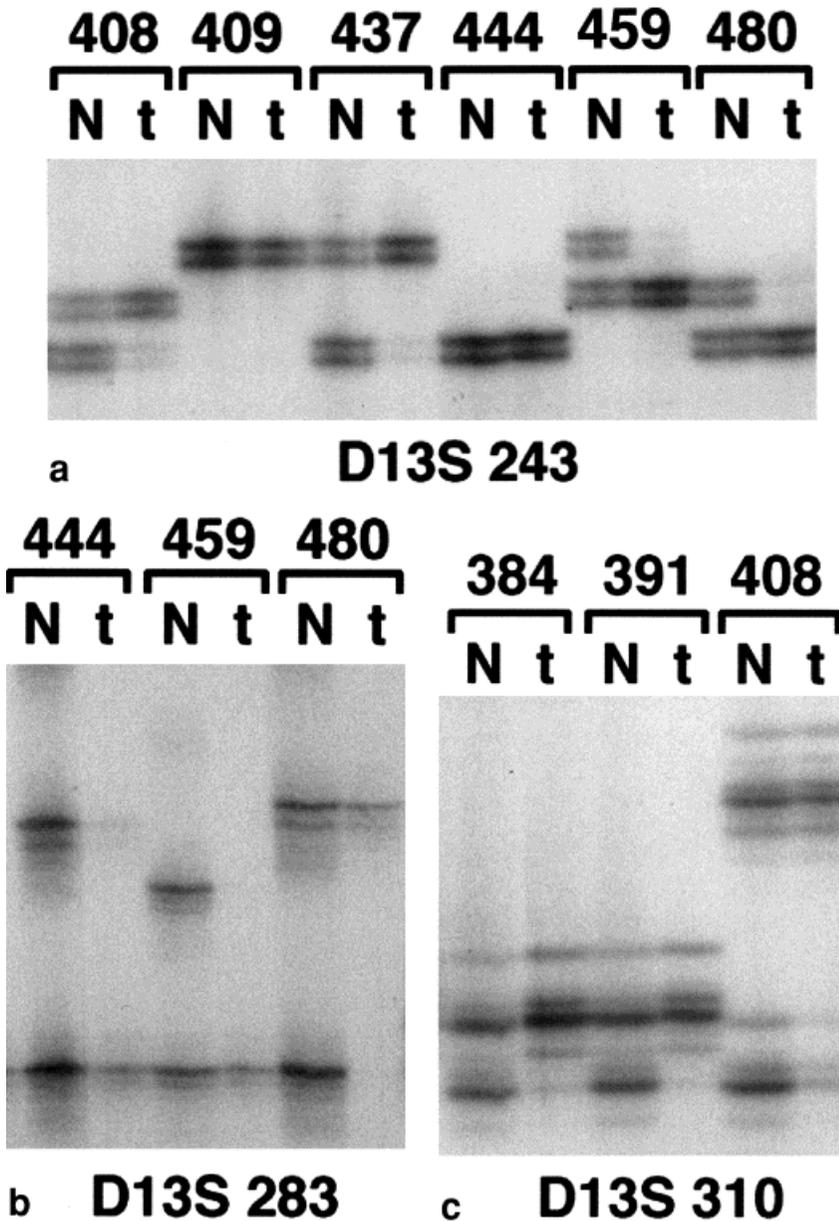


Figure 1. Allelic loss on markers D12S243 (a), D12S283 (b), and D12S310 (c). Individual case numbers are shown at the top. N is normal DNA from blood and t is DNA from tumor cells.

promoter region methylation or haplotype insufficiency), frequent LOH in the absence of mutations in the *RBI* and *BRCA2* genes in ESCC patients suggests that other unknown gene(s) on chromosome arm 13q may be involved in the development of ESCC. To narrow the search region for such genes, we analyzed 42 microsatellite markers spanning bands 13q11-q13 in 56 ESCC patients, including 34 with a family history of UGI cancer and 22 without a family history of any cancer. Patients seen in 1995 and 1996 at the Shanxi Cancer Hospital in Taiyuan, Shanxi Province, People's Republic of China, who were diagnosed 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 (NCI). For this study, a total of 56 patients were selected who had a histologic diagnosis of ESCC confirmed by pathologists at both the Shanxi Cancer Hospital and the NCI. None of the patients had had prior therapy. Of the 56 ESCC patients studied, the 34 patients in Group 1 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 stomach), and the 22 patients in Group 2 had no family history of any cancer (for a more detailed description of cases, see Huang et al., 2000).

