Increased Fragile Sites and Sister Chromatid Exchanges in Bone Marrow and Peripheral Blood of Young Cigarette Smokers

Chien-Song Kao-Shan, Robert L. Fine, Jacqueline Whang-Peng,¹ Elaine C. Lee, and Bruce A. Chabner

ABSTRACT

Cigarette smoking is considered to be the single most important acquired cause of cancer mortality. Studies of chromosome aberrations, sister chromatid exchanges, and fragile sites in peripheral blood or bone marrow are useful methods to detect the effects of the environmental mutagens or carcinogens found in cigarette smoke. The effects of smoking on the immature cells in the bone marrow have not been studied. Here, we examine the peripheral blood and bone marrow in 18 smokers (15 females and 3 males) with a median age of 25 years (range, 21-40) and an average cigarette use corresponding to 6 pack years. In both bone marrow cells and peripheral blood lymphocytes, we were able to show a significantly increased frequency of sister chromatid exchanges in smokers with a 5 or more cigarette pack year history, but not in those who smoked less than 5 pack years. We also found a higher frequency of sister chromatid exchanges in peripheral blood lymphocytes than in bone marrow cells. In addition, the peripheral lymphocytes of smokers demonstrated (a) a significantly higher frequency of fragile sites, (b) an increased number of metaphases with extensive breakage; and (c) elevated expression of fragile sites at the cancer breakpoints 3p14.2, 11q13.3, 22q12.2, and 11p13-p14.2 and at the oncogene sites bcl 1, erb B, erb A, and sis. Our results suggest that chromosomal DNA of peripheral blood lymphocytes is sensitive to cigarette smoking. Studies of the chromosomal changes in these cells provide an index of the mutagenic damage caused by these exogenous agents in individual patients and the ability of individuals to repair that damage, and might predict susceptibility to malignant events.

INTRODUCTION

Cigarette smoke is considered to be the single most important acquired cause of cancer mortality in the United States. Overall death rates from cancer are two times greater in cigarette smokers than in nonsmokers, with heavy smokers (over one pack a day) having a 3-4 times greater risk than nonsmokers. In smokers, the risk of lung cancer is increased more than for one pack a day) have a 3—4 times greater risk than nonsmokers. In addition, the peripheral lymphocytes of smokers increased the frequency of SCEs in peripheral blood lymphocytes more than in bone marrow cells. In the peripheral lymphocytes of smokers, we found a significantly higher frequency of fragile sites, an increased number of metaphases with extensive breakage, and the highest expression of fragile sites at the cancer breakpoints 3p14.2, 11q13.3, 22q12.2, and 11p13-p14.2 and at the oncogene sites bcl 1, erb B, erb A, and sis. Our results suggest that chromosomal DNA of peripheral blood lymphocytes is sensitive to cigarette smoking. Studies of the chromosomal changes in these cells provide an index of the mutagenic damage caused by these exogenous agents in individual patients and the ability of individuals to repair that damage, and might predict susceptibility to malignant events.

MATERIALS AND METHODS

The frequency of SCEs and chromosomal aberrations of the peripheral blood lymphocytes and bone marrow cells of eighteen smokers (15 females and 3 males), with a median age of 25 years (range, 21-40) was compared to that found for 20 nonsmokers (8 females and 12 males), with a median age of 25 years (range, 20-40). In addition, the frequency of chromosomal fragile sites of the peripheral blood lymphocytes was determined for 15 smokers and 15 nonsmokers. None of the volunteers was taking any medications or used alcohol, and none had known exposure to occupational or environmental hazards. The smokers were subgrouped according to their number of cigarette pack years (1 pack of cigarettes smoked per day x number of years smoked; 1 pack = 20 cigarettes): less than 5 pack years and equal to or greater than 5 pack years. The average was 6 cigarette pack years.

Sister Chromatid Exchange and Chromosomal Aberrations

Culturing, Harvesting, and Scoring. Bone marrow aspirations were obtained aseptically from the posterior iliac crest after informed consent was obtained. Four-5 drops of the bone marrow were cultured in 10 ml of RPMI 1640 medium supplemented with penicillin, streptomycin, and glutamine (GIBCO Laboratories, Grand Island, NY) and containing 20% fetal bovine serum. After 3 h initial incubation at 37°C, 5-bromo-2-deoxyuridine (10 μg/ml) was added to each culture (final concentration, 10 μg/ml); the culture was incubated for an additional 48 h in the dark in a 5% CO2 atmosphere.

