p53 Mutations in Squamous Cell Carcinoma of the Head and Neck Predominate in a Subgroup of Former and Present Smokers with a Low Frequency of Genetic Instability

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ABSTRACT

We examined the p53 mutational profile of 65 squamous cell carcinomas of the head and neck (SCCHNs) from patients living in northwest England. Twenty-three p53 mutations were detected in 20 samples (31%). GC→AT transitions were the predominant type of mutation. The p53 mutational profile of SCCHN tumors was similar to that of non-small cell lung tumors from patients within the same geographical area, supporting the idea of a common model for carcinogenesis in the upper respiratory tract. Statistical analysis showed that the incidence of p53 mutations among present and former smokers was significantly higher than that in nonsmokers (P < 0.02). In addition, p53 mutations were found to predominate in a group of SCCHN patients with low genetic damage, as indicated by the fractional allelic loss value. The above findings suggest an early initiating role for p53 and imply that at least two separate carcinogenic pathways may be involved in the development of SCCHN.

INTRODUCTION

Cigarette smoking and alcohol consumption are well-established risk factors for the development of SCCHN. The p53 tumor suppressor gene is commonly mutated in these tumors (3, 4) and appears to be one of the molecular targets of tobacco-related carcinogens. The initial association between cigarette smoking and altered p53 gene expression in SCCHN, showing a positive correlation between immunohistochemical detection of p53 (suggestive but not necessarily indicative of a p53 mutation) and a patient history of heavy smoking, was made by Field et al. (5–7). In addition, the majority of tumors from patients who had stopped smoking for more than 5 years prior to presentation immunostained for p53, suggesting that p53 gene alterations were an early event in the development of these cancers. The association between p53 mutations and smoking has recently been confirmed in both carcinomas and premalignant lesions of the head and neck (8–10).

The spectrum of mutations in tumors can provide clues to the carcinogens involved in their pathogenesis (11, 12). Mutations may be caused by both endogenous biological processes and exogenous carcinogens. Endogenous DNA alterations include the spontaneous deamination of 5mC at CpG dinucleotides, resulting in GC→AT transitions (13, 14). However, deamination of 5mC can be accelerated by exogenous factors like 1-nitropyrene (15) or UV light (16). Deletions and insertions are frequent types of p53 mutations and can also reflect both spontaneous and induced mutagenesis (17). Certain carcinogens appear to induce particular p53 mutations. Aflatoxin B1 has been associated with G→T transversions in hepatocellular carcinomas (18–20), and benzo[a]pyrene has been also shown to produce preferentially G→T transversions (21, 22). Physical carcinogens are also associated with selective targeting of the p53 gene. The involvement of UV light in inducing p53 mutations in skin carcinomas at sun-exposed sites is indicated by the presence of CC→TT double-base changes and C→T transitions at dipyrimidine sites (23, 24). A predominance of G→T transversions has also been recorded in nonsmall cell lung cancer from smokers, but rather than being clustered, these are distributed over many codons (25–27).

Here, the presence and profile of p53 mutations in SCCHN were correlated with the patients’ smoking history, use of alcohol, a range of clinicopathological parameters, and the degree of genetic damage, indicated by LOH analysis, in each tumor.

MATERIALS AND METHODS

Tissue Samples. Tumor and adjacent normal tissue samples were collected at the time of surgery and frozen immediately in liquid nitrogen. The clinical and follow-up details were taken from the case notes. The sites and numbers of tumors were: 13 oral, 15 laryngeal, 20 hypopharyngeal, 12 oropharyngeal, and 5 nodal metastases.

All patients were caucasians, living in Merseyside, northwest England. With respect to their smoking history, the patients were divided into four subgroups: 30 heavy smokers (≥20 cigarettes/day), 13 moderate smokers (<20 cigarettes/day), 8 former smokers (stopped smoking 5–18 years prior to presentation), and 14 nonsmokers.

DNA Extraction and PCR. The tumor specimens were microdissected to provide >70% tumor cells, and DNA was extracted using the Nucleon II kit (Scotlab, United Kingdom). The oligonucleotides used and conditions followed for the PCR amplification of the p53 exons 5–9 have been described previously (28). The reaction mixture contained 16 mM (NH4)2SO4, 67 mM Tris-HCl (pH 8.8), 0.1% Tween 20, 100 μM dNTPs, 0.4 μM each primer, 1.5–2.5 mM MgCl2, and 0.5 units of BIOPRO polymerase (BIOLINE, United Kingdom).