Information on demographic and cancer lifestyle risk factors and a detailed family history of cancer was obtained as previously described (Huang et al., 2000). Ten ml of venous blood was taken from each patient prior to surgery, and genomic DNA was extracted and purified using standard methods. Tumor tissue obtained during surgery was fixed in ethanol and embedded in paraffin. Tumor cells were microdissected under direct-light microscopic visualization using methods previously described (Emmert-Buck et al., 1996; Bonner et al., 1997; Huang et al., 2000).

Forty-two polymorphic microsatellite markers on chromosome arm 13q with heterozygosity rates ranging from 46–83% were used for this study (Human MapPairs™; Research Genetics, Huntsville, AL) (Table 1). These 42 markers, including 19 on the physical map and 23 on the genetic map (<http://cedar/genetics.soton.ac.uk/pub>), are located on bands 13q11-q13. 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 in duplicate as previously described (Huang et al., 2000). LOH was defined as either complete or near-complete loss of a band in the tumor sample in both PCR reactions relative to the corresponding normal DNA (Fig. 1). Overall, the large and small alleles observed in each patient were lost approximately 50% of the time, indicating that the LOH was not a technical artifact due to preferential amplification of an allele based on size. Convincing evidence of a homozygous deletion in a tumor sample was not observed in any of the 42 markers employed. We also looked for microsatellite instability, but observed it only in a single different marker in each of two different cases. The results were reviewed independently by three investigators (GL, NH, and ME-B). Discrepant cases were reevaluated and repeated if necessary, and the data were accepted and included in the analysis only if all three reviewers agreed on the results.

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 ($\geq 75\%$). All statistical analyses were performed using Statistical Analysis Sys-

tems (SAS) software (SAS Corp., Cary, NC). The frequency of allelic loss was compared in Groups 1 and 2 by Fisher's exact test. All *P* values were two-sided and were considered statistically significant if *P* < 0.05.

Of the 56 ESCC patients analyzed, 54 (96%) showed LOH at one or more loci on bands 13q11-q13, and 37 (66%) showed allelic loss at more than half of the informative loci. Of the 42 microsatellite markers tested on bands 13q11-q13, four markers (FLT1 and D13S893 on the physical map; D13S141 and D13S1226 on the genetic map) were uninformative in nearly all cases (Table 1). LOH in the other 38 markers ranged from 19–89%: seven markers showed a very high frequency loss, 23 a high frequency of loss, six a medium frequency of loss, and two a low frequency of loss. Marker D13S221 showed the highest frequency of LOH (89%), followed by markers D13S802 (83%), D13S787 (79%), and D13S1243 (79%) (Table 1).

We used the 17 markers with at least 10 informative cases from the physical map to define boundaries for chromosomal regions with a high frequency of LOH. Fifty-three of the 56 cases (95%) showed LOH for one or more of these markers. Figure 2 shows the results for each of these 18 loci in all 56 ESCC patients, including 10 cases who showed allelic loss for all informative markers, 43 cases with allelic loss at one or more but not all loci, and three cases with no allelic loss at any loci. Two deleted regions were identified. The first region (I) was at segment 13q12.11 and was defined by markers D13S787, D13S1243, D13S283, and D13S221 (LOH frequencies of 79%, 77%, 76%, and 89%, respectively), spanning a physical distance of 1.83 Mb. The second deletion region (II) was at 13q12.3-q13.1 and included markers D13S267, D13S220, and D13S219 (LOH frequencies of 83%, 71%, and 70%, respectively). Since D13S219 was the most distal marker evaluated and is part of deletion region II, we cannot fully define the telomeric end of this deletion region.