For cultures of peripheral lymphocytes, 0.5 ml of heparinized whole blood was added to 10 ml of RPMI 1640 medium containing 20% fetal bovine serum and 2% phytohemagglutinin. After 24 h of incubation at 37°C, 5-bromo-2-deoxyuridine (10 μg/ml) was added for an additional 48-h incubation in the dark in a 5% CO2 atmosphere. For studies of chromosomal aberrations, 72-h phytohemagglutinin-stimulated cultures were used in order that we would be able to examine the same population of cells as used for SCEs and fragile sites, although the number of aberrations found in these cultures may not reflect the true degree of DNA damage that has occurred since many of the cells may already be in second division.

The bone marrow cells and peripheral blood lymphocytes were harvested by adding Colcemid (0.2 μg/ml) for the final 90 min of incubation. Then the cells were exposed to a hypotonic solution (1:1 0.075 M KCl and 1% sodium citrate) for 20 min and fixed in a 3:1
mixture of absolute ethanol and glacial acetic acid. Air-dried slides
were made, aged for 3–7 days, and then stained either for sister
chromatid differentiation by the method of Goto et al. (9) or for
chromosomal aberrations using conventional Giemsa stain or G-band-
ing stain (10). Chromosome preparations of the bone marrow made
without culturing were also scored for aberrations (11).

The frequency of SCEs per metaphase was scored on the basis of 30
intact second-division cells per sample wherever possible. Student's t
test was used for statistical analysis. Fifty metaphases were scored for
chromosomal aberrations such as chromatid breaks, chromosome
breaks (a discontinuity in chromosome structure occurring across the
entire chromosome), fragments (acentric chromosome structures), di-
centric or ring chromosomes, chromatid exchanges, pulverization, or
extensive fragmentation; the percentage of cells having these aberra-
tions was calculated.

Fragile Sites

Culturing, Harvesting, and Scoring. Culture conditions for evaluation
of fragile sites were similar to those described above except that mini-
imum essential medium (Eagle's) with Earle's salts was used (GIBCO).
After 72 h of incubation at 37°C (5% CO2), fluorodeoxyuridine was
added to a final concentration of 1 × 10^-3 M (Sigma Chemical Co., St.
Louis, MO). After an additional 24 h, caffeine (Sigma; final concentra-
tion, 2.2 µg/ml) was added for the last 6 h of culture. The metaphases
were then exposed to Colcemid (final concentration, 0.0005 µg/ml) for
20 min, 0.075 M Ml for 10 min, and rapidly fixed in a cold, freshly
prepared mixture of absolute methanol and acetic acid in a ratio of 3:1.
Chromosome preparations were made using cold, wet slides. After
aging for 1 week, G-banding stains were made. The metaphases
were photographed and scored for the total number and chromosomal loca-
tion of the fragile sites [nomenclature for chromosome bands was
according to the ISCN recommendations (12)]. For those metaphases
with extensive fragmentation, the number of breaks was scored as >60.
The frequency of fragile sites, the chromosomal location of the ten
most frequent breakpoints, and the number of breakpoints at oncogene
locations, cancer breakpoints, and constitutive and heritable fragile
sites were determined. The 5 most frequent fragile sites at cancer
breakpoints and the 3 most frequent fragile sites at oncogene sites were
also determined. Student's t test was used for statistical analysis.

RESULTS

SCEs and Chromosomal Aberrations. The majority of cells
proliferating in culture from the bone marrow specimens was
nonlymphocytic. The frequencies of SCEs and chromosomal
aberrations in the bone marrow cells and peripheral blood
lymphocytes of both smokers and nonsmokers are shown in
Table 1. There was a significant increase in the mean number
of SCEs in both bone marrow cells (P < 0.001) and peripheral
blood lymphocytes (P < 0.005) of the smoker group when
compared to the nonsmoker group. There was no difference
between smokers and nonsmokers in the median percentage of
cells (bone marrow cells or peripheral blood) having chromo-
somal aberrations; however, 7 of 18 smokers had 6–14% aber-
rations and one of 20 nonsmokers had 8% aberrations in the
bone marrow. Analysis of G-banded metaphases demonstrated
that the chromosomal breakpoints for both groups were random
in nature. The frequency of SCEs in bone marrow cells and
peripheral blood lymphocytes for the nonsmoker group and for
the 2 smoker subgroups (<5 and ≥5 pack years) is shown in
Table 2. In both bone marrow cells and in peripheral blood
lymphocytes, there was a significantly increased frequency of
SCEs in the subgroup of smokers with 5 or more cigarette pack
years (P < 0.001), but there was no significant increase in SCEs
for the subgroup of smokers with less than 5 cigarette pack
years (P < 0.2 and < 0.5, respectively), over those in the
nonsmoker group. In addition, there was a higher frequency of
SCEs in peripheral blood lymphocytes than in bone marrow
cells in each of these groups.