SSCP Analysis and Solid-Phase Sequencing. Two to 4 μl of the PCR product were mixed with 10 μl of denaturing solution, consisting of 80% formamide, 100 mM NaOH, 1 mM EDTA, 0.1% bromphenol blue, and 0.1% xylene cyanol FF. Samples were then heated at 95°C for 3 min, chilled on ice, and loaded onto a 8–10% native polyacrylamide gel, containing 5–10% glycerol. Gels were run at 15°C and silver-stained after electrophoresis. DNA samples that showed altered mobility by SSCP analysis were reamplified using a 5′ biotinylated upstream primer. The strands of the PCR product were then separated using streptavidin-conjugated Dynabeads M-280 (Dynal, United Kingdom). Sequencing reactions were performed using Sequenase V 2.0 kit (Amersham, United Kingdom), and products were electrophoresed through a 6% denaturing polyacrylamide gel. Gels were then fixed, dried, and exposed to Kodak XAR-50 films.

Statistical Analysis. The Fisher’s χ2 and log–rank tests were used, with SAS Institute software for PCs, to determine whether any correlation existed between the presence of mutations and the clinicopathological data.
RESULTS

**p53 Mutational Profile in SCCHN.** We examined 65 SCCHNs for the presence of mutations within exons 5–9 of the p53 gene using SSCP and sequencing analysis. SSCP analysis was used to rapidly screen for p53 mutations in exons 5–9, and all samples presenting abnormal electrophoretic mobility were sequenced. Sequencing analysis revealed 23 mutations in 20 of the 65 SCCHN samples (30.7%); Table 1). Representative examples of these mutations are shown in Fig. 1. Mutations were detected in 11 of 30 (36.6%) heavy smokers, 4 of 13 (30.6%) moderate smokers, 4 of 8 (50%) former smokers, and 1 of 14 (7.1%) nonsmokers (Table 2). To reduce the possibility of missing mutations during SSCP analysis, five SSCP-negative tumor DNA samples for each exon were randomly picked and sequenced. No mutations were revealed by sequencing in any of the SSCP-negative samples examined.

Sequencing demonstrated 12 missense, 3 nonsense, 3 frameshift, and 2 silent mutations, as well as 3 mutations affecting splicing. The mutational profile found was: 5 deletions, 17 base substitutions, and 2 complex changes, i.e., GA→AT and ATGAC→TTTACC. The base substitutions consisted of 13 transitions (11 GC→AT and 2 AT→GC) and 3 transversions (2 GC→TA and 1 AT→TA). Six of 11 GC→AT transitions occurred at CpG dinucleotides (Table 1). All but one of the mutations were somatic, and no mutations were detected in the corresponding normal tissues.

**p53 Mutations Correlate with a Smoking History.** Statistical analysis indicated a significant difference between the presence of p53 mutations in smokers and nonsmokers (P < 0.02), whereas there were no significant differences among the three classes of smokers: heavy, moderate, and former smokers. No correlation was found between the p53 mutations and any of the various clinicopathological parameters tested (age, gender, tumor-node-metastasis stage, differentiation, site of tumor, nodes, drinking history, and survival), although trends for an association between p53 mutations and drinking and differentiation were observed (P = 0.1; Table 3).

**p53 Mutations Predominate in the Population with a Lower Frequency of Genetic Instability.** We have previously undertaken a detailed allelotype analysis of SCCHN using 145 microsatellite markers on 39 chromosome arms and calculated FALs (number of chromosomal arms on which allelic imbalance was observed, divided by the number of chromosomal arms for which markers were informative in the patients normal cells) for each tumor specimen (29). These patients were subdivided into two groups: FAL < 0.22 (median value) and FAL ≥ 0.22. The patients with FAL < 0.22 will be referred to as individuals with a LGI, and those patients with FAL ≥ 0.22 will be referred to as individuals with a HGI. The FALs were available for 34 of the specimens under investigation in this p53 mutational analysis, of which 18 were LGI and 16 HGI. In this group of 34 SCCHN patients, 11 patients had p53 mutations in their tumors. It is of note that the majority of the tumors with p53 mutations fell within the LGI group (9 of 18, 50%), whereas only 2 of 16 (13%) lie within the HGI group (Table 4). A correlation was found between p53 mutations in the LGI subgroup and the HGI subgroup (P = 0.02). The LOH details of patients with LGI were analyzed to ascertain whether specific genetic loci were associated with p53 mutations; however, none were found.

