THE RELATIONSHIP BETWEEN GENETIC SUSCEPTIBILITY TO HEAD AND NECK CANCER WITH THE EXPRESSION OF COMMON FRAGILE SITES

Ünal Egeli, PhD, Lütfi Özkan, MD, Berrin Tunca, PhD, Sibel Kahraman, MD, Gülşah Çeçener, MSc, Emel Ergül, BSc, Kayihan Engin, MD

1 Department of Medical Biology, Uludağ University Medical College, Bursa, Turkey. E-mail: egeli@uu20.bim.uludag.edu.tr
2 Department of Radiation Oncology, Uludağ University Medical College, Bursa, Turkey.
3 Department of Molecular Biology, Uludağ University College of Science, Bursa, Turkey.

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Abstract: Background. Numerous studies have recently been conducted to investigate genetic mechanisms in cancer causes and pathogenesis. Some of these studies have shown that there were certain specific chromosomal defects in normal cells of cancer patients and in their first-degree relatives. It was suggested that these individuals were susceptible to cancer development when compared with people without these defects.

Materials and Methods. Chromosomal anomalies, such as gaps, breaks, and acentric fragments, and fragile site expression rates were determined in peripheral blood lymphocyte cultures in 14 head and neck cancer patients, 17 first-degree relatives of these patients, and 20 healthy individuals as a control group in this study. RPMI 1640 medium, composed of aphidicolin, 5-bromodeoxyuridine, and caffeine were used for the induction of fragile sites.

Results. In cytogenetic and statistical evaluation, it was observed that both chromosomal aberration rates and fragile site expression frequencies in head and neck cancer patients, and in their first-degree relatives were significantly greater than the control group (p < .05). It was found that fragile site expression was site specific in head and neck cancer patients and in their first-degree relatives. These specific sites were determined to be 1p21-22, 1q21, 1q25, 2q21, 2q31-33, 3p14, 16q22-23, 18q21, and 22q12 sites.

Conclusions. These findings support studies showing that the fragile sites might be unstable factors in human genomes and their expression could be affected by some genetic factors, such as tumor suppressor genes and mismatch repair genes, and by some environmental factors, such as benzo (a) pyrene, dimethylnitrosamine, and dimethylsulfate. In conclusion, fragile sites may play an important role in the genetic tendency to head and neck cancer. Overexpression of these sites in normal lymphocytes may be used as a reliable marker to determine the genetic susceptibility in head and neck cancer patients and in their first-degree relatives. © 2000 John Wiley & Sons, Inc. Head Neck 22: 591–598, 2000.

Keywords: head and neck cancer; fragile sites; genetic susceptibility; chromosomal abnormalities

Head and neck cancers constitute 5% of all cancers and are responsible for mortality in 2% of all cancers. Almost half of head and neck cancer patients die of this disease or complications developing during the course of the disease. A second primary tumor can be detected within the aerodigestive region in 5% to 15% of cases with head and neck cancer. Although some etiologic factors,
some cases. Numerous studies have been re-
genetically detectable in lymphocyte cultures in
cancers and that such abnormalities are cytoge-
normalities are etiologically related to specific
genetics indicate that specific chromosomal ab-
11, and 13 chromosomes.1
been detected in some cases in 1, 3, 5, 7, 8, 9, 10,

has been proven to be specific for the head and
mainly a disease of genes. Although none of them
ported in recent years showing that cancer is
inducible fragile sites.11 Band 3p14 is also defined
most common of the constitutive aphidicolin-
cancer development.6–10 The FRA3B at 3p14.2 is the
most common of the constitutive aphidicolin-
inducible fragile sites.11 Band 3p14 is also defined
as a tumor suppressor gene called FHIT, as evi-
denced by deletions, rearrangements, and allele
loss found in several tumors, including lung, head
and neck, renal, nasopharyngeal, cervical, and
breast cancer.12–19

The aim of the study was to investigate fragile
site expression in patients with head and neck
cancer and their first-degree relatives and the
possible correlation with the development of head
and neck cancer.

MATERIALS AND METHODS
Fourteen patients, who had histopathologically
verified squamous cell carcinoma (epidermoid) of
the head and neck, were studied in this trial be-
fore the initiation of radiotherapy or cytotoxic
chemotherapy. In the selection of the subjects, we
ascertained that they had not received irra-
diation, had not taken medications or drugs, and had
not had a viral infection within the last 3 months.

