Evidence for a Familial Esophageal Cancer Susceptibility Gene on Chromosome 13

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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 extended 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 χ² 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) χ² 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...
chromosome band 13q12 as seen in Fig. 2, where the individual
and smoothed $\chi^2$ 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 per-
formed 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 conclu-
sion 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 chro-
mosome 13. Our current findings are compatible with a mech-
anistic model in which patients born with a functionally inac-
tivated (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. 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-posi-
tive patients carry a germ-line variant of a gene, not necessarily
located on chromosome 13q, that results in a generalized in-
crease 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 $\chi^2$ 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.


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 italic (had <5 informative cases).

References


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