Genetic Susceptibility to Head and Neck Squamous Cell Carcinoma

Jacqueline Cloos, Margaret R. Spitz, Stimson P. Schantz, T. C. Hsu, Zuo-feng Zhang, Hilde Tobi, Boudewijn J. M. Braakhuis, Gordon B. Snow*

Background: In addition to influences of exposure to carcinogenic compounds, the development of cancer may depend on an individual intrinsic cancer susceptibility. Biomarkers for cancer susceptibility can be powerful additions to epidemiologic analyses. Purpose: This multicenter, case-control analysis combines previously published data and new data to substantiate the value of mutagen sensitivity as a biomarker of susceptibility to head and neck squamous cell carcinoma and, more importantly, to gain insight into the interaction between susceptibility and exposure to carcinogens. Methods: Mutagen sensitivity (mean number of chromatid breaks per cell of cultured lymphocytes treated with bleomycin in the late S-G2 phase of the cell cycle) was determined in 313 patients with head and neck cancer and in 334 control subjects at two major U.S. medical institutions and one European institution, yielding a unique study population. The ages of the case and control subjects, as well as their history of use of tobacco and alcohol, were also recorded. The relationships between variables were analyzed by use of Student's t tests, Spearman's rank correlations, and multiple linear regression. For estimation of cancer risk, crude odds ratios (ORs) were measured and multiple logistic regression was performed. All P values were based on two-sided tests. Results: There were no differences across institutions in the distribution of mutagen sensitivity (Kruskal-Wallis test) for both case subjects and control subjects. Values for case subjects were consistently and significantly (P < .0001) higher than values for control subjects in the overall analyses. Age and tobacco or alcohol use did not influence the outcome in terms of mutagen-sensitivity values for either the case or the control subjects. A mean number of breaks per cell dichotomized at 1.0 was found to be the best predictor of a hypersensitive phenotype. For nonsensitive, heavy smokers, the OR was 11.5 (95% confidence interval [CI] = 5.0-26.6). This risk increased dramatically in mutagen-hypersensitive, heavy smokers to 44.6 (95% CI = 17.4-114.0). Multiple logistic regression analysis confirmed these results, and a significant trend was found (P < .01) for the dose-dependent increase in cancer risk by smoking. The consumption of alcohol potentiated the effects of smoking, resulting in an OR of 57.5 (95% CI = 17.5-188.0) in hypersensitive persons. Conclusions: Mutagen sensitivity was found to be a biomarker of cancer susceptibility. This study underscores the importance of utilizing both susceptibility markers and exposure data for the identification of persons at high risk of developing cancer. Implications: More accurate risk estimation can define susceptible subgroups who might be targeted for intensive behavioral interventions, surveillance through screening, and enrollment in chemoprevention programs. [J Natl Cancer Inst 1996;88:530-5]

Tobacco exposure and alcohol exposure are the major determinants of head and neck squamous cell carcinoma (HNSCC) (1). Since only a fraction of exposed individuals develops cancer, however, an intrinsic susceptibility to environmental genotoxic exposures has also been suggested as playing a role in carcinogenesis (2). Within the general population, there may exist varying degrees of DNA maintenance capability. To investigate this hypothesis, Hsu (3) developed an assay in which the number of induced chromatid breaks are scored in metaphase spreads of cultured lymphocytes after in vitro treatment with bleomycin during the late S-G2 phase of the cell cycle. It has been shown that mutagen sensitivity (defined as the mean number of breaks per cell [b/c]) determines a cancer susceptibility phenotype (4). A mean number of b/c equal to or greater than 1.0 has been defined as a hypersensitive phenotype, which has been suggested to be a risk factor for the development of HNSCC and lung cancer (5). Consideration of markers of individual susceptibility helps to refine the risk assessment process (6). These types of molecular epidemiologic studies, however, require large numbers of subjects. We have thus combined data from three similar studies—two of which have been reported in the literature (7,8)—conducted at The University of Texas M. D. Anderson Cancer Center (Houston, TX), the Memorial Sloan-Kettering Cancer Center (New York, NY), and the Free University Hospital (Amsterdam, The Netherlands) into a unique set of data. Using these data, we have tested the most...
optimal model to explain the relationship between carcinogen exposure and mutagen sensitivity as a risk for HNSCC.

