

Short Communication

Evidence for a Familial Esophageal Cancer Susceptibility Gene on Chromosome 13

Nan Hu, Alisa M. Goldstein, Paul S. Albert, Carol Giffen, Ze-Zhong Tang, Ti Ding, Philip R. Taylor¹, and Michael R. Emmert-Buck²

Center for Cancer Research [N. H., P. R. T., M. R. E.-B.], Division of Cancer Epidemiology and Genetics [A. M. G.], and Division of Cancer Treatment and Diagnosis [P. S. A.], National Cancer Institute, Bethesda, Maryland 20892; Information Management Services, Inc., Silver Spring, Maryland 20904 [C. G.]; and Shanxi Cancer Hospital, Taiyuan, Shanxi 030013, People's Republic of China [Z.-Z. T., T. D.]

Abstract

Previous segregation analyses of pedigrees from areas of China where esophageal squamous cell carcinoma (ESCC) rates are extraordinarily high suggested a Mendelian mode of transmission. We initiated a search for a major ESCC gene by conducting a genome-wide scan in ESCC tumors. Chromosome 13 showed loss of heterozygosity (LOH) in 95% of microsatellite markers, the highest frequency of LOH on any chromosome. In the current study, we established a high-resolution deletion map using 107 markers on 13q and compared LOH frequency by family history of upper gastrointestinal cancer. Overall allelic loss was significantly higher in those with a positive (*versus* negative) family history, suggesting the presence of an inherited tumor suppressor gene on 13q in ESCC.

Introduction

ESCC³ is one of the most common fatal tumors worldwide; however, its molecular etiology remains largely unknown. The disease occurs at a high rate in several distinct geographic regions, including Shanxi Province in northern China. Association (1, 2), familial aggregation (3, 4), cytogenetic (5), and segregation analysis (6) studies support a role for genetic susceptibility to esophageal cancer, although the exact mechanism is unclear. To search for genes involved in the development or progression of ESCC in patients from Shanxi Province, we previously performed a genome-wide scan of allelic deletion using 366 microsatellite markers distributed at 10-cM intervals over the 22 autosomal chromosomes (7). The data showed striking LOH throughout the genome and identified 14 separate regions with especially high frequencies of deletion. We ex-

tended this study by analyzing the 14 LOH hot spots in a larger tumor set, including those from patients with and without a family history of upper gastrointestinal cancer [*i.e.*, first-, second-, or third-degree relative with cancer of the esophagus, gastric cardia, or body of the stomach (8)]. The deletion patterns on all chromosomes were similar between the family history-positive and family history-negative groups, except for chromosome 13, where a suggestion of a higher frequency of LOH was observed in tumors from family history-positive patients (9). This result was potentially of importance because persons with a cancer syndrome can show a higher frequency of tumor LOH on the chromosome that harbors the responsible gene than sporadic counterpart tumors (10–13). Therefore, we investigated this finding in detail.

Materials and Methods

In the present study, allelic deletion patterns on chromosome 13 were evaluated in 56 patients with ESCC (34 family history-positive patients and 22 family history-negative patients) using 107 microsatellite markers spanning the entire length of the chromosome (see "Appendix" for the markers used). Details regarding the description of the characteristics of these cases, the methods used for biological specimen collection and processing, laser microdissection and DNA extraction, PCR reactions, and LOH reading and interpretation were as described previously (14). Among the 34 ESCC cases with a positive family history for upper gastrointestinal cancer, 31 had at least one esophageal cancer among their first-, second-, or third-degree relatives; whereas 3 had cardia cancer in first-degree relatives. Differences in the pattern of tumor LOH frequency between family history-positive and -negative patients were evaluated using a permutation test based on the 10% trimmed mean of the χ^2 test statistics comparing individual markers by family history status. The null distribution of no difference in the pattern of LOH frequency across family history groups was obtained by randomly permuting family history status 5000 times and evaluating the distribution of the mean (10% trimmed) χ^2 value. The permutation test provides a global test of the difference in the pattern of LOH frequency across family history status, thereby avoiding the inherent problem of multiple comparisons when testing differences at each marker location.

Results and Discussion

Only the 85 markers that were informative in at least five cases were used for our statistical analysis. Fig. 1 shows the LOH frequencies by family history status for these markers. The mean frequency of allelic loss for the 85 markers was 67% in the family history-positive cases but only 50% in the family history-negative cases, a difference that was statistically significant ($P = 0.03$, global permutation test). The largest difference in tumor LOH frequency between the family history-positive and family history-negative cases was observed on

Received 3/7/03; revised 6/2/03; accepted 6/16/03.