**DISCUSSION**

Here, we found that 31% of the head and neck tumors that were examined carried a mutation within the p53 gene. Moreover, the presence of p53 mutations was found to correlate with the patients’ smoking history; only 1 of 14 (7%) tumors from nonsmokers carried a p53 mutation, compared to 45% of tumors from smokers. There was no difference among the separate categories of smokers, indicating that even low degrees of exposure to tobacco carcinogens may have mutagenic effects on the p53 gene. In addition, the p53 mutational profile is similar in the three different classes of smokers, indicating that the amount of tobacco smoke consumed does not seem to determine the targeted nucleotide.

In the group of eight SCCHN patients who had stopped smoking for 5–18 years, 50% had a p53 mutation. These results are in agreement with the absence of a correlation between p53 mutation/expression data and the tumor-node-metastasis stage of the tumor and also add support to our original hypothesis that p53 mutations occur early in the development of SCCHN (5, 6).

It is of note that we found a low incidence of GC→TA transversions, normally associated with smoking-related cancers; in our study, 9% of the p53 mutations were GC→TA transversions, whereas 48% were GC→AT transitions (Table 5). On examining the most recent p53 database (30), there was also a preponderance of GC→AT transitions.

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**Table 1 p53 mutations in head and neck tumors**

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Site of tumor</th>
<th>Smoking status</th>
<th>Exon</th>
<th>Codon</th>
<th>Sequence change</th>
<th>Amino acid change</th>
</tr>
</thead>
<tbody>
<tr>
<td>0048</td>
<td>Oral</td>
<td>Stopped smoking</td>
<td>7-exon</td>
<td>261</td>
<td>6-bp deletion</td>
<td>Splicing/deletion of amino acid 261</td>
</tr>
<tr>
<td>0093</td>
<td>Oral</td>
<td>Stopped smoking</td>
<td>7</td>
<td>248</td>
<td>cgg→cag</td>
<td>Arg→Gln</td>
</tr>
<tr>
<td>0302</td>
<td>Hypopharynx</td>
<td>Stopped smoking</td>
<td>5</td>
<td>146</td>
<td>tgg→tg</td>
<td>Trp→Leu</td>
</tr>
<tr>
<td>0353</td>
<td>Mouth</td>
<td>Stopped smoking</td>
<td>6</td>
<td>279</td>
<td>1-bp deletion</td>
<td>Frameshift</td>
</tr>
<tr>
<td>0041</td>
<td>Larynx</td>
<td>Moderate smoking</td>
<td>7</td>
<td>248</td>
<td>cgg→cag</td>
<td>Arg→Trp</td>
</tr>
<tr>
<td>0318</td>
<td>Larynx</td>
<td>Moderate smoking</td>
<td>8</td>
<td>285</td>
<td>1-bp deletion</td>
<td>Frameshift</td>
</tr>
<tr>
<td>0348</td>
<td>Oropharynx</td>
<td>Moderate smoking</td>
<td>6</td>
<td>213</td>
<td>cga→ga</td>
<td>Arg→Stop</td>
</tr>
<tr>
<td>0359</td>
<td>Larynx</td>
<td>Moderate smoking</td>
<td>5</td>
<td>174</td>
<td>gta→ga</td>
<td>Silent</td>
</tr>
<tr>
<td>0057</td>
<td>Node</td>
<td>Heavy smoking</td>
<td>5</td>
<td>159</td>
<td>gcc→gct</td>
<td>Ala→Val</td>
</tr>
<tr>
<td>0062</td>
<td>Hypopharynx</td>
<td>Heavy smoking</td>
<td>5</td>
<td>173</td>
<td>gta→ga</td>
<td>Val→Met</td>
</tr>
<tr>
<td>0074</td>
<td>Oropharynx</td>
<td>Heavy smoking</td>
<td>6</td>
<td>214</td>
<td>1-bp deletion</td>
<td>Frameshift</td>
</tr>
<tr>
<td>0100</td>
<td>Larynx</td>
<td>Heavy smoking</td>
<td>8</td>
<td>266</td>
<td>gga→agt</td>
<td>Gly→Asp</td>
</tr>
<tr>
<td>0162</td>
<td>Hypopharynx</td>
<td>Heavy smoking</td>
<td>4</td>
<td>257</td>
<td>gta→agt</td>
<td>Val→Met</td>
</tr>
<tr>
<td>0217</td>
<td>Larynx</td>
<td>Heavy smoking</td>
<td>7</td>
<td>246/247</td>
<td>atgac→tttacc</td>
<td>Met-Asn→Phe-Trp</td>
</tr>
<tr>
<td>0308</td>
<td>Oropharynx</td>
<td>Heavy smoking</td>
<td>5</td>
<td>144</td>
<td>cag→tag</td>
<td>Gin→Stop</td>
</tr>
<tr>
<td>0358</td>
<td>Hypopharynx</td>
<td>Heavy smoking</td>
<td>8</td>
<td>285</td>
<td>gaa→aag</td>
<td>Glu→Lys</td>
</tr>
<tr>
<td>1082</td>
<td>Oral</td>
<td>Heavy smoking</td>
<td>4</td>
<td>152</td>
<td>ctc→ctg</td>
<td>Pro→Leu</td>
</tr>
<tr>
<td>1087</td>
<td>Oral</td>
<td>Heavy smoking</td>
<td>5</td>
<td>175</td>
<td>cgc→cac</td>
<td>Arg→His</td>
</tr>
<tr>
<td>1100</td>
<td>Oropharynx</td>
<td>Heavy smoking</td>
<td>5</td>
<td>248</td>
<td>cgg→cag</td>
<td>Arg→Trp</td>
</tr>
<tr>
<td>0136</td>
<td>Hypopharynx</td>
<td>Nonsmoking</td>
<td>7</td>
<td>248</td>
<td>cgg→cag</td>
<td>Arg→Trp</td>
</tr>
</tbody>
</table>