Of the 34 ESCC patients with a family history of UGI cancer (Group 1), 26 (76%) showed allelic loss for more than 50% of their informative loci, compared to only 11 of 22 (50%) of the patients without a family history of UGI cancer (Group 2) (*P* = 0.04). There were 38 markers that were informative in at least eight cases. A higher frequency of LOH was observed in Group 1 compared to Group 2 for 33 of these 38 markers (87%). For eight of these markers (three physical map markers and five genetic map markers), the difference in LOH frequency was statistically significant (Table 1). Five

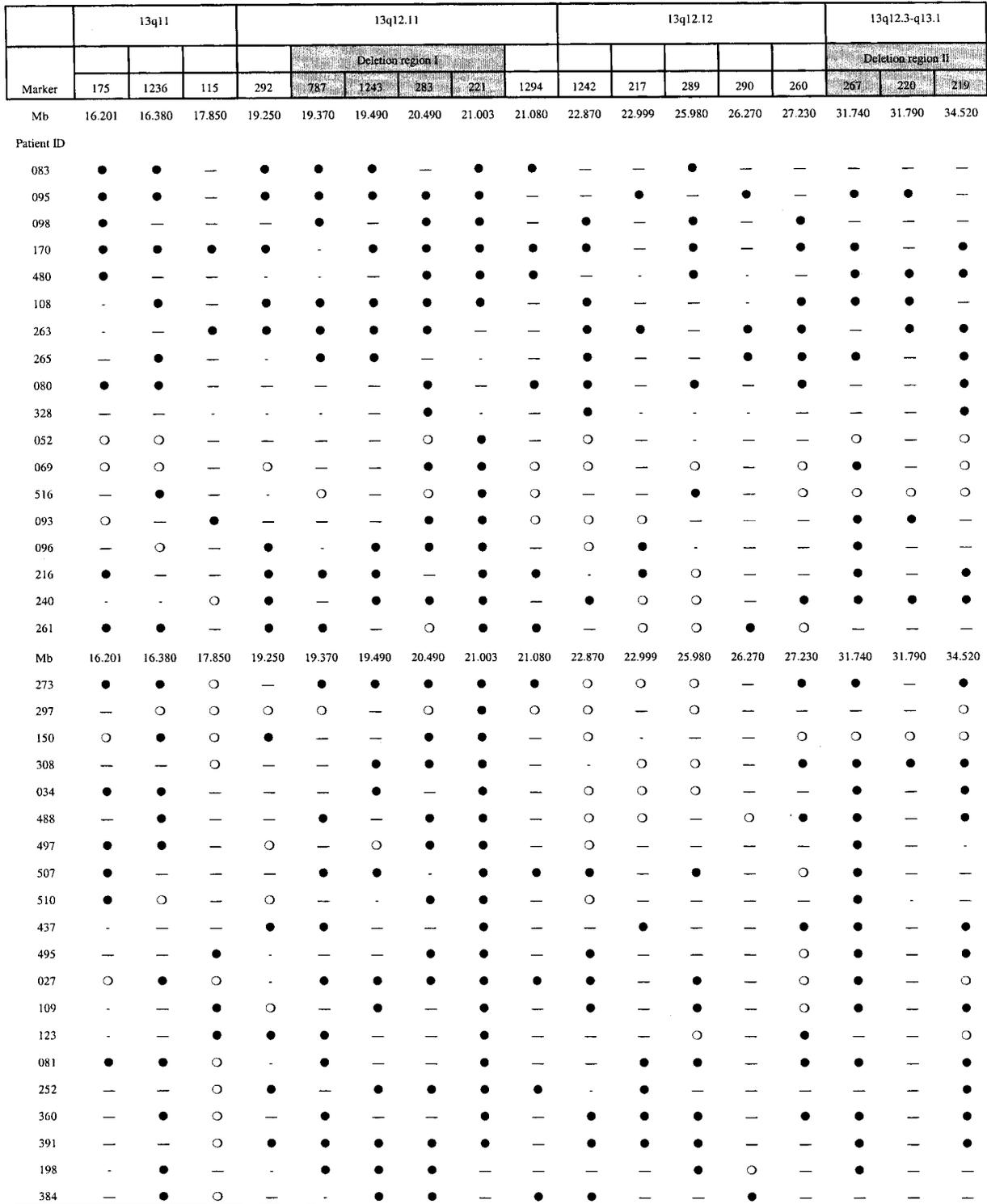


Figure 2. Deletion map for chromosome bands 13q11-q13 in esophageal squamous cell carcinoma patients.

of the 38 markers showed a lower frequency of LOH in Group 1 compared to Group 2, and for one of these markers (D13S246), this difference was significant (Table 1).