Subjects and Methods

Subjects

The three groups of HNSCC patients (from Houston, New York, or Amsterdam) consisted of previously untreated patients with histologically confirmed tumors in the mucosa of the upper aerodigestive tract (oral cavity, larynx, oropharynx, and hypopharynx).

The case subjects from Houston were registered at The University of Texas M. D. Anderson Cancer Center. The control subjects were recruited from the blood bank of that institution and were frequency matched by age, sex, and ethnicity to the case subjects. Information regarding cigarette-smoking and alcohol-drinking habits was collected from both case and control subjects by use of extensive self-administered questionnaires.

The same questionnaire was used for collection of subject data in New York, where the case subjects were recruited from the Memorial Sloan-Kettering Cancer Center. The control subjects were healthy volunteers identified from the Blood Bank Center of that institution and were matched by age, sex, and ethnicity to the case subjects.

The cancer patients from Amsterdam were registered at the Free University Hospital. The control subjects were not individually matched with regard to age, alcohol consumption, and tobacco exposure to the case subjects and were either cancer-free patients who had registered at the hospital or healthy volunteers. Using a standard question format, the treating physician obtained information regarding lifestyle (including cigarette-smoking and alcohol-drinking habits) from both case and control subjects through direct personal interview.

The matching of case and control subjects was not possible in this study, since the subjects from Amsterdam were not individually matched and, unfortunately, the control group was too heterogeneous to allow for evaluating the effect of these subjects after data collection and during the analysis. Moreover, some subjects were excluded from the analysis because of missing values. The number of subjects used for each separate analysis is given in the "Results" section.

The number of pack-years, a measure of cumulative smoking, was calculated as the number of years smoked multiplied by the number of packs of cigarettes smoked daily. The number of packs is calculated on the basis of 25 cigarettes per pack (the usual number of cigarettes in a pack in Europe). Each of the values of pack-years would be 20% greater if converted to the number of cigarettes per pack in the United States (i.e., 20 cigarettes per pack). Units per day was taken as a measure of daily alcohol consumption, in which 1 U is defined as one alcoholic beverage (equivalent to approximately 15 mL of ethanol).

Mutagen Sensitivity

The assay to determine mutagen sensitivity was described in detail previously (9). Briefly, 10 mL of whole blood was diluted 10 times in RPMI-1640 medium with 2 mM L-glutamine (Life Technologies, Inc., [GIBCO BRL], Paisley, Scotland) supplemented with 15% fetal calf serum (ICN Biomedicals, Ltd., Irvine, Scotland), 1.5% phytohemagglutinin (Wellcome Diagnostics, Dartford, England), and 100 U/mL each of penicillin and streptomycin (ICN Biomedicals Ltd.). After the cells were cultured for 3 days at 37 °C and in 5% CO₂, they were incubated for 5 hours with 30 μM/mL bleomycin (Lundbeck, Amsterdam; Bristol laboratories, Syracuse, NY). To arrest the cells at metaphase, 0.04 μg/mL Colcemid (Sigma Chemical Co., St Louis, MO) was added to the cultures 1 hour before the cells were harvested. This procedure yielded cells in metaphase that were damaged by the bleomycin in the late S-G₂ phase of the cell cycle. The cells were swollen in hypotonic solution (0.06 M KCl) and fixed in Carnoy’s fixative (3:1 vol/vol methanol:glacial acetic acid). After the drops containing cells were placed on wet slides, the metaphase spreads were air dried and stained with Giemsa solution (Merck, Darmstadt, Federal Republic of Germany). Before at least 50 metaphase spreads were scored for the presence of chromatid breaks, the slides were coded to ensure objective screening. The slides were scored as a measure of daily alcohol consumption, in which 1 U is defined as one alcoholic beverage (equivalent to approximately 15 mL of ethanol). Background levels of chromatid breaks with no damage induced by bleomycin, which were determined in previous studies, were very low (b/c values of about 0.06) and did not differ between patients and control subjects.

