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Chromosome Instability and Risk of Squamous Cell Carcinomas of Head and Neck

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Abstract

In 895 subjects with squamous cell carcinoma of the head and neck (SCCHN) and 898 cancer-free controls matched by age, sex, and ethnicity, we validated our previous finding that mutagen sensitivity as measured by the frequency of chromatid breaks in vitro induced by benzo[*a*]pyrene diol epoxide (BPDE) is an independent risk factor for SCCHN. Using a previously established concentration of 4 μ M BPDE to treat short-term cultured primary lymphocytes for 5 hours, we evaluated chromatid breaks in 50 well-spread metaphases for each blood sample. The mean frequency of BPDE-induced chromatid breaks was significantly higher in cases than in controls in non-Hispanic whites ($P = 0.0003$) but not in other ethnic groups ($P = 0.549$ for Hispanic Americans and 0.257 for African Americans). The odds ratio associated with risk of SCCHN for the frequency of chromatid breaks greater than median value of controls was 1.56 (95% confidence interval, 1.27–1.91) in non-Hispanic whites (767 cases and 763 controls) after adjustment for age, sex, smoking status, and drinking status. When the quartiles of the controls were used as the cutoff values, there was a dose response between the degree of mutagen sensitivity and risk of SCCHN in non-Hispanic whites ($P_{\text{trend}} = 0.0001$). However, none of these associations in non-Hispanic whites was identified in Hispanic Americans (69 cases and 70 controls) or African Americans (59 cases and 65 controls), possibly because of the small samples of these ethnic groups or ethnic difference in genetic variation, which needs to be confirmed in future studies.

Keywords

mutagen sensitivity; genetic susceptibility; molecular epidemiology; chromosome aberration; head and neck cancer

Introduction

Squamous cell carcinoma of the head and neck (SCCHN), which includes cancers of the oral cavity, pharynx, and larynx, accounts for 3% of all newly diagnosed cancers in the United States. Incidence rates and death rates of SCCHN have been decreasing since 1975, and it is estimated that 45,660 new cases of SCCHN will be diagnosed and 11,210 related deaths will occur in 2007 in the US (1). Tobacco use and alcohol consumption are well-established risk

factors for SCCHN (2), but only a fraction of smokers develop SCCHN, suggesting that genetic susceptibility factors contribute to the etiology of the disease. Therefore, establishing biomarkers for genetic susceptibility is important for identifying at-risk individuals in the general population who can be targeted for primary prevention and early detection of SCCHN.

Tobacco smoke contains numerous chemicals that are potentially carcinogenic. One of these tobacco-related chemicals is benzo[*a*]pyrene, a classic DNA-damaging carcinogen that is metabolized by phase I enzymes to an ultimate carcinogen, benzo[*a*]pyrene-7,8-diol 9,10-epoxide (BPDE), which can induce DNA damage, including both formation of DNA adducts through covalent binding or oxidation (3) and chromosomal aberrations through yet-to-be identified mechanisms (4), if not effectively repaired. The rare, recessive chromosomal instability syndromes, such as xeroderma pigmentosum, provide some clues for hypersensitivity to chemical-induced chromosomal aberrations, possibility due to various defects in DNA-repair mechanisms of the host cells, leading to high cancer risk (5). Suboptimal DNA-repair capacity and high levels of induced DNA adducts and chromosomal aberrations have been associated with enhanced susceptibility to several tobacco-related cancers, such as lung cancer (4,6–8) and SCCHN (9–11).

The mutagen sensitivity assay was developed by Dr. T. C. Hsu (12) to score the number of bleomycin-induced chromatid breaks per cell in cultured primary peripheral blood lymphocytes and has been shown to provide a useful biomarker for susceptibility to different types of cancer, including those of the thyroid, upper aerodigestive tract, lung, colon, and head and neck (12–17). In a pilot study of SCCHN (9), we used a modified assay with BPDE as the test mutagen, an ultimate tobacco metabolite etiologically related to SCCHN. We found that 60 patients newly diagnosed with SCCHN had a statistically significantly higher mean frequency of BPDE-induced chromatid breaks than 112 healthy cancer-free controls. In another case-control study of 71 subjects with oral premalignant lesions and 71 normal controls, high BPDE sensitivity was associated with a significantly elevated risk of oral premalignant lesions (18), a well-known risk factor for SCCHN (19,20).

The two above-mentioned studies both used peripheral blood lymphocytes as the surrogate tissue to represent the target tissues, i.e., oral epithelium. Such a representation had been questioned for some time until it was partially addressed by one published study that was designed specifically to address this issue (21). In that study, Cloos and co-workers compared bleomycin-induced damage in lymphocytes with that in primary oral fibroblasts and keratinocytes in 30 subjects, and found a correlation between the percentages of aberrant metaphases in peripheral blood lymphocytes and oral fibroblasts but not in keratinocytes. It is suggested that keratinocytes can be influenced by environmental factors and thus are limited in their application to the identification of genetic factors underlying induced metaphase aberrations (21). Although oral fibroblasts may be suitable for analyzing induced chromosomal instability such as mutagen sensitivity, peripheral blood lymphocytes are more readily available and amenable for large-scale association studies.