¹ To whom requests for reprints may be addressed, at Cancer Prevention Studies Branch, National Cancer Institute, 6116 Executive Boulevard, Room 705, Bethesda, MD 20892-8314. Phone: (301) 594-2932; Fax: (301) 435-8645; E-mail: ptaylor@mail.nih.gov.

² To whom requests for reprints may be addressed, at Pathogenetics Unit, Laboratory of Pathology, National Cancer Institute, Building 10, Room 2A33, 9000 Rockville Pike, Bethesda, MD 20892. Phone: (301) 496-2912; Fax: (301) 594-7582; E-mail: mbuck@helix.nih.gov.

³ The abbreviations used are: ESCC, esophageal squamous cell carcinoma; LOH, loss of heterozygosity.

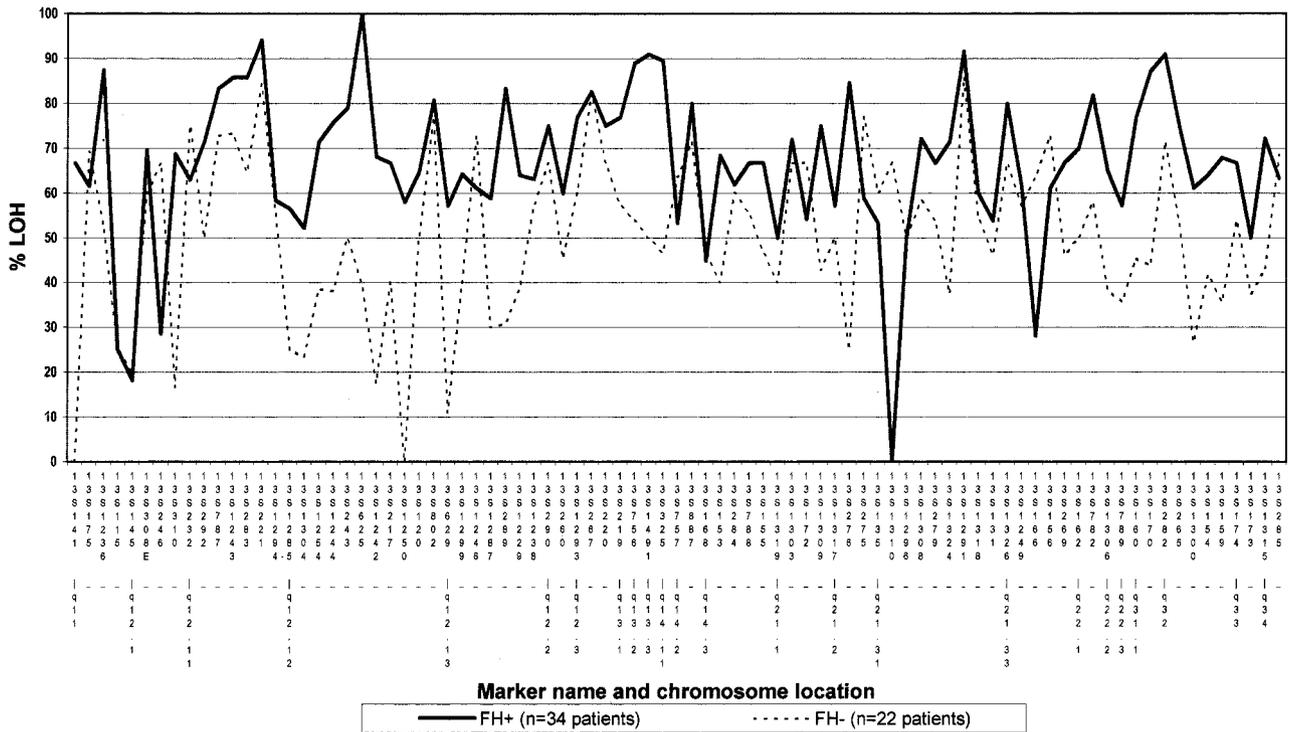


Fig. 1. LOH on chromosome 13 for cases with and without a positive family history of upper gastrointestinal cancer.

chromosome band 13q12 as seen in Fig. 2, where the individual and smoothed χ^2 values (via a nine-point running mean in which each point and its eight nearest neighbors are averaged at each marker location) for LOH frequency by family history status are plotted.

To further assess the significance of the difference in tumor LOH frequency between family history-positive and family history-negative cases on chromosome 13q, we performed a similar global permutation test on chromosome arm 17p using results from the 30 microsatellite markers reported previously (14) and found no difference by family history status ($P = 0.22$, global permutation test). Although we have not evaluated additional chromosome arms in this detailed manner, the data reported here and our previous analysis of the 14 LOH hot spots located throughout the genome support our conclusion that the substantially higher frequency of tumor LOH on chromosome 13 in the family history-positive cases compared with the family history-negative cases is found only on this chromosome.