Seventeen people, who were first-degree relatives
of these patients, and 20 healthy individuals as a
control group have also been included. Cytoge-
netic tests were performed at the Laboratory of
Genetics at the Department of Biology, Uludağ
University College of Science.

Heparinized whole peripheral blood samples
taken from head and neck cancer patients, their
relatives, and healthy individuals in the control
group were cultured in a medium composed of
RPMI 1640 medium (pH, 7.2), 15% fetal calf se-
erum, 6 μg/mL phytohemagglutinin L (PHA-L), 0.5
mg/mL L-glutamine, penicillin, and streptomycin
(100 IU and 100 μg/mL, respectively) at 37°C for
72 hours. To observe fragile sites more clearly, 0.2
mM aphidicolin, 5 μg/mL bromodeoxyuridine
(BrdU), and 2.2 mM caffeine were added to the
culture medium 5 hours before the harvest. Chro-
mosomes were inhibited in metaphase using col-
cemide an hour before the harvest. The routine
chromosome analysis method was used for the
harvest procedure. Three slides for each case
were stained with Giemsa and chromosomal ab-
errations were evaluated under light microscope
(Zeiss Axioplan, Germany) blindly. Thirty to 50
metaphase figures were evaluated for each case
according to mitotic index. After the location of
metaphases with gaps and breaks on the slides,
these preparates were destained with methanol
(Merck). High-resolution banding (HRB) was
then performed to detect the exact locations of
gaps and breaks.20,21 Strict criteria were used to
describe a common fragile site. The common frag-
ile sites were defined as the gaps and breaks lo-
cated at bands as described in HGM8 (1985),
HGM9 (1987), and ISCN (1985), and a site was ac-
cepted as fragile if it was detected in at least 3 cases
in both patient and relative groups tested.22–24

Statistical analysis were performed by use of
nonparametric Mann–Whitney and the logistic
regression analysis tests. Differences with a $p <
.05$ were accepted as statistically significant.

RESULTS
The mean age of the patients with head and neck
cancer who were included in this study was 56
years, with a range of 41 to 80 years, whereas it
was 37 years for their relatives (range, 24–60
years) and 43 years (range, 25–73 years) for the
healthy individuals used as control group.

The characteristics of patients, relatives, and
controls are shown in Tables 1 to 3. Ten patients
had laryngeal cancer, 1 had lip cancer, and 1 each
had an oral cavity tumor, hypopharynx, and oro-
pharynx tumor. All patients’ histopathologic findings were epidermoid carcinoma.

Results of cytogenetic analysis for the patients with head and neck cancer, their relatives, and the control group are also shown in Tables 1 to 3. Significance values \( p < .0001 \) derived from statistical analysis of the cytogenetic findings comparing the three groups are shown in Tables 1 to 3.

When cells with chromosomal aberrations were taken into consideration, the aberration rate was 0.06 in the control group, whereas it was 0.26 in patients with head and neck cancer and 0.22 in their relatives. The differences between the patients and the control group and the patients’ relatives and the control group were statistically significant \( p < .0001 \) and \( p < .0001 \), respectively. The difference between the patients and their relatives did not reach statistical significance \( p > .05 \).

The gap and break expression rate was 0.44 in the patients with head and neck cancer, whereas it was 0.29 in the patients’ relatives and 0.06 in the control group. The differences between the pa-