Here we report a large hospital-based case-control study of SCCHN, the largest study of its kind involving use of cultured peripheral blood lymphocytes, in which we further validated the findings of our pilot study by evaluating BPDE-induced chromosomal instability *in vitro* exposed lymphocytes of 895 SCCHN cases and 898 healthy and cancer-free controls.

Materials and Methods

Study Population

All patients included in this study were recruited from those registered in the Department of Head and Neck Surgery at The University of Texas M. D. Anderson Cancer Center between January 2000 and June 2006. They had newly diagnosed, histopathologically confirmed but untreated SCCHN. Of the eligible patients approached, ~95% agreed to participate in this study. The control subjects were recruited from among healthy visitors to the outpatient clinics at M. D. Anderson Cancer Center, who were accompanying cancer patients and were genetically unrelated to any of the case subjects in this study or to each other. Approximately 90% of eligible control subjects who were asked participated in the study. Control subjects were frequency-matched to the cases by age (± 5 years), sex, and ethnicity. After informed consent was obtained, each subject completed a questionnaire and donated a blood sample for the *in vitro* BPDE-induced mutagen sensitivity assay. The study protocol was approved by M. D. Anderson's institutional review board.

Mutagen Sensitivity Assay

BPDE-induced mutagen sensitivity assays were performed as described previously and evaluated as chromatid breaks per cell (9). Briefly, short-term cultures of 1 mL of fresh whole blood were established in 9 mL of RPMI 1640 medium supplemented with 20% fetal bovine serum at a final concentration of 112.5 $\mu\text{g/mL}$ phytohemagglutinin (Remel, Lenexa, KS) to stimulate T-lymphocyte growth in T-25 flasks. After ~67 h of culture at temperature 37°C with 5% CO₂, the cells were treated with BPDE ($\geq 98\%$ purity; NCI Chemical Carcinogen Repository, Midwest Research Institute, Kansas City, MO) at a final concentration of 4 μM and were allowed to grow for another 5 hours. The BPDE was dissolved in tetrahydrofuran (Sigma Chemical Co., St. Louis, MO) to create a 1 mM stock solution.

Mitotic arrest was induced with colcemid (Gibco BRL, Carlsbad, CA) at 0.06 $\mu\text{g/mL}$ 1 h before harvesting. We used conventional chromosome harvesting procedures: the cells were treated for 15 min with 60 mM hypotonic KCl solution and fixed three times for 5 min each with freshly prepared methanol:acetic acid solution (3:1 v/v), after which air-dried slides were prepared as previously described (12). The slides were then stained with 4% Giemsa (Biomedical Specialties, Santa Monica, CA) for 7 min. The number of chromatid breaks was scored from 50 well-spread metaphases per blood sample and averaged to the number of breaks per cell.

Statistical Analysis

The χ^2 test was used to compare differences in the distributions for categorical variables. Subjects who had smoked >100 cigarettes in their lifetime were defined as ever-smokers; those who had smoked ≤ 100 cigarettes in their lifetime were considered never-smokers. Among smokers, those who had quit smoking more than one year before enrollment in the study were considered former smokers, and the rest of smokers were considered current smokers. Those who had consumed alcoholic beverages at least once a week for more than one year were defined as ever users of alcohol, and the others were considered never users of alcohol. Among ever users of alcohol, those who had quit drinking alcoholic beverages more than one year before enrollment in the study were considered former users, and the rest were considered current users. The numbers of BPDE-induced chromatid breaks per cell were analyzed as a continuous variable. Student's *t* test and analysis of variance (ANOVA) were used to compare the differences between cases and controls or within subgroups for the continuous variables. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by unconditional logistic regression analysis with and without adjustment for other

covariates. Both median and quartile levels of BPDE-induced chromatid breaks per cell in the control subjects were used as cutoff values. Subjects with the values of BPDE-induced chromatid breaks per cell greater than the median were considered to be mutagen sensitive. We also performed an effect modification analysis with the unconditional logistic regression model and the additive model. Empirically, a more-than-additive interaction was indicated if $OR_{11} > OR_{10} + OR_{01} - 1$. When the test for multiplicative interaction was not rejected, further tests for additive interaction were performed by a bootstrapping test of goodness of fit of the null hypothesis of an additive model with no interaction against an alternative hypothesis that allows an additive interaction. To perform the hypothesis test for additive models, we implemented bootstrapping using STATA 8.2. All statistical analyses were two-sided and performed using SAS software (version 9.1; SAS Institute, Cary, NC) except for the additive models (StataCorp., College Station, TX).

Results

Included in this analysis were 895 SCCHN patients and 898 healthy controls who were matched by age (± 5 years), sex, and ethnicity. Table 1 summarizes frequency distributions of select variables between cases and controls. The age range and the median age were 18–90 and 56 years for the cases, respectively, and 20–85 years and 55 years for the controls, respectively. Among the 898 controls, the frequency distribution of chromatid breaks per cell was approximately normal; only two observations (outliers) skewed to the high values. There was a slight but significant trend of decreasing frequency of chromatid breaks per cell as age increased for controls (data not shown). Women tended to have slightly higher frequencies of chromatid breaks per cell than men regardless of ethnicity, smoking status, or drinking status in these controls (data not shown).