We hypothesize that patients in Shanxi Province with a positive family history of upper gastrointestinal cancer have a unique germ-line variant of a tumor suppressor gene on chromosome 13. Our current findings are compatible with a mechanistic model in which patients born with a functionally inactivated (or compromised) ESCC tumor suppressor gene will develop esophageal tumors associated with LOH of the wild-type allele as the second hit. In this model, the tumors are expected to have a high frequency of LOH at the responsible gene locus. In contrast, sporadic counterpart esophageal tumors in this population will develop through several possible genetic pathways, not necessarily including LOH at the gene that is associated with the familial syndrome. Thus, these cases are predicted to have a significant yet lower LOH frequency at the

familial gene locus. As an example of this kind of model, allelic deletion studies of multiple endocrine neoplasia type I (MEN1) have shown higher frequencies of tumor LOH at the *MEN1* gene locus on chromosome band 11q13 in tumors from affected kindreds compared with sporadic counterpart tumors (10–13). Candidate tumor suppressor genes on 13q12 include *BRCA2* (15) and *RNF6* (16).

An alternative hypothesis is that the family history-positive patients carry a germ-line variant of a gene, not necessarily located on chromosome 13q, that results in a generalized increase in genomic instability in ESCC tumors when compared with family history-negative patients. In this case, the elevated frequency of tumor LOH on chromosome 13q simply reflects the overall higher level of allelic deletion that is present throughout the genome. However, the data to date, including the global hot spot LOH analysis and the comparison between chromosomes 13q and 17p, suggest that the difference in tumor DNA deletion frequency between family history-positive and -negative cases is not a generalized phenomenon and is, in fact, unique to chromosome 13.

To test the hypothesis that ESCC patients with a positive family history carry a unique allelic variant on chromosome 13, we are evaluating the DNA sequence of candidate genes on this chromosome (15, 16). In addition, we plan to conduct linkage analysis using multiple case ESCC families ascertained from the same population as the patients in the current study.

Appendix

The 107 microsatellite markers evaluated in this study were D13S141, D13S175, D13S1236, D13S115, D13S145, D13S308E, D13S246, D13S310, D13S232, D13S292, D13S787, D13S1243, D13S283, D13S221, D13S1294, D13S1285, D13S1304, D13S1254, *FLT1*, D13S1244, D13S243, D13S625, D13S1242, D13S217, D13S1250, D13S120, D13S802, D13S629, D13S1299, D13S1246,