Fragile Sites. The number of fragile sites per metaphase in
peripheral blood lymphocytes for the smoker and nonsmoker
groups is shown in Table 3. The mean number of fragile sites
per cell in the smokers [17.86 ± 4.13 (SD)] was 2-fold greater
than that found in nonsmokers (8.89 ± 3.38) (P < 0.001). The
percentage of metaphases having extensive breaks (>60) was
also increased in the smokers (median, 6.38%) over those in
nonsmokers (median, 3.92%).

The percentage of breakpoints located at various sites is
shown in Table 4. There were no significant differences between

Table 1. SCEs and chromosomal aberrations in young smoker and nonsmoker groups

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Group</th>
<th>No. of subjects</th>
<th>SCEs/cell Mean ± SD</th>
<th>P</th>
<th>Chromosomal aberrations (%) Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone marrow</td>
<td>Smokers</td>
<td>18</td>
<td>5.03 ± 0.96</td>
<td>&lt;0.001</td>
<td>3</td>
<td>0–14</td>
</tr>
<tr>
<td></td>
<td>Nonsmokers</td>
<td>20</td>
<td>3.60 ± 0.89</td>
<td></td>
<td>2</td>
<td>0–8</td>
</tr>
<tr>
<td>Peripheral lymphocytes</td>
<td>Smokers</td>
<td>18</td>
<td>10.09 ± 2.35</td>
<td>&lt;0.005</td>
<td>2</td>
<td>0–8</td>
</tr>
<tr>
<td></td>
<td>Nonsmokers</td>
<td>20</td>
<td>7.72 ± 1.78</td>
<td></td>
<td>0</td>
<td>0–4</td>
</tr>
</tbody>
</table>

Table 2. Frequency of SCEs in bone marrow cells and peripheral blood lymphocytes from the nonsmoker group and individual smokers

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex/age (yr)</th>
<th>Smoking history, pack yr</th>
<th>Bone marrow* SCEs/cell (mean ± SD)</th>
<th>Peripheral blood lymphocytes* SCEs/cell (mean ± SD)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–20 (group)</td>
<td>M and F/20–40</td>
<td></td>
<td>3.60 ± 0.89</td>
<td>7.72 ± 1.78</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>F/24</td>
<td>0.75</td>
<td>4.74 ± 2.35</td>
<td>9.33 ± 4.44</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>M/27</td>
<td>2</td>
<td>3.90 ± 2.47</td>
<td>8.60 ± 2.87</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>F/22</td>
<td>2</td>
<td>5.20 ± 1.94</td>
<td>7.37 ± 2.80</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>M/20</td>
<td>3</td>
<td>3.73 ± 1.80</td>
<td>8.47 ± 3.57</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>F/22</td>
<td>3</td>
<td>4.33 ± 2.35</td>
<td>7.13 ± 2.33</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>M/27</td>
<td>3.5</td>
<td>4.03 ± 1.61</td>
<td>7.67 ± 2.84</td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td></td>
<td></td>
<td>4.32 ± 0.56</td>
<td>&lt;0.2</td>
<td>8.059 ± 0.84</td>
</tr>
<tr>
<td>27</td>
<td>F/30</td>
<td>5</td>
<td>5.33 ± 2.40</td>
<td>8.30 ± 2.73</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>F/22</td>
<td>5</td>
<td>4.03 ± 2.21</td>
<td>11.97 ± 4.44</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>F/26</td>
<td>5</td>
<td>5.60 ± 3.11</td>
<td>12.43 ± 5.13</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>M/26</td>
<td>6</td>
<td>3.67 ± 1.83</td>
<td>15.0 ± 4.37</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>F/22</td>
<td>7</td>
<td>4.70 ± 2.18</td>
<td>12.40 ± 4.30</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>F/21</td>
<td>7.5</td>
<td>5.90 ± 2.64</td>
<td>7.60 ± 2.59</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>M/23</td>
<td>7.5</td>
<td>5.00 ± 2.18</td>
<td>8.90 ± 4.74</td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>F/25</td>
<td>7.5</td>
<td>6.70 ± 2.81</td>
<td>12.50 ± 4.40</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>F/40</td>
<td>8</td>
<td>5.57 ± 2.65</td>
<td>10.06 ± 4.56</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>F/26</td>
<td>10</td>
<td>5.67 ± 2.54</td>
<td>12.93 ± 4.10</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>F/27</td>
<td>10</td>
<td>6.81 ± 2.71</td>
<td>9.71 ± 3.32</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>F/27</td>
<td>10</td>
<td>5.63 ± 3.05</td>
<td>11.73 ± 3.88</td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td></td>
<td></td>
<td>5.38 ± 0.94</td>
<td>&lt;0.001</td>
<td>11.13 ± 2.196</td>
</tr>
</tbody>
</table>