Because the distributions of smoking status, drinking status, and chromatid breaks by clinical category in the three ethnic groups were similar, we present these data combined together in Table 1. As shown in Table 1, there were more ever-smokers (73.3%) and ever users of alcohol (73.7%) among SCCHN patients than among controls (50.9% smokers and 55.7% alcohol drinkers); the differences between cases and controls were statistically significant in smoking status (never, former, and current) and drinking status (never, former, and current) ($P < 0.001$ for both). The risk of SCCHN were significantly associated with both smoking and drinking status with a trend between smoking status and risk of SCCHN (adjusted OR=1.39 [95% CI=1.09–1.78] for former smokers, and 3.97 [95% CI=3.08–5.13] for current smokers compared with never smokers, while compared with never users of alcohol, the risk of SCCHN was two-fold higher among former users or current users (Table 1); however, family history with any kind of cancers, was not associated with risk of SCCHN, suggesting a possible overmatching or selection bias that may have occurred, because the controls were selected from the visitors to a cancer hospital even though the controls were not genetically related to the cases.

Among the 895 patients, 26.4% had squamous cell carcinoma of the oral cavity, 50.3% of the oropharynx, 4.9% of the hypopharynx, and 18.4% of the larynx. There were 59.5% of patients with overall tumor stage IV, 17.9% with tumor stage III, 13.0% with tumor stage II, and 9.6% with tumor stage I. Consistent with our previous report in the pilot study, there were no significant differences in BPDE-induced chromatid breaks among the subgroups of tumor characteristics, including anatomic site ($P = 0.729$) and tumor stage (tumor [T], lymph node [N] and overall: $P = 0.796$, 0.298 , and 0.595 , respectively), suggesting that sensitivity to BPDE-induced chromatid breaks is unlikely to be a tumor marker (data not shown).

Overall, there was a statistically significant difference in BPDE-induced chromatid breaks per cell between the 895 cases (mean and standard deviation [SD], 0.50 ± 0.19) and the 898 controls (0.46 ± 0.18 ; $P = 0.0002$), but the differences in the mean values among the controls of the ethnic groups varied (0.46 ± 0.17 for 763 non-Hispanic whites, 0.49 ± 0.19 for 70 Hispanic Americans, 0.50 ± 0.23 for 65 African Americans), and the differences were statistically significant (ANOVA test: $P = 0.037$) (Table 2). Therefore, we performed the following analyses separately by each ethnic group.

After stratification by select variables listed in Table 1, we found significant differences in frequencies of BPDE-induced chromatid breaks between cases and controls for all subgroups of non-Hispanic whites except females, never smokers, and never users of alcohol (Table 2). For both Hispanic Americans and African Americans, we found no statistically significant differences between cases and controls overall, or in most of the strata listed in Table 2, because of their small sample sizes and relatively large variations in mutagen sensitivity; however, there appeared to be some noticeable differences, particularly by sex and family history of cancer (Table 2). There were no differences by age or smoking status either in cases and controls overall or by ethnic groups. For example, subjects aged 55 years or younger tended to have higher frequency of BPDE-induced chromatid breaks than those aged older than 55 in both cases and controls of non-Hispanic whites and Hispanic Americans but not African Americans, but none of these differences were statistically significant (Table 2). In non-Hispanic white controls, never users of alcohol were more sensitive to BPDE, with a higher frequency of BPDE-induced chromatid breaks (0.48 ± 0.19 for 311 never users of alcohol) than ever users of alcohol (0.42 ± 0.16 for 133 former users of alcohol and 0.45 ± 0.15 for 319 current users of alcohol), and the difference was statistically significant (ANOVA test: $P = 0.011$). We could not identify similar patterns among cases nor in other ethnic groups, partly because of their small sample sizes, but there appeared to be a nonsignificant trend for alcohol effects in the African-American controls (0.56 ± 0.26 for 39 never users of alcohol, 0.48 ± 0.14 for 9 former users of alcohol, and 0.39 ± 0.15 for 17 current users of alcohol) (Table 2). Detailed smoking and alcohol information did not show significant relations between smoking pack-year or alcohol amount with frequency of BPDE-induced chromatid breaks (data not shown).