LOH studies have demonstrated that loss of chromosome arm 13q occurs commonly in several human tumor types. LOH studies on the entire long arm of chromosome 13 were recently reported

| Marker | 13q11 | | | 13q12.11 | | | | | 13q12.12 | | | | | 13q12.3-q13.1 | | | |
|--------|--------|--------|--------|----------|-------------------|--------|--------|--------|----------|--------|--------|--------|--------|---------------|--------------------|--------|--------|
| | 175 | 1236 | 115 | 292 | Deletion region I | | | | 1294 | 1242 | 217 | 289 | 290 | 260 | Deletion region II | | |
| | 175 | 1236 | 115 | 292 | 787 | 1243 | 283 | 221 | 1294 | 1242 | 217 | 289 | 290 | 260 | 267 | 220 | 219 |
| Mb | 16.201 | 16.380 | 17.850 | 19.250 | 19.370 | 19.490 | 20.490 | 21.003 | 21.080 | 22.870 | 22.999 | 25.980 | 26.270 | 27.230 | 31.740 | 31.790 | 34.520 |
| 408 | — | — | ○ | — | ● | — | ● | — | — | ● | — | — | — | — | ● | — | — |
| 444 | ● | ● | ○ | — | — | — | ● | — | ○ | ○ | ● | ● | ● | ● | — | — | ● |
| 459 | — | ● | — | ○ | — | ● | ● | — | — | — | — | ● | ● | — | ● | — | ● |
| 186 | — | — | ○ | — | ● | ● | ● | — | ● | — | ● | ● | — | — | ● | — | — |
| 021 | ● | ● | ○ | ● | ● | — | ● | — | — | — | — | — | — | ○ | ● | — | ● |
| 200 | — | ○ | — | — | — | ○ | ○ | ○ | — | ○ | — | ● | — | ○ | — | ○ | ○ |
| 247 | — | — | ● | ○ | ○ | ○ | ○ | ○ | — | ○ | — | — | — | — | — | ○ | ○ |
| 322 | ○ | ○ | ○ | ○ | — | ● | ○ | ○ | ○ | ○ | — | — | — | ○ | ○ | — | ○ |
| 057 | ● | ● | ○ | ● | ● | — | — | — | — | — | — | ● | — | ○ | ● | ● | ● |
| 138 | — | — | — | — | — | ● | — | — | — | ○ | — | ● | — | ● | — | — | ● |
| 152 | — | — | ○ | — | — | — | — | — | — | ○ | — | — | ● | ● | ● | ● | ● |
| 235 | — | ○ | — | — | — | — | — | — | — | ● | — | — | — | — | ● | — | ● |
| 118 | ○ | ● | — | — | ● | — | — | — | — | ○ | ● | ○ | ● | ○ | ● | — | ● |
| 340 | ○ | ○ | ○ | — | ○ | — | ○ | — | — | ○ | ○ | ○ | — | — | — | — | ● |
| 113 | — | — | — | ○ | — | ○ | ○ | — | ○ | ● | ○ | — | — | ○ | ● | — | — |
| 066 | ○ | — | ○ | ○ | ○ | — | — | ○ | — | ○ | — | — | ○ | — | ○ | — | ○ |
| 208 | — | ○ | ○ | — | ○ | ○ | — | — | ○ | ○ | — | — | — | — | — | ○ | — |
| 409 | — | — | — | — | — | ○ | — | — | ○ | — | ○ | ○ | ○ | — | ○ | — | ○ |
| LOH % | 65 | 70 | 25 | 62 | 79 | 79 | 76 | 89 | 57 | 46 | 55 | 60 | 71 | 57 | 83 | 71 | 70 |

● allelic loss ○ allelic retention — noninformative - data not available

Figure 2. (Continued.)

with 18 microsatellite markers in esophageal cancer (Harada et al., 1999), 26 microsatellite markers in prostate cancer (Hyytinen et al., 1999), 18 markers in breast cancer (Eiriksdottir et al., 1998), and 13 markers in hepatocellular carcinoma (Kuroki et al., 1995). In order to define the distribution and extent of chromosome arm 13q loss more clearly, we extended our previous work by analyzing 42 microsatellite markers located on bands 13q11-q13, representing the most detailed LOH testing on chromosome region 13q11-q13 reported to date in patients with ESCC.

