Environmental Tobacco Smoking, Mutagen Sensitivity, and Head and Neck Squamous Cell Carcinoma[1]

Zuo-Feng Zhang,[2] Hal Morgenstern, Margaret R. Spitz, Donald P. Tashkin, Guo-Pei Yu, Tao C. Hsu, and Stimson P. Schantz

Department of Epidemiology, University of California Los Angeles School of Public Health, and Jonsson Comprehensive Cancer Center, Los Angeles, California 90095-1772 [Z-F. Z., H. M.]; Department of Epidemiology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030 [M. R. S.]; Department of Medicine, University of California Los Angeles School of Medicine, Los Angeles, California 90095 [D. P. T.]; New York Eye and Ear Infirmary, New York, New York 10003 [G-P. Y., S. P. S.]; and Department of Cell Biology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030 [T. C. H.]

Abstract

Although active tobacco smoking has been considered a major risk factor for head and neck cancer, few studies have evaluated environmental tobacco smoke (ETS) and its interaction with mutagen sensitivity on the risk of head and neck cancer. We investigated the relationship between ETS and head and neck cancer in a case-control study of 173 previously untreated cases with pathologically confirmed diagnoses of squamous cell carcinoma of the head and neck and 176 cancer-free controls at Memorial Sloan-Kettering Cancer Center between 1992 and 1994. A structured questionnaire was used to collect ETS exposure and other covariates including a history of active tobacco smoking and alcohol use. ETS measures include a history of ETS exposure at home and at workplace. The associations between passive smoking and head and neck cancer were analyzed by Mantel-Haenszel methods and logistic regression models. Additive and multiplicative models were used to evaluate effect modifications between ETS and mutagen sensitivity. The crude odds ratio (OR) for ETS exposure was 2.8 [95% confidence intervals (CI), 1.3–6.0]. Controlling for age, sex, race, education, alcohol consumption, pack-years of cigarette smoking, and marijuana use, the risk of squamous cell carcinoma of the head and neck was increased with ETS (adjusted OR, 2.4; 95% CI, 0.9–6.8). Dose-response relationships were observed for the degree of ETS exposure; the adjusted ORs were 2.1 (95% CI, 0.7–6.1) for those with moderate exposure and 3.6 (95% CI, 1.1–11.5) for individuals with heavy exposure (*P* for trend = 0.025), in comparison with those who never had ETS exposures. These associations and the dose-response relationships were still present when the analysis was restricted to nonactive smoking cases and controls (crude OR, 2.2; 95% CI, 0.6–8.4). Crude odds ratios were 1.8 for those with moderate ETS exposure and 4.3 for individuals with heavy ETS exposure among nonsmoking cases and controls (*P* for trend = 0.008). More than multiplicative interaction was suggested between passive smoking and mutagen sensitivity. This study suggests that ETS exposure may increase the risk of head and neck cancer with a dose-response pattern. Our analysis indicated that passive smoking may interact with mutagen sensitivity and other risk factors to increase the risk of head and neck cancer. Our results need to be interpreted with caution because of potential residual confounding effects of active tobacco smoking and other methodological limitations. Future large-scale studies are warranted to confirm our findings.

Introduction

ETS[3] is generated by exhalations of smokers, plus emissions from cigarettes, cigars, or pipes between puffs. Approximately 80% of ETS comes from SS and 20% from exhaled MS. Carcinogens in SS are much higher in concentration than in MS, such as *N*-nitrosamines (20 fold), 4-aminobiphenyls (30-fold), 2-naphthylamine (30-fold), aniline (30-fold), benzene (5-fold), benzo(*a*)pyrene (3-fold), and 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (2-fold). Strong evidence linking ETS to carcinoma of the lung cancer has been documented by a series of well-designed and conducted epidemiological studies, as summarized by Dockery and Trichopoulos (1). ETS was classified as a human carcinogen (group A) by the United States Environmental protection Agency in 1993 (2). A recent large-scale multicenter epidemiological investigation by Boffetta *et al.* from the International Agency for Research on Cancer suggested a moderately elevated risk of lung cancer among nonsmokers exposed to ETS as adults at home or in the workplace, with risk tending to rise with the amount of ETS exposure (3, 4).

The association between ETS and lung cancer and the similarities between the carcinogenic properties of passively and actively inhaled tobacco smoking suggest the possible role of environmental tobacco smoke or passive smoking on the development of squamous cell carcinoma of the head and neck. Although tobacco smoking and alcohol drinking have been identified as major risk factors for head and neck cancer, few

Received 9/8/99; revised 7/7/00; accepted 7/21/00.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] Supported in part by Grant ES-06718 from the National Institute of Environmental Health Services; Grants CA-51845, and CA16042–24 from the National Cancer Institute; Grant DA/CA11386 from the National Institute on Drug Abuse, NIH, Department of Health and Human Services; by a seed grant by UCLA Jonsson Cancer Center Foundation; and by the Weisman Fund.
[2] To whom requests for reprints should be addressed, at Department of Epidemiology, University of California Los Angeles School of Public Health, 71-225 CHS, Box 951772, Los Angeles, California 90095-1772. Phone: (310) 825-8418; Fax: (310) 206-6039; E-mail: zfzhang@ucla.edu.