We performed univariate and multivariate logistic regression analyses to estimate associations between mutagen sensitivity and risk of SCCHN. When BPDE-induced chromatid breaks were fitted into the logistic regression model as a continuous variable, the OR for risk associated with each 0.1 increment of chromatid breaks per cell was 1.12 (95% CI, 1.05–1.18) for non-Hispanic whites. When we used the median value of BPDE-induced chromatid breaks in controls as the cutoff point, those cases with chromatid breaks above the median (0.43 breaks/cell) were at 1.5-fold increased risk of SCCHN (OR, 1.54; 95% CI, 1.26–1.88) than those who had a frequency of chromatid breaks lower than the median, and this risk was essentially not altered after adjustment for the potential covariates in the regression model (OR, 1.56; 95% CI, 1.27–1.91; $P < 0.0001$) (Table 3). After the data were divided by quartiles of the control values, the ORs for risk of SCCHN increased as the frequency of chromatid breaks per cell values increased (0.98 [95% CI, 0.72–1.33] for the second quartile, 1.49 [1.12–1.98] for the third quartile, and 1.59 [1.20–2.11] for the fourth quartile; trend test: $P = 0.0001$, compared with the lowest quartile) with adjustment for the covariates (Table 3). This significant association was not observed in other ethnic groups, but the finding needs to be verified in future studies with large samples of these minority groups.

Finally, we evaluated possible joint-effect modification or interaction between select risk factors and BPDE-induced chromatid breaks on risk of SCCHN in non-Hispanic whites only (Table 4). Although the results of the trend tests were statistically significant for all

combinations of these variables ($P < 0.0001$), we did not find any statistical evidence of multiplicative interactions between these covariates and chromatid breaks. However, on an additive scale, age and alcohol interacted significantly with BPDE-induced chromatid breaks on risk of SCCHN ($P = 0.032$ and 0.044 , respectively) (Table 4).

Discussion

In this expanded study, we confirmed a previously reported association between *in vitro* BPDE-induced mutagen sensitivity and the risk of SCCHN (9). Since Dr. T. C. Hsu developed the mutagen sensitivity assay with bleomycin as an effective inducer of chromosomal aberrations *in vitro*, this assay has been widely used in epidemiologic studies to assess genetic susceptibility to various types of cancer. The assay has been modified with a variety of etiologically related challenge mutagens to evaluate cancer susceptibility in association studies, such as BPDE for tobacco-related cancers of the lung (4,6–8) and SCCHN (9–11), gamma-radiation for glioma (22,23), and ultraviolet light or 4-nitroquinoline-1-oxide for skin cancer (24,25). These studies have consistently showed that *in vitro* induced mutagen sensitivity phenotype is a risk factor for developing cancers.

Because the above-mentioned studies used a case-control study design, it is questionable whether mutagen sensitivity is a tumor marker or a marker of risk (or genetic susceptibility). In the present study, the frequency of BPDE-induced chromatid breaks was not associated with tumor/clinical factors (tumor stage and cancer site) in the 895 cases; being a risk factor of cancer, mutagen sensitivity could be determined genetically. Indeed, it has been shown that mutagen sensitivity may be a heritable component (21,26). In a twin study of 460 individuals, for example, the contribution of non-shared environment to the mutagen sensitivity phenotype was estimated to be approximately 31.8% to 39.7% (26).

It is well known that both genetic and environmental factors play roles in the development of cancers. In an earlier study, Cloos et al. found that alcohol consumption increased the mutagen sensitivity of oral keratinocytes as measured by bleomycin-induced chromatid breaks (21). In primary lymphocytes used in the present study, we did not observe any evidence of interaction of the mutagen sensitivity with smoking but borderline with drinking status. The present large study did confirm our finding in an earlier pilot study of 60 SCCHN patients and 112 controls that BPDE-induced mutagen sensitivity is an independent risk factor for SCCHN. This is consistent with the result reported by Cloos et al. that bleomycin-induced mutagen sensitivity was a genetic susceptibility biomarker for risk of SCCHN in an early study of 313 patients and 334 controls (15,27), in which neither age nor tobacco or alcohol use was found to affect the mutagen sensitivity values in either controls or cases, although SCCHN patients with multiple primary tumors appeared to be more sensitive than those who had a single primary tumor (27,28).

It is hypothesized that the *in vitro* mutagen sensitivity phenotype may be determined by host cells' DNA-repair capacity and related genetic factors (29), because spontaneous chromosomal aberrations and hypersensitivity to chemical-induced chromosomal aberrations are often observed in some rare, recessive chromosomal instability or breakage syndromes (30–32), including Fanconi's anemia, Bloom's syndrome, ataxia telangiectasia, Nijmegen breakage syndrome, Werner syndrome, Rothmund-Thompson syndrome, and xeroderma pigmentosum (5). These syndromes are characteristic of inherited DNA-repair defects in different pathways, carrying a high risk of cancer; however, few reported population-based studies have provided a direct support for this hypothesis. In a recent pilot study of the correlation between *in vitro* BPDE-induced DNA damage and repair (measured by the comet assay) and chromosomal aberrations in primary lymphocytes of 136 cancer-free controls, we found that there was a weak correlation ($r = 0.163$; $P = 0.009$) between

measurements of Olive tail moment (single DNA strand breaks) and frequencies of chromatid breaks per cell (i.e., mutagen sensitivity) (25).

There were few minorities recruited in this study, and the observed non-significant difference in BPDE-induced chromatid breaks between cases and controls of minority groups may be due to low study power (the post-hoc power for Hispanic Americans was 8.7% and 22.3% for American Americans). In addition, there may be some ethnic difference in allele frequencies of many genes that are involved in repair of chromosomal damage, including those involved in NER and BER pathways, as reported in the HapMap (<http://www.hapmap.org/>).