* Samples cultured for 2 days.
† Cultured for 3 days.

Table 3. Number of fragile sites found in each metaphase of peripheral blood lymphocytes

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of subjects</th>
<th>No. of fragile sites/cell Mean ± SD</th>
<th>Cells with extensive breaks (%) &gt;60 (individual)</th>
<th>P</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smokers</td>
<td>15</td>
<td>17.86 ± 4.13</td>
<td>&lt;0.001</td>
<td>6.38</td>
<td>0–14</td>
<td></td>
</tr>
<tr>
<td>Nonsmokers</td>
<td>15</td>
<td>8.89 ± 3.38</td>
<td></td>
<td>3.92</td>
<td>0–14</td>
<td></td>
</tr>
</tbody>
</table>
INCREASED FRAGILE SITES AND SCEs IN SMOKERS

Table 4 Percentage of breakpoints at various chromosomal sites in peripheral blood lymphocytes

<table>
<thead>
<tr>
<th>Group</th>
<th>Oncogene sites</th>
<th>Cancer sites</th>
<th>Constitutive fragile sites</th>
<th>Heritable fragile sites</th>
<th>Sex chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% breakpoints: median (range)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smokers</td>
<td>17.52 (15.53–21.70)</td>
<td>32.09 (26.83–35.78)</td>
<td>48.09 (39.41–60.34)</td>
<td>10.33 (6.28–13.12)</td>
<td>5.44 (3.15–9.56)</td>
</tr>
<tr>
<td>Nonsmokers</td>
<td>17.96 (13.08–23.13)</td>
<td>39.49 (30.0–47.74)</td>
<td>57.11 (51.48–68.85)</td>
<td>10.89 (8.03–14.94)</td>
<td>2.18 (0.65–6.31)</td>
</tr>
</tbody>
</table>

Table 5 Most frequent fragile sites found at oncogene site in peripheral blood lymphocytes

<table>
<thead>
<tr>
<th>Oncogene</th>
<th>Chromosome location</th>
<th>No. of subjects*</th>
<th>No. of sites/cell (mean ± SD)</th>
<th>No. of subjects*</th>
<th>No. of sites/cell (mean ± SD)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>bcl 1</td>
<td>11q13</td>
<td>12</td>
<td>0.155 ± 0.09</td>
<td>11</td>
<td>0.301 ± 0.15</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>erb B</td>
<td>7p12-p14</td>
<td>6</td>
<td>0.078 ± 0.052</td>
<td>9</td>
<td>0.238 ± 0.088</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>erb A</td>
<td>17q11-q21</td>
<td>1</td>
<td>0.043 ± 0.04</td>
<td>7</td>
<td>0.240 ± 0.11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>sis</td>
<td>22q12.3-q13.1</td>
<td>0</td>
<td>0.028 ± 0.031</td>
<td>7</td>
<td>0.192 ± 0.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>N-ras</td>
<td>1p22-p31</td>
<td>9</td>
<td>0.146 ± 0.079</td>
<td>3</td>
<td>0.180 ± 0.097</td>
<td>&lt;0.4</td>
</tr>
<tr>
<td>ets</td>
<td>11q23-24</td>
<td>8</td>
<td>0.087 ± 0.056</td>
<td>0</td>
<td>0.070 ± 0.048</td>
<td></td>
</tr>
</tbody>
</table>

* Numbers of subjects in whom this was ranked as one of the most frequent fragile sites at the oncogene sites in either smoker or nonsmoker groups.

Mean numbers calculated from the total 15 subjects.

Fig. 1. Comparison of the incidence of the 10 most frequent fragile sites found for smoker and nonsmoker groups.