Therefore, data representing "spontaneous" breaks were no longer included in the determination of mutagen sensitivity. All data on subjects in the United States were scored in Houston at the Department of Cell Biology. All data on subjects in The Netherlands were scored by the investigator from Amsterdam (J. Cloos), who had been trained in these analyses by Dr. Hsu and his colleagues.

Statistics

The Mann–Whitney U test and Student’s t test were used to compare means between the groups. Both Spearman’s rank correlations and linear regression were performed to study the correlations between relevant variables. Variation in the data between the three institutions was evaluated by use of the Kruskal–Wallis test. To estimate cancer risk, we calculated crude odds ratios (ORs) and 95% confidence intervals (CIs). To estimate the joint influence of assessed parameters on mutagen sensitivity, we performed multiple linear regression. We used unconditional multiple logistic regression to analyze the relationship between a hypersensitive phenotype and the influence of exposure to carcinogens on cancer risk. Using the multiple logistic regression, we also tested for trend by including the classifying predictor as a continuous variable. This procedure is equivalent to the trend test in the one-dimensional case.

All P values were determined on the basis of two-sided tests. All analyses were performed by the use of the following computer programs: Statistical Package for the Social Sciences (SPSS, version 4.0; SPSS Inc., Chicago, IL) and BMDP (Statistical Software Inc., Los Angeles, CA).

Results

Table 1 summarizes selected demographic characteristics of the case and control subjects by study site. There was a significant difference across the study sites by age of the case and control subjects. The mean age of the Amsterdam case subjects was higher than that of the other two groups. The mean age of the American control subjects was lower than that of the other two control groups. The subjects in all three case groups smoked heavily (mean of 35 pack-years compared with a mean of 14 pack-years for the U.S. control subjects and 21 pack-years for the Amsterdam control subjects). Alcohol consumption was higher in the Amsterdam control group than in the other two control groups. There were no differences across institutions in the distribution of mutagen sensitivity (Kruskal–Wallis test) for both case and control subjects. The b/c values for case subjects were consistently and significantly (P < .0001) higher than those for control subjects in the overall analysis.

For control subjects, no correlations were found in the combined data between b/c values and pack-years, daily tobacco consumption, unit years, daily alcohol intake, or age. For the HNSCC patient group, however, a very small correlation (r = .18; P = .01) was found between b/c values and age. The mean values for the patients and control subjects differed significantly (Mann–Whitney U test) with regard to age, pack-years, daily alcohol intake, and mutagen sensitivity. Although the absolute difference between the average b/c levels of patients and control subjects was not large, our analysis clearly shows a difference in the frequency distribution of b/c values between the two populations (Fig. 1). We considered several models using b/c as a continuous variable or dichotomized at a chosen threshold. It is curious that, in every model, the variant dichotomized at 1.0 turned out to be the best predictor. Therefore, in the text that follows, we will consider only two groups: subjects with a b/c
sensitivity was measured as the mean number of bleomycin-induced chromatid breaks per cell in cultured lymphocytes of the subjects.

Multiple linear regression was performed to estimate the joint influence of age, tobacco use, and alcohol consumption on the mutagen-sensitivity value. We also performed a stratified analysis to assess the interaction between mutagen sensitivity and several smoking and alcohol categories (Table 2). We found an interaction between mutagen sensitivity and heavy smoking or alcohol consumption. This pattern appeared to persist in the site-specific analyses; however, the numbers of patients in several groups were too small for a precise estimation of risks. We also noted a strong increase in risk for those persons who were mutagen sensitive and who were also exposed to both tobacco and alcohol. However, there was only one nonsmoking patient who drank more than 2 U of alcohol per day. Thus, we could not evaluate the influence of drinking in nonsmoking subjects.

Unconditional multiple logistic regression analysis confirmed the results that were obtained by use of the crude ORs (Fig. 2). The hypersensitivity phenotype without tobacco exposure was associated with an elevated (although not statistically significant) risk (OR = 2.6). Mutagen hypersensitivity, however, had a large and statistically significant (P for trend <.01) impact on cancer risk for those subjects exposed to tobacco in a dose-dependent relation. An important observation was that addition of "hospital" as a variable did not change the outcome, indicating that the study site did not influence the analyses (data not shown).