In a large population-based case-control study of breast cancer with 2311 cases (894 African Americans, 1417 whites) and 2022 controls (788 African Americans, 1234 whites) in North Carolina, specific combinations of NER genotypes, including *XPD* 312 Asp/Asn, *XPD* 751 Lys/Gln, *XPG* 1104 Asp/His, *XPC* 939 Lys/Gln, *RAD23B* 249 Ala/Val, and *ERCC6* 1213 Arg/Gly, modified the association of breast cancer and smoking among African American but not white women (33). It has been also reported that polymorphisms in the NER pathway modulated mutagen sensitivity (34). Therefore, ethnic difference in associations between chromosome instability and risk of SCCHN in our study may be due to genetic variation among ethnic groups in addition to the small sample sizes for minorities. However, this ethnic difference needs to be validated in additional studies.

The present study has several limitations. The overmatching on family history of cancer is a potential limitation of our hospital-based case-control study, particularly in a cancer hospital setting. This could bias the results towards the null, if the controls carried some unknown genetic factors that were associated with mutagen sensitivity and risk of cancer. It is obvious that the mutagen sensitivity measured in surrogate tissue (blood lymphocytes) may not truly represent that of the target tissue cells; however, it has been shown that the mutagen sensitivity measured in blood lymphocytes may be close to that of fibroblasts in the target tissues (21). Furthermore, the molecular mechanism that underlies the formation of chromatid breaks induced by BPDE *in vitro* remains unclear.

In conclusion, this large hospital-based study has confirmed the findings of our previously published pilot study that BPDE-induced chromatid breaks are a genetic susceptibility marker for risk of developing SCCHN. The molecular mechanisms underlying the formation of BPDE-induced chromatid breaks as well as the correlation between induced chromatid breaks in surrogate and target tissues warrant further investigations.

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References

1. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. *CA Cancer J Clin.* 2007; 57:43–66. [PubMed: 17237035]
2. Blot WJ, McLaughlin JK, Winn DM, et al. Smoking and drinking in relation to oral and pharyngeal cancer. *Cancer Res.* 1988; 48:3282–7. [PubMed: 3365707]
3. Gelboin HV. Benzo[alpha]pyrene metabolism, activation and carcinogenesis: role and regulation of mixed-function oxidases and related enzymes. *Physiol Rev.* 1980; 60:1107–66. [PubMed: 7001511]