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transitions in head and neck tumors (Table 6). Despite the observed difference in the distribution frequency of p53 mutations between our study and the database, statistical analysis showed no significant difference. It is of note that more than half of the GC→AT transitions in this study occurred at CpG dinucleotides, suggesting an increased endogenous mutagenesis. However, we cannot exclude the possibility that these mutations are the result of carcinogens (e.g., nitrosamines), which are known to produce such transitions (15, 31, 32).

The above results are of particular importance when compared to the p53 mutational profile of tumor samples from patients with lung cancer, also recruited from the Merseyside region in the United Kingdom (33), in whom a similar pattern (GC→AT) was observed (Table 5), suggesting that the Merseyside population may be exposed to specific carcinogens in this geographic area. A similar GC→AT transition preponderance has been found in squamous cell carcinomas of the esophagus (34, 35), indicating that there may be a common model of carcinogenesis in the upper aerodigestive tract. This similarity of the p53 mutation pattern in lung and head and neck tumors in our study is in contrast with a study from Law et al. (36), who found variation in the p53 mutation spectra between the lower and upper respiratory tract.

We have undertaken the largest allelotype to date in any cancer (29) and have shown a correlation between the FAL and survival, as well as positive nodes at pathology, thus indicating that the accumulation of genetic damage, as provided by allelotype data, provides a powerful molecular indicator of tumor behavior and clinical outcome in SCCHN. Thus, it is of note that we have now demonstrated a correlation between p53 mutations and FALs. FALs were available for 34 of the specimens under investigation in this p53 mutational analysis. Nine of 11 patients with p53 mutations fell within the LGI group, whereas only 2 lay within the HGI group. It may be argued that genetic damage can be measured by a FAL score; then, the patients’ tumors with p53 mutations had less genetic damage (allelic imbalance or LOH) than the majority of tumors from patients with a high FAL score and no p53 mutations. This implies that there are most likely two separate carcinogenic pathways involved in the development of SCCHN, based on the FAL/p53 status. Inverse correlation between

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**Table 2** Distribution of p53 mutations according to the patients’ smoking history

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Heavy smokers (n = 30)</th>
<th>Moderate smokers (n = 13)</th>
<th>Former smokers (n = 8)</th>
<th>Nonsmokers (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC→AT</td>
<td>6</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>GC→TA</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>AT→GC</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>AT→TA</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Deletion/insertion/other</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

Total no. of mutations: 12 in 11 samples (4 in 4 samples, 6 in 4 samples, 1 in 1 sample)

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**Table 3** Analysis of the p53 mutation incidence in relation to the patients’ smoking and drinking history