Table 6 Most frequent fragile sites found at cancer breakpoints in peripheral blood lymphocytes

<table>
<thead>
<tr>
<th>Cancer Site</th>
<th>Nonsmokers</th>
<th>Smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects*</td>
<td>No. of sites/cell (mean ± SD)</td>
<td>No. of subjects*</td>
</tr>
<tr>
<td>3p14.2</td>
<td>15</td>
<td>0.438 ± 0.12</td>
</tr>
<tr>
<td>11q13.3</td>
<td>9</td>
<td>0.155 ± 0.09</td>
</tr>
<tr>
<td>1q21.3-q23</td>
<td>14</td>
<td>0.253 ± 0.126</td>
</tr>
<tr>
<td>22q12.2</td>
<td>0</td>
<td>0.028 ± 0.031</td>
</tr>
<tr>
<td>11p13-p14.2</td>
<td>3</td>
<td>0.072 ± 0.068</td>
</tr>
<tr>
<td>3q21.3</td>
<td>7</td>
<td>0.102 ± 0.03</td>
</tr>
<tr>
<td>1p13.2</td>
<td>5</td>
<td>0.124 ± 0.152</td>
</tr>
</tbody>
</table>

* Number of subjects in whom this ranked as one of the most frequent cancer breakpoints in either smokers or nonsmokers.

Mean numbers calculated from the total 15 subjects.

the 2 groups except those on the X chromosomes, which exhibited 2.5 times more fragile sites in smokers than in nonsmokers. The 10 most frequent breakpoints for both groups are shown in Fig. 1. In both groups, the 4 most frequently found fragile sites were 3p14.2, 11q13.3, 7q32.3, and 1q21.3; these sites are the same as those for all age groups described previously (13). The remaining frequently occurring fragile sites were located at 7q21.2, 1p21.2, 1p31.2, 1q25.1, 11q23.3, 2p13, 3q21.3, and 7q31.2 in young nonsmokers; and at Xp22.31, 16q23.2, 7p14.2, 17q21.3, 2q32.12, and 13q13.2 in young smokers.

Table 5 shows the most frequent breakpoints located at known oncogene sites (7, 14) along with the group mean numbers of fragile sites per metaphase. Breakpoints at bcl 1, erb B, erb A, and sis were found frequently in smokers, while breakpoints at bcl 1, N-ras, and ets were frequent in nonsmokers. Significantly elevated mean numbers of breaks at the bcl 1 (P < 0.005), erb B (P < 0.001), erb A (P < 0.001), and sis (P < 0.001) were seen in smokers when compared to nonsmokers. However, there was no significant difference between mean numbers of breakpoints at N-ras and ets in both smokers and nonsmokers.

The most frequent fragile sites found at cancer breakpoints for both the smoker and nonsmoker groups are listed in Table 6. The 5 most common cancer breakpoints in smokers were 3p14.2, 11q13.3, 1q21.3-q23, 22q12.2, and 11p13-p14.2. The
frequency of these fragile sites was significantly elevated in smokers when compared to nonsmokers, except for site 1q21.3- q23 \( (P < 0.5) \).

DISCUSSION

The positive correlation between cigarette smoking and the incidence of cancer is largely derived from retrospective and prospective epidemiological studies as well as from laboratory or animal experiments. Analysis of tobacco smoke shows the presence of almost 4000 compounds, many of which may act as initiators, promoters, and/or cocarcinogens with the major carcinogenic activity resulting from polycyclic compounds (15).

The frequency of SCEs reflects the damage occurring through 2 cycles of DNA replication and may serve as a sensitive index for mutagenicity and/or carcinogenicity of environmental chemicals (3, 16). In our studies of bone marrow cells and peripheral blood lymphocytes, we were able to demonstrate a significant increase in SCEs in smokers with a history of 5 or more cigarette pack years but not in those with less than 5 cigarette pack years. We also found a higher frequency of SCEs in peripheral blood lymphocytes than in bone marrow cells. An explanation for this finding may be that circulating lymphocytes, with an average life span of 4.4 years (17–19), may accumulate more DNA damage than do bone marrow cells, and this damage may manifest itself as SCEs during cell divisions. The imbalance in the male to female ratio between the 2 groups should not have biased this study since the SCE frequency is similar for both sexes for both peripheral blood lymphocytes (20) and bone marrow cells (21). Thus our studies indicate that in smokers with a history of 5 or more cigarette pack years, it is not necessary to examine bone marrow in order to identify possible damage by carcinogenic agents, since there is a significant increase in the frequency of SCEs in peripheral blood lymphocytes.