4. Wei Q, Gu J, Cheng L, et al. Benzo(a)pyrene diol epoxide-induced chromosomal aberrations and risk of lung cancer. *Cancer Res.* 1996; 56:3975–9. [PubMed: 8752166]
5. de Boer J, Hoeijmakers JH. Nucleotide excision repair and human syndromes. *Carcinogenesis.* 2000; 21:453–60. [PubMed: 10688865]
6. Li D, Wang M, Cheng L, Spitz MR, Hittelman WN, Wei Q. In vitro induction of benzo(a)pyrene diol epoxide-DNA adducts in peripheral lymphocytes as a susceptibility marker for human lung cancer. *Cancer Res.* 1996; 56:3638–41. [PubMed: 8705998]
7. Wei Q, Cheng L, Amos CI, et al. Repair of tobacco carcinogen-induced DNA adducts and lung cancer risk: a molecular epidemiologic study. *J Natl Cancer Inst.* 2000; 92:1764–72. [PubMed: 11058619]
8. Li D, Firozi PF, Wang LE, et al. Sensitivity to DNA damage induced by benzo(a)pyrene diol epoxide and risk of lung cancer: a case-control analysis. *Cancer Res.* 2001; 61:1445–50. [PubMed: 11245449]
9. Wang LE, Sturgis EM, Eicher SA, Spitz MR, Hong WK, Wei Q. Mutagen sensitivity to benzo(a)pyrene diol epoxide and the risk of squamous cell carcinoma of the head and neck. *Clin Cancer Res.* 1998; 4:1773–8. [PubMed: 9676854]
10. Li D, Firozi PF, Chang P, et al. In vitro BPDE-induced DNA adducts in peripheral lymphocytes as a risk factor for squamous cell carcinoma of the head and neck. *Int J Cancer.* 2001; 93:436–40. [PubMed: 11433411]
11. Li D, Wang LE, Chang P, El-Naggar AK, Sturgis EM, Wei Q. In vitro benzo[a]pyrene diol epoxide-induced DNA adducts and risk of squamous cell carcinoma of head and neck. *Cancer Res.* 2007; 67:5628–34. [PubMed: 17575128]
12. Cherry LM, Hsu TC. Bleomycin-induced chromosome damage in lymphocytes of medullary thyroid carcinoma patients and their family members. *Anticancer Res.* 1983; 3:367–72. [PubMed: 6200055]
13. Spitz MR, Fueger JJ, Beddingfield NA, et al. Chromosome sensitivity to bleomycin-induced mutagenesis, an independent risk factor for upper aerodigestive tract cancers. *Cancer Res.* 1989; 49:4626–8. [PubMed: 2472882]
14. Spitz MR, Hsu TC, Wu X, Fueger JJ, Amos CI, Roth JA. Mutagen sensitivity as a biological marker of lung cancer risk in African Americans. *Cancer Epidemiol Biomarkers Prev.* 1995; 4:99–103. [PubMed: 7537995]
15. Cloos J, Spitz MR, Schantz SP, et al. Genetic susceptibility to head and neck squamous cell carcinoma. *J Natl Cancer Inst.* 1996; 88:530–5. [PubMed: 8606381]
16. Fireman Z, Shabtai F, Lurie B. Chromosome sensitivity to bleomycin-induced mutagenesis in lymphocytes from colorectal cancer patients under 40 years of age. *Dis Colon Rectum.* 1994; 37:1317–20. [PubMed: 7527751]
17. Ankathil R, Jyothish B, Madhavan J, Nair MK. Deficient DNA repair capacity: a predisposing factor and high risk predictive marker in familial colorectal cancer. *J Exp Clin Cancer Res.* 1999; 18:33–7. [PubMed: 10374674]
18. Wu X, Lippman SM, Lee JJ, et al. Chromosome instability in lymphocytes: a potential indicator of predisposition to oral premalignant lesions. *Cancer Res.* 2002; 62:2813–8. [PubMed: 12019158]
19. Vokes EE, Weichselbaum RR, Lippman SM, Hong WK. Head and neck cancer. *N Engl J Med.* 1993; 328:184–94. [PubMed: 8417385]
20. Lippman SM, Benner SE, Hong WK. Cancer chemoprevention. *J Clin Oncol.* 1994; 12:851–73. [PubMed: 8151328]
21. Cloos J, Reid CB, van der Sterre ML, et al. A comparison of bleomycin-induced damage in lymphocytes and primary oral fibroblasts and keratinocytes in 30 subjects. *Mutagenesis.* 1999; 14:87–93. [PubMed: 10474828]
22. Bondy ML, Wang LE, El-Zein R, et al. Gamma-radiation sensitivity and risk of glioma. *J Natl Cancer Inst.* 2001; 93:1553–7. [PubMed: 11604478]
23. Bondy ML, Kyritsis AP, Gu J, et al. Mutagen sensitivity and risk of gliomas: a case-control analysis. *Cancer Res.* 1996; 56:1484–6. [PubMed: 8603389]
24. Wang LE, Xiong P, Strom SS, et al. In vitro sensitivity to ultraviolet B light and skin cancer risk: a case-control analysis. *J Natl Cancer Inst.* 2005; 97:1822–31. [PubMed: 16368944]

25. Wang LE, Hsu TC, Xiong P, et al. 4-Nitroquinoline-1-oxide-induced mutagen sensitivity and risk of nonmelanoma skin cancer: a case-control analysis. *J Invest Dermatol.* 2007; 127:196–205. [PubMed: 16841033]
26. Wu X, Spitz MR, Amos CI, et al. Mutagen sensitivity has high heritability: evidence from a twin study. *Cancer Res.* 2006; 66:5993–6. [PubMed: 16778168]
27. Cloos J, Braakhuis BJ, Steen I, et al. Increased mutagen sensitivity in head-and-neck squamous-cell carcinoma patients, particularly those with multiple primary tumors. *Int J Cancer.* 1994; 56:816–9. [PubMed: 7509776]
28. Cloos J, Snow GB, Braakhuis BJ. Determination of genetic susceptibility for development of squamous epithelial carcinomas of the head and neck with bleomycin-induced chromosome instability. *Laryngorhinootologie.* 1995; 74:742–7. [PubMed: 8579674]
29. Wu X, Gu J, Spitz MR. Mutagen sensitivity: a genetic predisposition factor for cancer. *Cancer Res.* 2007; 67:3493–5. [PubMed: 17440053]
30. Schroeder TM. Genetically determined chromosome instability syndromes. *Cytogenet Cell Genet.* 1982; 33:119–32. [PubMed: 6749441]
31. Taylor AM. Chromosome instability syndromes. *Best Pract Res Clin Haematol.* 2001; 14:631–44. [PubMed: 11640873]
32. Duker NJ. Chromosome breakage syndromes and cancer. *Am J Med Genet.* 2002; 115:125–9. [PubMed: 12407692]
33. Mechanic LE, Millikan RC, Player J, et al. Polymorphisms in nucleotide excision repair genes, smoking and breast cancer in African Americans and whites: a population-based case-control study. *Carcinogenesis.* 2006; 27:1377–85. [PubMed: 16399771]
34. Lin J, Swan GE, Shields PG, et al. Mutagen sensitivity and genetic variants in nucleotide excision repair pathway: genotype-phenotype correlation. *Cancer Epidemiol Biomarkers Prev.* 2007; 16:2065–71. [PubMed: 17932354]

Table 1
Frequency distributions of select variables in patients with SCCHN and cancer-free controls