### Table 1. Characteristics and chromosome aberrations of patients with head and neck cancer.

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Smoking habit (packet/day)</th>
<th>Site</th>
<th>Histologic grade</th>
<th>Stage</th>
<th>Metaphase No.</th>
<th>Aberrant Cell</th>
<th>Gap + break/cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>45</td>
<td>2</td>
<td>SG larynx</td>
<td>1</td>
<td>IV</td>
<td>50</td>
<td>0.28</td>
<td>0.68</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>58</td>
<td>—</td>
<td>G larynx</td>
<td>2</td>
<td>IV</td>
<td>40</td>
<td>0.17</td>
<td>0.20</td>
</tr>
<tr>
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<td>Oral cav.</td>
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<td>IV</td>
<td>50</td>
<td>0.32</td>
<td>0.48</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>50</td>
<td>1</td>
<td>G larynx</td>
<td>2</td>
<td>I</td>
<td>30</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>59</td>
<td>2</td>
<td>SG larynx</td>
<td>2</td>
<td>IV</td>
<td>30</td>
<td>0.40</td>
<td>0.30</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>67</td>
<td>2.5</td>
<td>SG larynx</td>
<td>1</td>
<td>IV</td>
<td>30</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
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<td>SG larynx</td>
<td>3</td>
<td>IV</td>
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<td>0.42</td>
<td>1.00</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>65</td>
<td>1</td>
<td>SG larynx</td>
<td>3</td>
<td>IV</td>
<td>40</td>
<td>0.28</td>
<td>0.31</td>
</tr>
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<td>9</td>
<td>M</td>
<td>53</td>
<td>1</td>
<td>Lower lip</td>
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<td>III</td>
<td>40</td>
<td>0.05</td>
<td>0.08</td>
</tr>
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<td>10</td>
<td>M</td>
<td>80</td>
<td>—</td>
<td>Hypopharynx</td>
<td>3</td>
<td>IV</td>
<td>30</td>
<td>0.40</td>
<td>0.53</td>
</tr>
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<td>11</td>
<td>M</td>
<td>51</td>
<td>1</td>
<td>Oropharynx</td>
<td>2</td>
<td>IV</td>
<td>50</td>
<td>0.52</td>
<td>0.88</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>41</td>
<td>1</td>
<td>SG larynx</td>
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<td>IV</td>
<td>40</td>
<td>0.35</td>
<td>0.43</td>
</tr>
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<td>13</td>
<td>M</td>
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<td>SG larynx</td>
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<td>III</td>
<td>30</td>
<td>0.23</td>
<td>0.23</td>
</tr>
<tr>
<td>14</td>
<td>M</td>
<td>55</td>
<td>1</td>
<td>G larynx</td>
<td>1</td>
<td>I</td>
<td>50</td>
<td>0.16</td>
<td>0.94</td>
</tr>
</tbody>
</table>

Mean ± SD 55.5 ± 9.67 1.18 ± 0.72 0.26* ± 0.15 0.44* ± 0.33

* \( P < 0.0001 \) (statistically significant at \( p < .0001 \) when compared with control group).

### Table 2. Characteristics and chromosome aberrations of relatives of patients with head and neck cancer.

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Relationships with patient</th>
<th>Smoking habit (packet/day)</th>
<th>Metaphase No.</th>
<th>Aberrant Cell</th>
<th>Gap + break/cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>42</td>
<td>Father</td>
<td>—</td>
<td>30</td>
<td>0.13</td>
<td>0.17</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>40</td>
<td>Father</td>
<td>0.1</td>
<td>30</td>
<td>0.20</td>
<td>0.17</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>36</td>
<td>Father</td>
<td>0.5</td>
<td>30</td>
<td>0.10</td>
<td>0.07</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>27</td>
<td>Father</td>
<td>—</td>
<td>30</td>
<td>0.17</td>
<td>0.07</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>42</td>
<td>Brother</td>
<td>0.5</td>
<td>30</td>
<td>0.23</td>
<td>0.33</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>29</td>
<td>G. father</td>
<td>—</td>
<td>30</td>
<td>0.17</td>
<td>0.17</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>24</td>
<td>G. father</td>
<td>—</td>
<td>30</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>35</td>
<td>G. father</td>
<td>0.5</td>
<td>30</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
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<td>Uncle</td>
<td>—</td>
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<td>0.35</td>
<td>0.80</td>
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<td>Father</td>
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<td>0.13</td>
</tr>
<tr>
<td>11</td>
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<td>Father</td>
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<td>0.30</td>
<td>0.26</td>
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<tr>
<td>12</td>
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<td>Father</td>
<td>0.25</td>
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<td>0.47</td>
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<tr>
<td>13</td>
<td>M</td>
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<td>Brother</td>
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<td>0.10</td>
</tr>
<tr>
<td>14</td>
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<td>Father</td>
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<td>0.50</td>
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<tr>
<td>17</td>
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<td>55</td>
<td>Father</td>
<td>1</td>
<td>30</td>
<td>0.28</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Mean ± SD 36.76 ± 10 0.32 ± 0.52 0.22* ± 0.13 0.29* ± 0.27