Discussion

Intrinsic susceptibility and exposure to carcinogens can act in concert to modulate cancer risk (2,6,12). At the extreme end of the susceptibility spectrum are patients with ataxia telangiectasia (AT) who have a genetic predisposition to develop cancer, because they possess an elevated chromosomal instability. The ATM gene has recently been cloned (13), and the protein that it encodes is involved in the DNA damage surveillance network (14). Cells from heterozygous carriers of the ATM gene have been shown to be very sensitive to in vitro radiation in the G2 phase of the cell cycle (15,16), and these individuals are thought to be at increased risk of developing breast cancer after they are exposed to low amounts of radiation (17). On the basis of gene frequency, it can be calculated that about one in 100 persons has the AT-heterozygous genotype (18). This calculation would imply that these individuals are more susceptible to cancer because they have a latent chromosomal instability that becomes apparent only after exposure to DNA-damaging compounds.

There is a growing body of evidence that sensitivity to G2 phase damage is also predictive of the risk of developing common cancers that were previously thought to be due mainly to exposure to carcinogens. It has been reported that DNA breaks

![Fig. 1. Frequency distribution in which the case subjects and control subjects were subdivided according to several mutagen-sensitivity classes. Mutagen sensitivity was measured as the mean number of bleomycin-induced chromatid breaks per cell in cultured lymphocytes of the subjects.](https://academic.oup.com/jnci/article-abstract/88/8/530/915120/fig1)
induced in G2, such as those induced by radiation (16,19,20) or bleomycin (8,11), are of particular importance. Since radiation and bleomycin produce similar types of damage, the two assays using these damage inducers may be expected to give similar results. It is an interesting concept that a latent instability (mutator) phenotype is linked to an intrinsic cancer susceptibility.

An approach used to detect this type of cancer susceptibility is the mutagen-sensitivity assay. In this assay, chromosomal instability is measured in peripheral blood lymphocytes after damage is inflicted in vitro with bleomycin in the S-G2 phase of the cell cycle. A hypersensitivity phenotype has been found to be a risk factor for such common cancers as HNSCC, lung cancer, and colon cancer (11).

To investigate cancer susceptibility, a multidisciplinary approach is necessary (21). Classical epidemiology using only environmental exposure data is no longer adequate to assess cancer risk and requires the incorporation of specific biomarkers that have to be measured in the laboratory (22). For the analysis of all these variables, large numbers of subjects are required. In our study, the grouping of data from three different institutions resulted in a unique population of subjects for whom mutagen sensitivity was assessed.

Analysis of the combined data confirmed a significant difference in the b/c values between patients and control subjects, as previously reported (10,11). Age, cigarette pack-years, or daily alcohol intake did not influence the b/c score. There is a notable degree of concordance of the mutagen-sensitivity data across the three combined studies.

The most important finding of our study is that assessment of mutagen sensitivity greatly improved the risk estimation when exposure characteristics were also considered. In smokers, mutagen hypersensitivity increased cancer risk in a dose-dependent and interactive pattern. The unconditional multiple logistic regression analysis shows that, at a low mutagen-sensitivity level, exposure to carcinogens has less impact on cancer risk. This phenomenon was also indicated in a study in which the mutagen sensitivity was measured in elderly men who never developed a tumor, but who had been heavily exposed to both alcohol and tobacco (23). It is interesting that 98% of the subjects in this latter population had a nonsensitive phenotype. The low impact of mutagen sensitivity on subjects who were not consumers of alcohol and tobacco indicates that endogenous DNA damage may not play a large role in carcinogenesis for these persons.

Other investigators (1,24) have described the potentiating effect of alcohol on the effects of tobacco smoking that we found.