<table>
<thead>
<tr>
<th>Tobacco consumption</th>
<th>Alcohol consumption</th>
<th>No tobacco use</th>
<th>Tobacco and alcohol use (MS + HS + SS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSa</td>
<td>NS</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>MSb</td>
<td>MS</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>SS</td>
<td>SS</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>MS + HS</td>
<td>MS + HS</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>No. of tumors (n = 65)</td>
<td>No. of p53 mutations (n = 20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 (7)</td>
<td>1</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>13 (31)</td>
<td>4</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>8 (50)</td>
<td>4</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>43 (35)</td>
<td>2</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>13 (30)</td>
<td>1</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>11 (2)</td>
<td>2</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>32 (10)</td>
<td>2</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>2 (50)</td>
<td>4</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>25 (40)</td>
<td>4</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>36 (33)</td>
<td>4</td>
<td>1</td>
<td>10</td>
</tr>
</tbody>
</table>

a No alcohol data on seven individuals.

b NS, nonsmoker; MS, moderate smoker; HS, heavy smoker; SS, stopped smoker; ND, nondrinker; MD, moderate drinker; HD, heavy drinker; SD, stopped drinker.

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Table 4 Comparison of p53 mutations with fractional allele loss data

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Patient age (yr)</th>
<th>Site</th>
<th>Smoking history</th>
<th>Drinking history</th>
<th>p53 mutation</th>
<th>FAL*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0128</td>
<td>61</td>
<td>Hypopharynx</td>
<td>Nonsmoker</td>
<td>No data</td>
<td>Exon 7</td>
<td>0.00</td>
</tr>
<tr>
<td>0041</td>
<td>71</td>
<td>Larynx</td>
<td>Moderate</td>
<td>Heavy</td>
<td>Exon 7</td>
<td>0.05</td>
</tr>
<tr>
<td>0353</td>
<td>71</td>
<td>Oral</td>
<td>Stopped smoking</td>
<td>Stopped drinking</td>
<td>Exons 5/8</td>
<td>0.10</td>
</tr>
<tr>
<td>0302</td>
<td>50</td>
<td>Hypopharynx</td>
<td>Moderate</td>
<td>Nonsmoker</td>
<td>Exon 5</td>
<td>0.13</td>
</tr>
<tr>
<td>0310</td>
<td>65</td>
<td>Hypopharynx</td>
<td>Heavy</td>
<td>Heavy</td>
<td>Exon 5</td>
<td>0.14</td>
</tr>
<tr>
<td>1086</td>
<td>43</td>
<td>Oropharynx</td>
<td>Heavy</td>
<td>Moderate</td>
<td>Exon 5</td>
<td>0.15</td>
</tr>
<tr>
<td>0339</td>
<td>68</td>
<td>Larynx</td>
<td>Heavy</td>
<td>High</td>
<td>Exon 5</td>
<td>0.17</td>
</tr>
<tr>
<td>0359</td>
<td>54</td>
<td>Larynx</td>
<td>Moderate</td>
<td>Heavy</td>
<td>Exon 5</td>
<td>0.19</td>
</tr>
<tr>
<td>0204</td>
<td>72</td>
<td>Larynx</td>
<td>Heavy</td>
<td>Heavy</td>
<td>Exon 5</td>
<td>0.20</td>
</tr>
<tr>
<td>1087</td>
<td>00</td>
<td>Oral</td>
<td>Heavy</td>
<td>Heavy</td>
<td>Exon 5</td>
<td>0.20</td>
</tr>
<tr>
<td>0350</td>
<td>64</td>
<td>Hypopharynx</td>
<td>Heavy</td>
<td>Moderate</td>
<td>Exon 5</td>
<td>0.20</td>
</tr>
<tr>
<td>0358</td>
<td>53</td>
<td>Hypopharynx</td>
<td>Nonsmoker</td>
<td>Moderate</td>
<td>Exon 5</td>
<td>0.20</td>
</tr>
<tr>
<td>0336</td>
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<td>Hypopharynx</td>
<td>Heavy</td>
<td>Moderate</td>
<td>Exon 5</td>
<td>0.20</td>
</tr>
<tr>
<td>0318</td>
<td>62</td>
<td>Larynx</td>
<td>Nonsmoker</td>
<td>Moderate</td>
<td>Exon 5</td>
<td>0.20</td>
</tr>
<tr>
<td>0361</td>
<td>71</td>
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<td>Nonsmoker</td>
<td>Moderate</td>
<td>Exon 5</td>
<td>0.20</td>
</tr>
<tr>
<td>0340</td>
<td>45</td>
<td>Hypopharynx</td>
<td>Moderate</td>
<td>Heavy</td>
<td>Exon 5</td>
<td>0.20</td>
</tr>
<tr>
<td>0315</td>
<td>55</td>
<td>Oral</td>
<td>Nonsmoker</td>
<td>Heavy</td>
<td>Intron 4</td>
<td>0.21</td>
</tr>
<tr>
<td>1062</td>
<td>00</td>
<td>Oral</td>
<td>Heavy</td>
<td>Nondrinker</td>
<td>Intron 4</td>
<td>0.22</td>
</tr>
<tr>
<td>0215</td>
<td>71</td>
<td>Larynx</td>
<td>Stopped smoking</td>
<td>Nondrinker</td>
<td>Intron 4</td>
<td>0.22</td>
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<tr>
<td>0347</td>
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<td>Larynx</td>
<td>Heavy</td>
<td>Moderate</td>
<td>Intron 4</td>
<td>0.22</td>
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<td>73</td>
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<td>Heavy</td>
<td>Moderate</td>
<td>Intron 4</td>
<td>0.23</td>
</tr>
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<td>0305</td>
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<td>Nonsmoker</td>
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<td>Intron 4</td>
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<td>Heavy</td>
<td>Intron 4</td>
<td>0.30</td>
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<td>0224</td>
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<td>Exon 6</td>
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<td>00</td>
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</tr>
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<td>58</td>
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<td>Heavy</td>
<td>Moderate</td>
<td>Intron 4</td>
<td>0.45</td>
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<td>93</td>
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<td>Nondrinker</td>
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<td>Hypopharynx</td>
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<td>Heavy</td>
<td>Intron 4</td>
<td>0.56</td>
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<td>0341</td>
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<td>Heavy</td>
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<td>0.56</td>
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<tr>
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<td>Heavy</td>
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<td>0.56</td>
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<td>Hypopharynx</td>
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<tr>
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<td>Stopped smoking</td>
<td>Heavy</td>
<td>Intron 4</td>
<td>0.56</td>
</tr>
</tbody>
</table>