* \( P < 0.0001 \) (statistically significant at \( p < 0.0001 \) when compared with control group).
patients with head and neck cancer and the control
group and the patients’ relatives and the control
group were statistically significant ($p < .0001$) by
Mann–Whitney test. The difference between the
patients and their relatives was not significant ($p
> .05$). In addition, when our findings were com-
pared between patients and relatives by the logis-
tic regression analysis test, they were not statis-
tically significant to similar results of the Mann–
Whitney test ($p > .05$). Localizations of break
points in patients and relatives are given in Fig-
ure 1. It was observed that chromosomal breaks
condensed on chromosomes 3, 2, and 1 in the pa-
tients and relatives groups and on chromosomes

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Smoking habit (pack/day)</th>
<th>Metaphase No.</th>
<th>Aberrant cell</th>
<th>Gap + break/cell</th>
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</thead>
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<tr>
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<td>0.07</td>
</tr>
<tr>
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<td>0.07</td>
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<td>F</td>
<td>48</td>
<td>—</td>
<td>30</td>
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</tr>
</tbody>
</table>

Mean ± SD 42.85 ± 12.09 0.58 ± 0.71 0.06 ± 0.07 0.06 ± 0.08

FIGURE 1. Distribution of chromosome breaks in all the three groups.
1, 2, and 5 in the control group (Tables 1 and 2 and Figs. 1 and 2).

As a result of fragile site evaluation, which was done according to the criteria described in Materials and Methods section, 1p21-22, 1q21, 1q25, 2q21, 2q31-33, 3p14, 16q22-23, 18q21, and 22q12 sites are defined as fragile sites in head and neck cancer. Fragile site expression rate was 0.13 totally in the patients with head and neck cancer, whereas it was 0.11 in the patients' relatives and 0.012 in the control group. The differences between the patients and their relatives and the healthy controls were statistically significant ($p < .0001$ in both groups). In addition fra(3)(p14) and (2)(q31-33) were determined to be most frequent in both the patient and relative groups. The rate of fra(3)(p14) was 0.042 in patients and relatives and 0.003 in controls. Although the differences were significant between patients and relatives and controls ($p < .001$, $p < .005$, respectively), they were not statistically significant ($p > .05$) when patients and relatives were compared with each other. The rate of (2)(q31-33) was 0.020 in patients, 0.012 in relatives, and 0.00 in controls ($p < .001$). Similarly, this site was not statistically significant when experiment groups were compared with each other. The comparisons with the control group of these two sites in the patients and relatives were statistically significant. These results were significant because the FHIT putative tumor suppressor gene and the hPMS1 mismatch repair gene were localized in these sites, respectively.

Moreover, the rates of chromosome aberrations, fra(3)(p14) and total fragile sites of smokers in the healthy controls and the patients and relatives were compared with each other. The increase in chromosomal aberrations and fra(3)(p14) was significant when smoking patients and relatives were compared with a smoking control group. Although there was a significant increase statistically in terms of total fragile site rate in the smoker patients when compared with the smokers in the controls, the frequency of total fragile site expression was not defined to be statistically significant when the smoker relatives were compared with the smoking controls, although the frequency of total fragile site expression of the relatives was more than total fragile sites in the control group (Table 4). We think that this situation may be a result of too few subjects.

FIGURE 2. Localization of fragile sites observed in patients with head and neck cancer and their relatives. The solid circles indicate gap or break points. The asterisks indicate break points of one subject.
DISCUSSION

Our results indicate that chromosomal aberrations and fragile site expression rates in the patients with head and neck cancer and their relatives are significantly greater than the healthy individuals used as the control group. These individuals may be considered predisposed to head and neck cancer. The results also suggested that common fragile sites might really be the preferential points of breakage and that the expression of these sites might be primary contributors to chromosomal defects of the human genome.

Previously, Dave et al.⁴ found that chromosome breaks increased in lymphocyte cultures of patients with head and neck cancer and melanoma by use of bleomycin and 4NQO. These investigators indicate that chromosome breaks condensed on 3 and 7 chromosomes in patients with head and neck cancer. Our results have shown that chromosomal breaks cluster on 3, 2, and 1 from high to low. The target chromosome was chromosome 3 in both Dave et al’s results and our findings. Therefore, our results are consistent with Dave et al’s findings for chromosomal aberrations and chromosome 3.