Two deleted regions were identified in ESCC patients in this study. The first deleted region spans a distance of 1.83 Mb on segment 13q12.1. Based on the MIM gene map, this region contains several interesting candidate genes, including: *ED2* (ectodermal dysplasia 2) (Radhakrishna et al., 1997); *GJB2* (gap junction protein beta-2, also known as *CX26* or connexin 26 gap junction protein) (Mignon et al., 1996); *GJA3* (gap junction protein alpha-3, also known as *CX46* or connexin 46 gap junction protein) (Mignon et al., 1996); and *ZNF198* (zinc finger protein 198) (Xiao et al., 1998). The *ED2* gene is involved in hidrotic ectodermal

dysplasia (HED), an autosomal dominant disorder affecting the skin and its derivatives. Multiple cutaneous squamous cell carcinomas of the palmar tissue and nail bed have been reported in these patients (Campbell and Keokarn, 1966). The *GJB2* and *GJB3* genes encode the gap junction proteins connexin 26 and connexin 46, respectively. Connexins are transmembrane proteins that form channels allowing rapid transport of ions or small molecules between cells and are expressed in many different tissues. *GJB2* gene mutations can inactivate connexin 26 and are associated with autosomal recessive nonsyndromic deafness (Kelsell et al., 1997). The *ZNF198* gene has been associated with a specific chromosome translocation, t(8;13)(p11;q11-q12), which is found in both lymphoma and leukemia patients (Xiao et al., 1998), and has been identified on the rearranged band. This translocation can result in *ZNF198/FGFR1* (fibroblast growth factors receptor-1), a fusion gene that contributes to progression of leukemia/lymphoma by constitutive activation of tyrosine kinase function. Although these genes are not tumor suppressor genes, two (*ED2* and *ZNF198*) are related to tumors and two (*ED2* and *GJB2*) are associated with

hereditary diseases. Determining the possible roles of these genes in ESCC will require further analysis.

The second deleted region was located on segments 13q12.3-q13.1. Two of the markers that were frequently lost in this region (D13S260 and D13S267) are known to flank *BRCA2*. While several studies have shown a low frequency of *BRCA2* mutations in esophageal cancer patients (Montesano et al., 1996; Harada et al., 1999), the close proximity of *BRCA2* to this deletion interval necessitates that it be analyzed further in this group of patients. Another candidate gene in the interval is *GTF3A* (general transcription factor IIIA), whose developmentally regulated protein is involved in the assembly of active chromatin (Arakawa et al., 1995). Arakawa et al. (1995) reported that the zinc finger protein domains in *GTF3A* resembled those in the Wilms tumor protein, the transcriptional repressor YY1, and the *MYC*-associated zinc finger protein MAZ.

Eight of the 42 microsatellite markers we examined were lost significantly more often in patients with a positive family history of UGI cancer than in those without a family history. Only three of these markers were on our physical map, however, and none were in either of the two deletion regions we identified. This suggests that putative genes in these deletion regions are involved in both familial and sporadic ESCC.

It is evident from the high LOH rates observed at many loci throughout the genome that ESCC is genetically unstable. Compared to other tumor types that we have studied, this instability is striking and suggests that loss of function of genes that maintain genomic integrity (e.g., inactivation of mitotic arrest deficiency genes) is fundamental to initiation and/or progression of ESCC (Emmert-Buck et al., 1995, 1997). Our studies have shown that LOH not only occurs frequently throughout the genome in ESCC, but often occurs in an intermittent pattern, i.e., multiple interspersed regions of allelic loss and retention in relatively small genomic regions. A similar pattern has never been observed in LOH studies of other tumor types (i.e., breast, prostate, or endocrine) that we have performed using the same methods as in the present report. The widespread instability complicates precise mapping of tumor suppressor genes using LOH methods and dictates caution in interpreting results. Nonetheless, the high rates of allelic loss that were identified in the minimal deletion intervals using a large number of patients and a dense concentration of microsatellite markers is highly

suggestive that tumor suppressor genes important in ESCC are located within these regions.