[3] The abbreviations used are: ETS, environmental tobacco smoke; SS, sidestream smoke; MS, mainstream smoke; OR, odds ratio; CI, confidence interval.

clinical or epidemiological studies have been conducted to examine the possible effects of passive smoking on the risk of head and neck cancer. One of those studies was a case report involving a 69-year-old nonsmoking woman with squamous carcinoma of the left tonsil. Her illness was suspected to be related to her husband's 40 years of smoking (5). In another study of the possible role of passive smoking in the development of squamous cell head and neck cancer (SCHNC), a group of 59 non-smoking cases with squamous cell head and neck cancer were retrospectively studied at the Cleveland Clinic Foundation (6). When compared with the individuals without ETS exposure, an elevated risk of head and neck cancer was noted for those exposed to ETS both in the home and in the workplace. The authors concluded that a cause and effect relationship between passive smoking and head and neck cancer cannot be clearly established on the basis of their study because of the small sample size, the limited data collection, and inadequate adjustment for potential confounding effects in their analysis.

The aim of the present analysis was to examine the association between ETS and head and neck cancers, controlling for other known risk factors for the disease, including cigarette smoking and alcohol drinking. We will also explore the possible interactions between ETS and mutagen sensitivity, and with other known risk factors for head and neck cancer. Mutagen sensitivity is considered a predisposition marker of cancer risk (7, 8). Defects in one or more steps of the DNA repair process may play a significant role in environmental carcinogenesis, and the extent of such defects may be partially responsible for susceptibility or resistance to environmental mutagens (9). Mutagen sensitivity tests are indirect indicators of DNA repair competence. Bleomycin, a radiomimetic agent, was used as the test mutagen, an assay developed to evaluate the rates of induced chromosome breakage as a crude indicator of the response to a genotoxic agent (10, 11).

Patients and Methods

Subject Selection. Untreated new patients with a histologically confirmed diagnosis of first primary squamous cell carcinoma of the head and neck, seen at Memorial Sloan-Kettering Cancer Center from 1992 to 1994, were considered as cases in this study. We have approached 192 patients, and 173 agreed to participate. Eleven tumor sites were classified by the American Joint Committee on Cancer criteria. Age and sex frequency-matched controls were identified for this study. Controls were without a history of cancer and were identified from the Blood Bank Center of Memorial Sloan-Kettering Cancer Center during the same period. We approached 196 blood donors, and 176 agreed to participate the study.

Data Collection and Variables. The study was approved by the Institutional Research Board on Human Subjects of Memorial Sloan-Kettering Cancer Center. All cases and controls were asked to sign an informed consent form if they agreed to participate in the study, to complete a structured questionnaire, and to donate a sample of blood. The questionnaire requested information on the following variables: age, gender, race, year and place of birth, religion, family income, and education; average number of tobacco cigarettes smoked/day, years of smoking, age at initiation of smoking; exposure to ETS (at home and at work); alcohol consumption, types and frequency of alcohol consumption; occupational and environmental exposures; family history of cancer; sexual history; medical history; and oral hygienic history. In addition, all subjects were asked if they had ever used marijuana. If they responded yes, subjects were asked the average number of times they smoked per day and the number of years of marijuana use. For ETS exposure, all subjects were asked: (*a*) "Have you ever been regularly exposed to other people's cigarette smoke at home?" and (*b*) "Have you ever been regularly exposed to other people's cigarette smoke at work?" There were three choices for each question: never, occasionally, and regularly. In addition, a question regarding partner's or spouse's smoking was asked: "Does your current partner/spouse smoke? (yes/no)". A total of 155 cases (89.6%) and 166 controls (94.3%) had complete data on passive smoking at home and at work. Individuals with missing data on passive smoking were excluded from analysis. Those reported either occasional or regular exposure to ETS at home or work were categorized as ever exposed to ETS, and those who reported no exposure to ETS both at home and at work were defined as never exposed to ETS. The degree of ETS exposure was defined according to history of passive smoking both at home and at work. "Never exposed to ETS" was defined as those individuals who were exposed to ETS neither at home nor at work, "heavily exposed to ETS" was defined as those individuals who were exposed to ETS both at home and at work, and "moderately exposed to ETS" included those who were either exposed to ETS at home or at work.