* Median FAL = 0.22.

p53 mutations and genomic instability has been also reported for gastric (37) and colorectal (38) carcinomas, whereas a direct correlation between p53 mutations and genomic instability has been shown in bladder (39), ovarian (40), and breast (41) tumors.

This set of data may also be viewed from the perspective of a smoking history. Forty % of heavy and former smokers were found to have p53 mutations, and there were 9 heavy and former smokers in the LGI and in the HGI subgroups. Five of 9 individuals in LGI group contained p53 mutations, whereas only 1 of 9 had p53 mutations in the HGI group (P < 0.05), thus dividing these smokers into two subgroups, which most likely reflects different carcinogenic initiating events. These results suggest that about one-half of heavy/former smokers contain p53 mutations and that the majority of these individuals have minimal amount of genetic damage, as assessed by their FAL score, thus suggesting that p53 mutations are early initiating events in SCCHN and also accelerate their progression through carcinogenesis. However, the other group of heavy/former smokers had no p53 mutations but had a very large genetic burden, based on their high FAL scores. Furthermore, there are two groups of individuals who develop SCCHN and have no p53 mutations but may also be segregated on the basis of their FAL scores. In the future, epidemiological information on the patients’ environmental/industrial exposure to carcinogens, as well as genetic susceptibility data, may further refine the separation of these four patient subgroups.

Table 6 Comparison between p53 mutation types in head and neck tumors and cell in the database of Hollstein et al. (30)

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Tumors</th>
<th>Cell lines</th>
<th>Tumors</th>
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<td>GC→AT</td>
<td>110*</td>
<td>25</td>
<td>35</td>
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<tr>
<td>GC→TA</td>
<td>58</td>
<td>18</td>
<td>6</td>
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<tr>
<td>AT→GC</td>
<td>41</td>
<td>13</td>
<td>1</td>
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<td>26</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>GC→CG</td>
<td>36</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>AT→CG</td>
<td>10</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Deletion/insertion/other</td>
<td>45</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>326</td>
<td>34</td>
<td>23</td>
</tr>
</tbody>
</table>

* GC→AT compared with all other types of p53 mutations: χ², P = 0.25.

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p53 Mutations in Squamous Cell Carcinoma of the Head and Neck Predominate in a Subgroup of Former and Present Smokers with a Low Frequency of Genetic Instability

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