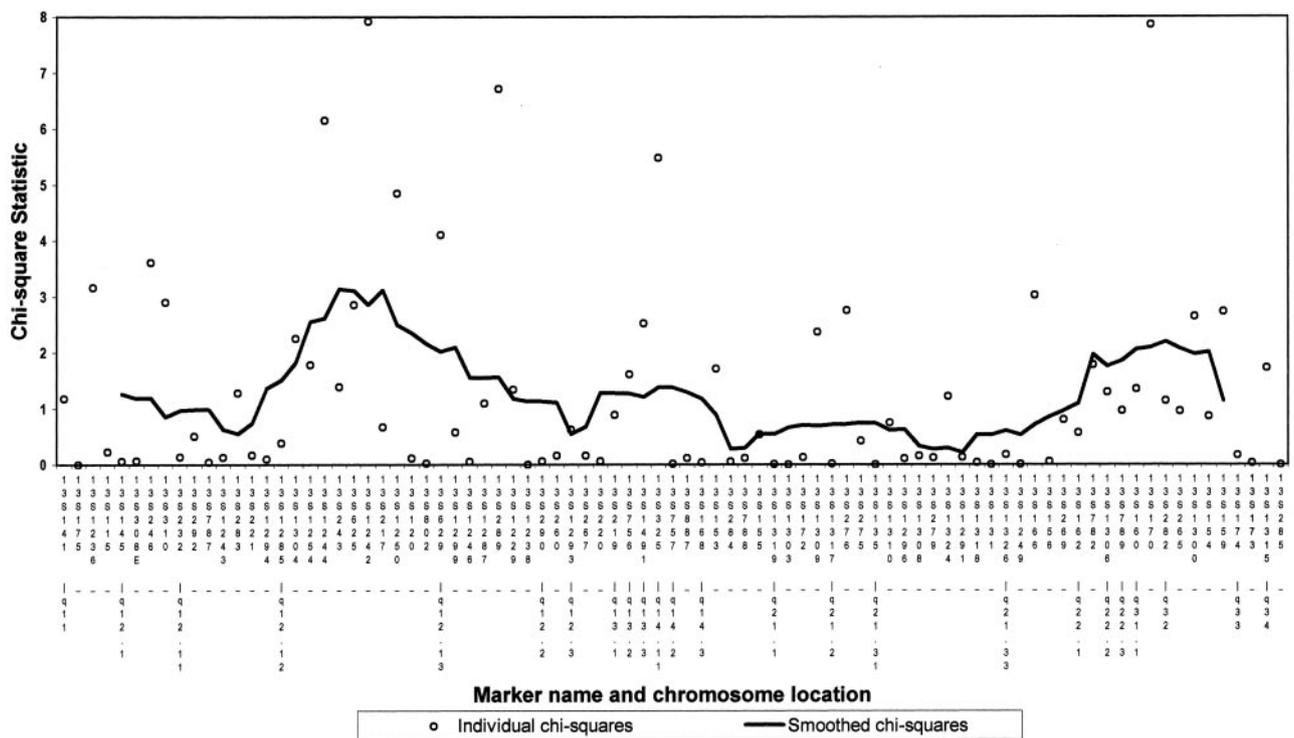


Fig. 2. Individual and smoothed χ^2 statistics (via a nine-point running mean in which each point and its eight nearest neighbors are averaged at each marker location) comparing LOH frequencies in family history-positive versus -negative cases.

D13S1287, D13S289, D13S1229, D13S1238, D13S290, *D13S893*, *D13S1226*, D13S260, D13S1293, D13S267, D13S220, D13S219, **D13S1169**, **D13S1465**, D13S756, D13S1491, **D13S1466**, **D13S898**, D13S325, **D13S1203**, D13S757, D13S887, **D13S1161**, **D13S1180**, D13S168, **D13S917**, D13S153, **D13S1178**, **D13S1171**, **D13S1150**, **D13S1168**, D13S284, D13S788, D13S155, **D13S1184**, **D13S1207**, **D13S1182**, D13S1319, D13S1303, D13S172, **D13S906**, D13S1309, D13S1317, D13S276, D13S275D13S135, D13S1310, D13S1296, D13S1308, D13S279, D13S1324, D13S1291, D13S1318, D13S131, **D13S913**, **D13S1181**, D13S1326, D13S1249, D13S166, D13S156, D13S269, D13S162, D13S782, D13S1306, **D13S747**, D13S789, D13S160, D13S170, D13S282, D13S265, D13S1300, D13S154, D13S159, D13S174, D13S173, D13S1315, and D13S285.

A more complete listing of these markers can be found online.⁴

Markers that were dropped in our analysis include the 20 markers in **bold** (homozygous in all 56 cases), and the 2 markers in *italics* (had <5 informative cases).

References

- Hu, N., Dawsey, S. M., Wu, M., and Taylor, P. R. Family history of oesophageal cancer in Shanxi Province, China. *Eur. J. Cancer*, 27: 1336, 1991.
- Guo, W., Blot, W. J., Li, J. Y., Taylor, P. R., Liu, B. Q., Wang, W., Wu, Y. P., Zheng, W., Dawsey, S. M., and Li, B. A nested case-control study of oesophageal and stomach cancers in the Linxian nutrition intervention trial. *Int. J. Epidemiol.*, 23: 444–450, 1994.
- Li, G., and He, L. A survey of the familial aggregation of esophageal in Yangcheng County, Shanxi Province. In: M. Wu and D. W. Nebert (eds), *Genes and Disease, Proceedings of the First Sino-American Human Genetics Workshop*, pp. 43–47. Beijing: Science Press, 1986.
- Hu, N., Dawsey, S. M., Wu, M., Bonney, G. E., He, L. J., Han, X. Y., Fu, M., and Taylor, P. R. Familial aggregation of esophageal cancer in Yangcheng County, Shanxi Province, China. *Int. J. Epidemiol.*, 21: 877–882, 1992.
- Wu, M., Hu, N., and Wang, X. Q. Genetic factor in the etiology of esophageal cancer and the strategy of its prevention in high-incidence areas of North China. In: H. T. Lynch and T. Hirayama (eds.), *Genetic Epidemiology for Cancer*, pp. 187–200. Boca Raton, FL: CRC Press Inc., 1989.