Variables	Cases (n = 895)		Controls (n = 898)		P*	OR (95% CI)†
	n	(%)	n	(%)		
Age (years)					0.493	
≤ 55	439	(49.1)	455	(50.7)		
> 55	456	(50.9)	443	(49.3)		
Sex					0.231	
Male	674	(75.3)	654	(72.8)		
Female	221	(24.7)	244	(27.2)		
Ethnicity					0.859	
Non-Hispanic white	767	(85.7)	763	(85.0)		
Hispanic American	69	(7.7)	70	(7.8)		
African American	59	(6.6)	65	(7.2)		
Smoking status					<0.0001	
Never	239	(26.7)	441	(49.1)		1.00
Former	291	(32.5)	310	(34.5)		1.39 (1.09–1.78)
Current	365	(40.8)	147	(16.4)		3.97 (3.08–5.13)
Drinking status					<0.0001	
Never	235	(26.3)	398	(44.3)		1.00
Former	206	(23.0)	149	(16.6)		2.12 (1.60–2.81)
Current	454	(50.7)	351	(39.1)		2.10 (1.68–2.63)
Family history of cancer						
Yes	516	(57.7)	511	(56.9)	0.748	1.00
No‡	379	(42.3)	387	(43.1)		1.01 (0.83–1.23)

* Two-sided χ^2 tests for differences between cases and controls.

† Adjusted in a logistic regression including age, sex, ethnicity, smoking status, drinking status, and family history of cancer, accordingly.

‡ Including 5 patients and 3 controls missing information on family history of cancer.

Table 2
Differences in chromatid breaks per cell between patients with SCCN and cancer-free controls

Variables	Non-Hispanic white						Hispanic American						African American					
	Cases (n = 767)			Controls (n = 763)			Cases (n = 69)			Controls (n = 70)			Cases (n = 59)			Controls (n = 65)		
	n	Chromatid breaks per cell	Mean ± SD	n	Chromatid breaks per cell	Mean ± SD	P*	n	Chromatid breaks per cell	Mean ± SD	n	Chromatid breaks per cell	Mean ± SD	n	Chromatid breaks per cell	Mean ± SD	P*	
Total	767	0.49 ± 0.18	763	0.46 ± 0.17	0.0003	69	0.51 ± 0.20	70	0.49 ± 0.19	0.549	59	0.55 ± 0.23	65	0.50 ± 0.23	0.257			
Age (years)																		
≤ 55	385	0.50 ± 0.18	355	0.47 ± 0.19	0.012	33	0.52 ± 0.18	50	0.52 ± 0.19	0.916	21	0.54 ± 0.20	50	0.49 ± 0.18	0.341			
> 55	382	0.48 ± 0.19	408	0.45 ± 0.15	0.013	36	0.51 ± 0.22	20	0.44 ± 0.18	0.230	38	0.56 ± 0.24	15	0.54 ± 0.35	0.855			
Sex																		
Male	575	0.49 ± 0.18	577	0.45 ± 0.16	0.0004	56	0.52 ± 0.21	39	0.45 ± 0.16	0.061	43	0.56 ± 0.23	38	0.48 ± 0.18	0.085			
Female	192	0.50 ± 0.18	186	0.47 ± 0.21	0.245	13	0.49 ± 0.16	31	0.55 ± 0.21	0.377	16	0.53 ± 0.22	27	0.54 ± 0.28	0.886			
Smoking status																		
Never	208	0.48 ± 0.16	358	0.46 ± 0.19	0.127	22	0.48 ± 0.19	47	0.49 ± 0.21	0.930	9	0.70 ± 0.23	36	0.50 ± 0.26	0.039			
Former	259	0.50 ± 0.20	277	0.45 ± 0.17	0.002	21	0.54 ± 0.19	13	0.54 ± 0.18	0.931	11	0.56 ± 0.28	20	0.54 ± 0.17	0.822			
Current	300	0.48 ± 0.18	128	0.45 ± 0.16	0.054	26	0.52 ± 0.23	10	0.47 ± 0.11	0.431	39	0.52 ± 0.21	9	0.45 ± 0.24	0.437			
Drinking status																		
Never	207	0.49 ± 0.16	311	0.48 ± 0.19	0.419	19	0.49 ± 0.17	48	0.49 ± 0.19	0.965	9	0.52 ± 0.26	39	0.56 ± 0.26	0.640			
Former	167	0.50 ± 0.20	133	0.42 ± 0.16	0.0004	19	0.52 ± 0.22	7	0.52 ± 0.22	0.981	20	0.55 ± 0.24	9	0.48 ± 0.14	0.413			
Current	393	0.49 ± 0.19	319	0.45 ± 0.15	0.006	31	0.53 ± 0.22	15	0.50 ± 0.18	0.640	30	0.56 ± 0.22	17	0.39 ± 0.15	0.006			
Family history of cancer																		
Yes	454	0.48 ± 0.18	460	0.45 ± 0.16	0.007	36	0.51 ± 0.22	20	0.42 ± 0.12	0.055	26	0.50 ± 0.22	31	0.54 ± 0.23	0.547			
No [‡]	313	0.50 ± 0.19	303	0.46 ± 0.18	0.016	33	0.51 ± 0.19	50	0.52 ± 0.20	0.842	33	0.59 ± 0.23	34	0.47 ± 0.23	0.041			

* Two-sided Student's *t* tests for the differences in chromatid breaks per cell between cases and controls.