In summary, detailed LOH mapping identified two commonly deleted regions on chromosome arm 13q in ESCC patients, one located on segment 13q12.11 and the other located on 13q12.3-q13. These findings suggest that there may be one or more as yet unidentified tumor suppressor genes on chromosome arm 13q that play a role in the development of ESCC.

REFERENCES

- Arakawa H, Nagase H, Hayashi N, Ogawa M, Nagata M, Fujiwara T, Takahashi E, Shin S, Nakamura Y. 1995. Molecular cloning, characterization, and chromosomal mapping of a novel human gene (*GTF3A*) that is highly homologous to *Xenopus* transcription factor IIIA. *Cytogenet Cell Genet* 70:235-238.
- Bonner RF, Emmert-Buck MR, Cole KA, Pohida T, Chuaqui RF, Zhuang ZP, Goldstein SR, Liotta LA. 1997. Laser capture microdissection: molecular analysis of tissue. *Science* 278:1481-1483.
- Boynton RF, Huang Y, Blount PL, Reid BJ, Raskind WH, Haggitt RC, Newkirk C, Resau JH, Yin J, McDaniel T, Meltzer SJ. 1991. Frequent loss of heterozygosity at the retinoblastoma locus in human esophageal cancers. *Cancer Res* 51: 5766-5769.
- Campbell G, Keokam TK. 1966. Squamous cell carcinoma of the nail bed in epidermal dysplasia. *Bone Joint Surg* 48:98-99.
- Eiriksdottir G, Johannesdottir G, Ingvarsson S, Bjornsdottir IB, Jonasson JG, Agnarsson BA, Hallgrímsson J, Gudmundsson J, Egilsson V, Sigurdsson H, Barkardottir RB. 1998. Mapping loss of heterozygosity at chromosome 13q: loss at 13q12-q13 is associated with breast tumour progression and poor prognosis. *Eur J Cancer* 34:2076-2081.
- Emmert-Buck MR, Vocke CD, Pozzatti RO, Duray PH, Jennings SB, Florence CD, Zhuang Z, Bostwick DG, Liotta LA, Linehan WM. 1995. Allelic loss on chromosome 8p12-21 in microdissected prostatic intraepithelial neoplasia (PIN). *Cancer Res* 55:2959-2962.
- Emmert-Buck MR, Bonner RF, Smith PD, Chuaqui RF, Zhuang ZP, Goldstein SR, Weiss RA, Liotta LA. 1996. Laser capture microdissection. *Science* 274:998-1001.
- Emmert-Buck MR, Lubensky IA, Dong Q, Chandrasekharappa C, Guru SC, Manickam P, Keseter M, Olufemi S-E, Agarwal S, Burns AL, Spiegel AM, Collins FS, Marx SJ, Zhuang Z, Liotta LA, Debelenko LV. 1997. Localization of the multiple endocrine neoplasia Type I (*MEN1*) gene based on tumor deletion mapping. *Cancer Res* 57:1855-1858.
- Harada H, Tanaka H, Shimada Y, Shinoda M, Imamura M, Ishizaki K. 1999. Lymph node metastasis is associated with allelic loss on chromosome 13q12-13 in esophageal squamous cell carcinoma. *Cancer Res* 59:3724-3729.
- Hu N, Roth MJ, Emmert-Buck MR, Tang ZZ, Polymeropolous M, Wang QH, Goldstein AM, Han XY, Dawsey SM, Ding T, Giffen C, Taylor PR. 1999. Allelic loss in esophageal squamous cell carcinoma patients with and without family history of upper gastrointestinal tract cancer. *Clin Cancer Res* 5:3476-3482.
- Hu N, Roth MJ, Polymeropolous M, Tang ZZ, Emmert-Buck MR, Wang QH, Goldstein AM, Feng SS, Dawsey SM, Ding T, Zhuang ZP, Han XY, Reid T, Giffen C, Taylor PR. 2000. Identification of novel regions of allelic loss from a genomewide scan of esophageal squamous cell carcinoma in a high-risk Chinese population. *Genes Chromosomes Cancer* 27:217-228.
- Huang Y, Boynton RF, Blount PL, Silberstein RJ, Yin J, Tong Y, McDaniel TK, Newkirk C, Resau JH, Sridhara R, Reid BJ, Meltzer SJ. 1992. Loss of heterozygosity involves multiple tumor suppressor genes in human esophageal cancers. *Cancer Res* 52: 6525-6530.
- Huang J, Hu N, Goldstein AM, Emmert-Buck MR, Tang ZZ, Roth MJ, Wang QH, Dawsey SM, Han XY, Ding T, Li G, Giffen C, Taylor PR. 2000. High frequency allelic loss on chromosome 17p13.3-p11.1 in esophageal squamous cell carcinomas from a high incidence area in northern China. *Carcinogenesis* 21:2019-2026.
- Hyytinen ER, Frierson HF Jr, Boyd JC, Chung LWK, Dong JT. 1999. Three distinct regions of allelic loss at 13q14, 13q21-22, and 13q33 in prostate cancer. *Genes Chromosomes Cancer* 25:108-114.