Table 2. Risk estimates for mutagen sensitivity by tobacco and alcohol exposure

<table>
<thead>
<tr>
<th>Stratification</th>
<th>No. of subjects</th>
<th>Crude odds ratios (95% confidence intervals)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>All</td>
</tr>
<tr>
<td>Tobacco use only†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smoked</td>
<td>104</td>
<td>1</td>
</tr>
<tr>
<td>&lt;25 pack-years</td>
<td>167</td>
<td>2.7 (1.4-4.9)</td>
</tr>
<tr>
<td>≥25 pack-years</td>
<td>192</td>
<td>13.1 (7.1-24.0)</td>
</tr>
<tr>
<td>Alcohol consumption only‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-2 U/day</td>
<td>271</td>
<td>1</td>
</tr>
<tr>
<td>2.5-5 U/day</td>
<td>105</td>
<td>1.8 (1.2-2.9)</td>
</tr>
<tr>
<td>≥5 U/day</td>
<td>70</td>
<td>7.2 (3.8-13.6)</td>
</tr>
<tr>
<td>Tobacco use and alcohol consumption</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smoked, &lt;2 U of alcohol/day</td>
<td>70</td>
<td>1</td>
</tr>
<tr>
<td>Never smoked, ≥2 U of alcohol/day</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Smoker, &lt;2 U of alcohol/day</td>
<td>168</td>
<td>4.6 (2.1-9.9)</td>
</tr>
<tr>
<td>Smoker, ≥2 U of alcohol/day</td>
<td>150</td>
<td>14.2 (6.5-31.0)</td>
</tr>
</tbody>
</table>

* b/c = number of breaks per cell.
† Pack-years have been calculated on the basis of the European pack size of 25 cigarettes per pack, and each of the values of pack-years would be 20% greater if converted to the number of cigarettes per pack in the United States (i.e., 20).
‡ Units of alcohol per day. One unit contains approximately 15 mL of ethanol.
§ The number of subjects in the group of never smokers who drank more than 2 U of alcohol per day was too low for the accurate estimation of odds ratios.

Fig. 2. Impact of mutagen sensitivity on cancer risk as determined by use of unconditional multiple logistic regression. The 95% confidence intervals (CIs) of the risk estimates for nonsensitive smokers were 0.95-5.32 for moderate smokers and 3.0-26.7 for heavy smokers. A mutagen-hypersensitive phenotype (number of breaks per cell ≥21) in never smokers elevated the risk to 2.64, though not to a significant degree (95% CI = 0.91-7.62). For this hypersensitive subject group, 95% CIs were 3.9-24.2 and 17.3-114.0 for moderate and heavy smokers, respectively.
in this population. This finding can be explained by the assumption that alcohol, although not a carcinogen, can temporarily inhibit DNA repair capacity (25).

This multi-institutional and interdisciplinary study emphasizes the importance of intrinsic susceptibility in estimating carcinogenic risk. Mutagen sensitivity is a model system that measures response to genotoxic assaults, a phenotype that may reflect susceptibility to cancer, especially in exposed individuals. This study also emphasizes the concept that genetic predisposition may play an important role in the development of HNSCC (26). One important implication of this mutagen-sensitivity assay for HNSCC patients is that this assay may serve to predict which patients have the highest risk of developing second primary tumors (27,28) and who can be targeted for more intense follow-up (29) and behavioral interventions and chemoprevention studies (30). In addition to the high number of chromatid breaks observed when cells from genetically susceptible individuals are tested with the assay, it was recently found that the induced chromatid breaks are not random but occur at specific, targeted sites (31,32). Functional studies of plasmid reconstruction in lymphoblastoid cell lines have indicated that repair fidelity may be impaired in mutagen-hypersensitive persons (33), as has been described for AT patients (34).

We encourage other investigators in this field of research to include the mutagen-sensitivity assay in their studies of susceptibility markers. Although controversy exists with regard to whether the scoring of chromatid breaks may be subject to interobserver variation (35), the assay can be performed easily in all types of laboratories when standardized scoring procedures are used. Moreover, we emphasize the importance of including any relevant exposure factors in the risk estimations of intrinsic susceptibility markers for cancer. Our study underscores the profound importance of mutagen sensitivity in the determination of an individual's susceptibility to develop cancer, particularly those cancers such as HNSCC and lung cancer, whose etiology can be strongly influenced by environmental genotoxic exposures.

References

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