Although there was no significant difference in the median values of chromosomal aberrations between the two groups in either bone marrow or peripheral blood, 7 of 18 smokers showed 6–14% aberrations in the bone marrow. Chromosomal breakpoints were found to be random in the peripheral blood and the bone marrow for both groups.

We also found a significantly higher frequency of fragile sites \( (P < 0.001) \) and an increased number of metaphases with extensive breakage in the lymphocytes of smokers. The effect of fluorodeoxyuridine on DNA replication, one leading to chromosomal breakage and extensive fragmentation during cell division is well known (22). However, this effect is dose related and the concentration used to elicit the expression of fragile sites \( (1 \times 10^{-7} \text{M}) \) does not result in increased breakage or fragmentation. Yunis and Sorenge (7) presented evidence that many constitutive fragile and heritable fragile sites are located at or near the chromosomal breakpoints at which specific structural chromosome defects occur in leukemia, lymphoma, and malignant tumors. He proposed that chromosomal rearrangements might be facilitated by the presence of these heritable fragile or constitutive fragile sites. In our study, the 5 most frequent cancer breakpoints in smokers were 3p14.2, 11q13.3, 1q21.3-q23, 22q12.2, and 11p13-p14.2. One of these, 3p14.2, was the most frequent fragile site in every individual; however, the expression of this fragile site was elevated in smokers \( (P < 0.001) \). The breakpoint at 3p14.2 coincides with a constitutive fragile site and is associated with chromosomal structural abnormalities specific for several neoplasms including del(3)(p14,p23) in small cell lung cancer (23), t(3;11)(p13-14;p15) in familial renal cell carcinoma (24), and t(3;8)(p14.2;q24.1) in hereditary renal carcinoma (25). The expression of fragile sites at the second most frequent cancer breakpoint, 11q13.3, was also elevated in smokers \( (P < 0.005) \). This breakpoint involves a heritable fragile site and is associated with abnormalities t(11;14)(q13.3;q32.3) found in non-Hodgkin’s lymphoma (26, 27) as well as t(11;14)(q13;q32) which occurs in B-cell lymphoproliferative disorders, multiple myeloma, and plasma cell leukemia (28–31). For 2 other frequent cancer breakpoints, 11p13-14.2 and 22q12.2, the mean numbers of fragile sites were significantly increased in smokers \( (P < 0.01 \text{ and } < 0.001, \text{respectively}) \). The cancer breakpoint 11p13-14.2 is associated with T-cell acute lymphocytic leukemia as t(11;14)(p13-14;q13) (7, 31), and 22q12.2 is associated with Ewing’s sarcoma and neuroepithelioma as t(11;22) (q24;q12) (32), and with Burkitt’s lymphoma as t(8;22)(q23;q12) (34, 35). Another frequent cancer breakpoint, 1q21.3-q23, is associated with pre-B acute lymphocytic leukemia occurring as t(1;19)(q21-23;q13) (7, 36). However, the expression of fragile sites at this cancer breakpoint was not significantly elevated in smokers \( (P < 0.5) \).

In our study the frequency of fragile sites was significantly elevated in smokers over those in nonsmokers at 4 oncogene sites, bel I, erb B, erb A, and sis. Oncogene bel I, mapped to chromosome 11q13, is normally regulated at this site but is activated when it is translocated to the immunoglobulin H region at 14q32, e.g., in chronic B-cell lymphocytic leukemia (37). Oncogene erb B located at 7p12-p14 includes a constitutive fragile site 7p14.2. Oncogene erb A has been mapped to 17q11-q21, which is near c-fra 17q23.1. Oncogene sis located at 22q12.3-q13.1 is close to c-fra 22q12.2, and is associated with Ewing’s sarcoma, neuroepithelioma, and Burkitt’s lymphoma. At the present time there is no evidence for the involvement of erb A, erb B, and sis genes in neoplasia (38).

The essential steps leading to chromosomal rearrangement after breakage are not known, but increased frequencies of SCEs and breakage sites (fragile sites) may eventually result in increased incidence of chromosomal structural rearrangements and alteration in expression of either constitutional genes or oncogenes and may lead toward malignant transformation.

Follow-up studies of frequency of fragile sites and the location of these sites at chromosomal cancer breakpoints, and protooncogenes in young smokers may permit further clarification of the relationship between exposure to such environmental agents as tobacco smoke and the development of neoplasms.

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