- Kelsell DP, Dunlop J, Stevens HP, Lench NJ, Liang JN, Parry G, Mueller RF, Leigh IM. 1997. Connexin 26 mutations in hereditary non-syndromic sensorineural deafness. *Nature* 387:80–83.
- Kuroki T, Fujiwara Y, Nakamori S, Imaoka S, Kanematsu T, Nakamura Y. 1995. Evidence for the presence of two tumour-suppressor genes for hepatocellular carcinoma on chromosome 13q. *Br J Cancer* 72:383–385.
- Lee WH, Bookstein R, Hong F, Young LJ, Shew JY, Lee EY. 1987. Human retinoblastoma susceptibility gene: cloning, identification, and sequence. *Science* 235:1394–1399.
- MacGee TL, Yandell DW, Dryjia TP. 1989. Structure and partial genomic sequence of human retinoblastoma susceptibility gene. *Gene* 80:119–128.
- Maesawa C, Tamura G, Suzuki Y, Ogasawara S, Ishida K, Satio K, Satodate R. 1994. Aberrations of tumor-suppressor genes (p53, apc, mcc, and Rb) in esophageal squamous-cell carcinoma. *Int J Cancer* 57:21–25.
- Mignon C, Fromaget C, Mattei MG, Gros D, Yamasaki H, Mesnil M. 1996. Assignment of connexin 26 (GJB2) and 46 (GJA3) genes to human chromosome 13q11-q12 and mouse chromosome 14D1-E1 by in situ hybridization. *Cytogenet Cell Genet* 72:185–186.
- Montesano R, Hollstein M, Hainaut P. 1996. Genetic alterations in esophageal cancer and their relevance to etiology and pathogenesis: a review. *Int J Cancer* 69:225–235.
- Radhakrishna U, Blouin JL, Mehenni H, Methra TY, Sheth FJ, Sheth JJ, Solanki JV, Antonarakis SE. 1997. The gene for autosomal dominant hidrotic ectodermal dysplasia (Clouston syndrome) in a large Indian family maps to the 13q11-q12.1 pericentromeric region. *Am J Med Genet* 71:80–86.
- Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, Collins N, Gregory S, Gumbs C, Micklem G. 1995. Identification of the breast cancer susceptibility gene BRCA2. *Nature* 378:789–792.
- Xiao S, Nalabolu SR, Aster JC, Ma J, Abruzzo L, Jaffe ES, Stone R, Weissman SM, Hudson TJ, Fletcher JA. 1998. FGFR1 is fused with a novel zinc-finger gene, ZNF198, in the t(8;13) leukemia/lymphoma syndrome. *Nat Genet* 18:84–87.