- Carter, C. L., Hu, N., Wu, M., Lin, P. Z., Murigande, C., and Bonney, G. E. Segregation analysis of esophageal cancer in 221 high-risk Chinese families. *J. Natl. Cancer Inst. (Bethesda)*, 84: 771–776, 1992.
- Hu, N., Roth, M. J., Polymeropolous, M., Tang, Z. Z., Emmert-Buck, M. R., Wang, Q. H., Goldstein, A. M., Feng, S. S., Dawsey, S. M., Ding, T., Zhuang, Z. P., Han, X. Y., Ried, T., Giffen, C., and Taylor, P. R. 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, 2000.
- Hu, N., Roth, M. J., Emmert-Buck, M. R., Tang, Z. Z., Polymeropolous, M., Wang, Q. H., Goldstein, A. M., Han, X. Y., Dawsey, S. M., Ding, T., Giffen, C., and Taylor, P. R. Allelic loss in esophageal squamous cell carcinoma patients with and without family history of upper gastrointestinal tract cancer. *Clin. Cancer Res.*, 5: 3476–3482, 1999.
- Li, G., Hu, N., Goldstein, A. M., Tang, Z. Z., Roth, M. J., Wang, Q. H., Dawsey, S. M., Han, X. Y., Ding, T., Huang, J., Giffen, C., Taylor, P. R., and Emmert-Buck, M. R. Allelic loss on chromosome bands 13q11-q13 in esophageal squamous cell carcinoma. *Genes Chromosomes Cancer*, 31: 390–397, 2001.
- Emmert-Buck, M. R., Lubensky, I. A., Dong, Q., Manickam, P., Guru, S. C., Kester, M. B., Olufemi, S. E., Agarwal, S., Burns, A. L., Spiegel, A. M., Collins, F. S., Marx, S. J., Zhuang, Z., Liotta, L. A., Chandrasekharappa, S. C., and Debelenko, L. V. Localization of the multiple endocrine neoplasia type I (MEN1) gene based on tumor loss of heterozygosity analysis. *Cancer Res.*, 57: 1855–1858, 1997.
- Chandrasekharappa, S. C., Guru, S. C., Manickam, P., Olufemi, S. E., Collins, F. S., Emmert-Buck, M. R., Debelenko, L. V., Zhuang, Z., Lubensky, I. A., Liotta, L. A., Crabtree, J. S., Wang, Y., Roe, B. A., Weisemann, J., Boguski, M. S., Agarwal, S. K., Kester, M. B., Kim, Y. S., Heppner, C., Dong, Q., Spiegel, A. M., Burns, A. L., and Marx, S. J. Positional cloning of the gene for multiple endocrine neoplasia-type 1. *Science (Wash. DC)*, 276: 404–407, 1997.
- McKeeby, J. L., Li, X., Zhuang, Z., Vortmeyer, A. O., Huang, S., Pirner, M., Skarulis, M. C., James-Newton, L., Marx, S. J., and Lubensky, I. A. Multiple leiomyomas of the esophagus, lung, and uterus in multiple endocrine neoplasia type 1. *Am. J. Pathol.*, 159: 1121–1127, 2001.
- Friedman, E., Sakaguchi, K., Bale, A. E., Falchetti, A., Streeten, E., Zimmering, M. B., Weinstein, L. S., McBride, W. O., Nakamura, Y., and Brandi, M. L.

⁴ http://cedar.genetics.soton.ac.uk/public_html/read.html.

Clonality of parathyroid tumors in familial multiple endocrine neoplasia type 1. *N. Engl. J. Med.*, 321: 213–218, 1989.

14. Huang, J., Hu, N., Goldstein, A. M., Emmert-Buck, M. R., Tang, Z. Z., Roth, M. J., Wang, Q. H., Dawsey, S. M., Han, X. Y., Ding, T., Li, G., Giffen, C., and Taylor, P. R. High frequency allelic loss on chromosome 17p13.3-p11.1 in esophageal squamous cell carcinomas from a high incidence area in northern China. *Carcinogenesis (Lond.)*, 21: 2019–2026, 2000.
15. Hu, N., Li, G., Li, W. J., Wang, C., Goldstein, A. M., Tang, Z. Z., Roth, M. J., Dawsey, S. M., Huang, J., Wang, Q. H., Ding, T., Giffen, C., Taylor, P. R., and Emmert-Buck, M. R. Infrequent mutation in the *BRCA2* gene in esophageal squamous cell carcinoma. *Clin. Cancer Res.*, 8: 1121–1126, 2002.
16. Lo, H. S., Hu, N., Gere, S., Lu, N., Su, H., Goldstein, A. M., Taylor, P. R., and Lee, M. P. Identification of somatic mutations of the *RNF6* gene in human esophageal squamous cell carcinoma. *Cancer Res.*, 62: 4191–4193, 2002.