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It has been well documented that tumor suppressor gene loss might play a major role in cancer development. Our findings indicated that fra(3)(p14) was expressed most frequently in all common fragile sites. Therefore, fragility on the 3p14 band that we detected could be of great significance. In a recent study, Sozzi et al.³² reported a new putative tumor suppressor gene, named FHIT, located above the mentioned site. We consider that this site may be one of the primary sites for most human cancers because fra(3)(p14) expression is observed in cancers such as lung, breast, stomach, colon, rectum, and ovary.²⁶,²⁸⁻³¹,³³,³⁴ Moreover, several investigators determined homozygous deletions within this site by molecular genetic studies in squamous cell carcinoma of the head and neck.¹³,³⁵⁻³⁷ Virgilio et al.³⁷ determined that 55% of head and neck squamous cell carcinoma cell lines expressed aberrant FHIT transcripts and that one or both FHIT alleles were partially deleted in many of these cell lines. This indicates that loss of FHIT function is likely to be important for the development of the

<table>
<thead>
<tr>
<th>Groups</th>
<th>Aberrant cell (mean ± SD)</th>
<th>Gap + break/cell (mean ± SD)</th>
<th>fra(3)(p14) (mean ± SD)</th>
<th>Total fragile sites (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Patients</td>
<td>0.26 ± 0.16</td>
<td>0.45 ± 0.35</td>
<td>0.039 ± 0.056</td>
<td>0.120 ± 0.150</td>
</tr>
<tr>
<td>B. Relatives</td>
<td>0.24 ± 0.16</td>
<td>0.24 ± 0.19</td>
<td>0.022 ± 0.023</td>
<td>0.087 ± 0.078</td>
</tr>
<tr>
<td>C. Controls</td>
<td>0.07 ± 0.09</td>
<td>0.08 ± 0.11</td>
<td>0.0037 ± 0.011</td>
<td>0.023 ± 0.036</td>
</tr>
<tr>
<td>Compared groups and p values:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A with C</td>
<td>&lt;.005</td>
<td>&lt;.005</td>
<td>&lt;.05</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>B with C</td>
<td>&lt;.05</td>
<td>&lt;.05</td>
<td>&lt;.05</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>A with B</td>
<td>&gt;.05</td>
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<td>&gt;.05</td>
<td>&gt;.05</td>
</tr>
</tbody>
</table>
tumorigenic phenotype. However, the biologic function of the FHIT protein, an Ap3A hydrolase, is not known.\(^{38}\) FHIT protein in the cell and its mechanism of action may provide important knowledge toward understanding of the biologic mechanisms underlying many different types of human cancer.

As we also had defined in our previous studies, it has been well established that smoking plays a major role in the development of lung and head and neck cancers.\(^{26}\) Smoking causes chromosomal damage and fragile site expression. In our study 65% of patients, 45% of relatives, and 25% of controls were smokers (Table 1). Tobacco smoke contains several carcinogens such as benzo(a)pyrene (BP), benzo(a)pyrene diol epoxide (BPDE, the metabolic product of BP), dimethylsulfate, and dimethylnitrosamine. These carcinogens especially attack 3p14 and 3p21 sites.\(^{10,39}\) In this situation, we have also believed that mutagen-induced chromosome aberrations were not random and might reflect the inherited genetic susceptibility of specific loci to damage by carcinogens. The short arm of chromosome 3 may be a hot spot for such damage and deletion of 3p may be a particularly useful genetic marker for genetic predisposition to head and neck cancers. Therefore, we have also suggested that mutagen-induced chromosome aberrations are not random and may reflect the inherited genetic susceptibility of specific chromosome loci to damage by several carcinogens.

In this connection, we believe that fragile site expression may be a suitable marker for predisposition to cancer development and may be used for early diagnosis of cancer. However, these studies must be supported by molecular genetic studies.

**REFERENCES**

13. On ST, Pong KM, Bader SA, Minna JD, Le Beau MM, McKeehan TW, Rassool FV. Precise localization of the FHIT gene to the common fragile site at 3p14.2 (FRA3B) and characterization of homozygous deletions within FRA3B that affect FHIT transcription in tumor cell lines. Genes, Chromosomes Cancer 1997;20:16–23.