[‡] Including 5 patients and 3 controls missing information on family history of cancer.

Table 3
Logistic regression analysis of chromatid breaks per cell in patients with SCCHN and cancer-free controls

Chromatid breaks per cell	Non-Hispanic white			Hispanic American			African American		
	Cases (n = 767)	Controls (n = 763)	Odds ratio (95% CI) [§]	Cases (n = 69)	Controls (n = 70)	Odds ratio (95% CI) [§]	Cases (n = 59)	Controls (n = 65)	Odds ratio (95% CI) [§]
0.1 increment [*]	767 (100%)	763 (100%)	1.12 (1.05–1.18)	69 (100%)	70 (100%)	1.06 (0.86–1.31)	59 (100%)	65 (100%)	1.11 (0.92–1.34)
By median [†]									
≤ 0.43	302 (39.4%)	382 (50.1%)	1.00	26 (37.7%)	32 (45.7%)	1.00	19 (32.2%)	27 (41.5%)	1.00
> 0.43	465 (60.6%)	381 (49.9%)	1.56 (1.27–1.91)	43 (62.3%)	38 (54.3%)	1.54 (0.69–3.46)	40 (67.8%)	38 (58.5%)	1.91 (0.77–4.73)
By quartile [‡]									
≤ 0.34	168 (21.9%)	214 (28.1%)	1.00	14 (20.3%)	14 (20.0%)	1.00	14 (23.7%)	14 (21.5%)	1.00
0.35–0.43	134 (17.5%)	168 (22.0%)	0.98 (0.72–1.33)	12 (17.4%)	18 (25.7%)	0.65 (0.20–2.13)	5 (8.5%)	13 (20.0%)	0.39 (0.08–1.82)
0.44–0.55	221 (28.8%)	187 (24.5%)	1.49 (1.12–1.98)	16 (23.2%)	18 (25.7%)	1.19 (0.36–3.89)	12 (20.3%)	17 (26.2%)	1.10 (0.31–3.90)
>0.55	244 (31.8%)	194 (25.4%)	1.59 (1.20–2.11)	27 (39.1%)	20 (28.6%)	1.26 (0.41–3.83)	28 (47.5%)	21 (32.3%)	1.57 (0.51–4.84)
Trend test [§]			P = 0.0001			P = 0.448			P = 0.233

* Chromatid breaks per cell were fitted in a logistic regression model as a continuous variable.

[†] Median of the control values.

[‡] Quartiles of the control values.

[§] Adjusted in a logistic regression including age, sex, smoking status, and drinking status.

Multivariable logistic regression analysis of interactions between the frequency of BPDE-induced chromosome breaks and select variables for SCCHN in non-Hispanic whites

Table 4

Variables	Chromatid breaks per cell*	Non-Hispanic white			
		No. of cases/controls	OR (95% CI) [†]	P _{trend} [‡]	P _{interaction} [§]
Age (y)					
> 55	Low	162/207	1.00	<0.0001	0.258/0.032
≤ 55	Low	140/175	1.50 (0.98–2.30)		
> 55	High	220/201	1.39 (1.04–1.85)		
≤ 55	High	245/180	2.61 (1.73–3.94)		
Sex					
Male	Low	230/292	1.00	<0.0001	0.985/0.377
Female	Low	72/90	1.22 (0.84–1.76)		
Male	High	345/285	1.55 (1.22–1.97)		
Female	High	120/96	1.88 (1.34–2.62)		
Smoking status					
Never	Low	80/184	1.00	<0.0001	0.546/0.184
Ever	Low	222/198	2.32 (1.66–3.24)		
Never	High	128/174	1.69 (1.19–2.40)		
Ever	High	337/207	3.42 (2.48–4.72)		
Drinking status					
Never	Low	78/145	1.00	<0.0001	0.426/0.044
Ever	Low	224/237	1.42 (1.01–2.01)		
Never	High	129/166	1.37 (0.95–1.98)		
Ever	High	336/215	2.33 (1.66–3.28)		

* Chromatid breaks per cell were dichotomized by the median of the controls' values (≤ 0.43, > 0.43).

[†] The ORs and 95% CIs were calculated using logistic regression models with adjustment for age (continuous), sex, smoking (ever and never), and drinking status (ever and never), accordingly.

[‡] P-values (two-sided) for trend tests were calculated using logistic regression models with adjustment for age (continuous), sex, smoking (ever and never), and drinking status (ever and never), accordingly.

[§] The interaction between each of variables and BPDE-induced chromatid breaks per cell was first tested for the departure from a multiplicative model, followed by the test for the departure from an additive model, if no evidence was found for a multiplicative interaction, with adjustment for age (continuous), sex, smoking (ever and never), drinking status (ever and never), and dichotomized chromatid breaks per cell as the main effects, accordingly. P-values were two-sided.