Mutagen Sensitivity Assay. A total of 91 patients and 131 controls provided a blood specimen for the assessment of mutagen sensitivity. The mutagen sensitivity assay used in this study has been described in detail previously (12). A peripheral blood sample (10 ml or less) was collected from cases and controls in a heparinized tube prior to initiation of lymphocyte culture. The standard lymphocyte culture procedure used RPMI 1640, supplemented with 15% FCS and phytohemagglutinin, in a ratio of blood:medium of 1:9. At 67 h of incubation, one set of cultures was treated with bleomycin (0.03 units/ml) for 5 h. Colcemid (0.04 mg/ml) was added in the last hour to induce mitotic arrest prior to harvesting. A conventional cell harvesting procedure followed. The cells were treated with hypotonic KCl (0.975 M KCl) solution for 15–20 min, fixed, washed with a freshly prepared mixture of methanol and acetic acid (3:1), and air-dried on wet slides. The slides were stained with Giemsa solution without banding. Fifty well-spread metaphases were examined from coded slides. Chromatid aberrations recorded were frank chromatid breaks or exchanges. Bleomycin tends to induce few chromatid exchanges (which, if present, are considered as two breaks). Chromatid gaps or attenuated regions were disregarded. The frequency of breakage was expressed as breaks/cell. The reliability of cytogenetic scoring has been evaluated previously by comparing four separate blood samples from a respective donor with a minimum interval between samples of 1 week (9).

Statistical Analysis. The effects of ETS on the risk of head and neck cancer were estimated with ORs and their 95% CIs, derived from logistic regression analysis (13). Dummy variables were used in logistic regression analysis to estimate ORs for each category of exposure. Trend tests for ordered variables were performed by assigning the score j to the jth exposure level of a categorical variable (where $j = 1, 2, \ldots$) and treating the categorical variable as an interval predictor in unconditional logistic regression. We have selected several potential confounders or effect modifiers in our analysis. In addition to active tobacco smoking and alcohol drinking, we considered marijuana smoking as a possible confounder or a effect modifier in our analysis because marijuana smoking was associated with head and neck cancer (14). Three models were used to assess ETS effects: (*a*) no covariates (crude analysis); (*b*)

statistical adjustment for pack-years of cigarette smoking (continuous variable); (*c*) statistical adjustment for pack-years of cigarette smoking plus age (continuous variable), sex (male *versus* female), race (white *versus* non-white), education (≤high school, college education, postgraduate education), history of marijuana use (yes *versus* no), and heavy alcohol drinking (≥100 drinks/month *versus* <100 drinks/month;). Stratified analysis was used to assess departures from additive or multiplicative effects between ETS and other known risk factors for head and neck cancer, including cigarette smoking, alcohol drinking, and mutagen sensitivity.

Results

The overall prevalence of ETS exposure was 83.7% in controls and 93.6% in cases. Higher ETS exposure was found in 95.7% of patients who had laryngeal cancer, 95.7% of pharyngeal cancer, and 92.7% of carcinoma of the oral cavity. The distributions of ETS exposure, stratified by demographic characteristics, active cigarette smoking, alcohol drinking, mutagen sensitivity, and marijuana use, among cases and controls and the distributions of ETS exposure in cases stratified by sites of head and neck tumor are shown in Table 1. No obvious differences for passive smoking were found in terms of age, gender, and race. Education was strongly associated with ETS exposure; high prevalence of the ETS exposures was found in people with lower education (less than or equal to high school) in controls, but not in cases. Cigarette smoking was generally independent of ETS exposure in both cases and controls, although the prevalence of ETS exposure was elevated with increased pack-years of smoking, cigarettes smoked/day, and years of smoking in controls. Heavy alcohol drinking and mutagen hypersensitivity were not related to ETS exposure in cases or controls. Marijuana use was associated with passive smoking in cases but not in controls.

The estimated crude OR for the effect of lifetime ETS exposure (ever *versus* never) on the risk of head and neck cancer was 2.8 (95% CI, 1.3–6.0). Adjusting for age, gender, race, education, heavy alcohol drinking, pack-years of cigarette smoking, and marijuana use, the OR was 2.4 (95% CI, 0.9–6.8; Table 2). Strong dose-response relationships were observed for the effects of the degree of ETS exposure. The adjusted ORs were 2.1 for those who had moderate ETS exposure and 3.6 for those who had heavy ETS exposure (P for trend test = 0.025). When ETS exposure at home, ETS exposure at work, and spouse smoking were analyzed separately and after controlling for potential confounding effects, the ETS effect for each variable was weaker in comparison to results of crude analysis.

The observed associations between ETS exposure and head and neck cancer in never-cigarette-smokers were similar to those in cigarette smokers (Table 3). The crude OR for the effect of lifetime ETS exposure was 2.2 (95% CI, 0.6–8.4) for never-smokers and 3.1 (95% CI, 1.1–8.3) for ever-smokers. Strong dose-response relationships were also observed for the effects of the degree of ETS exposure among never-smokers and ever-smokers. The crude ORs were 1.8 for those who had moderate ETS exposure and 4.3 for those who had heavy ETS exposure (P for trend test = 0.0082) among never-smokers. The crude ORs were 2.5 and 5.3 for moderate and heavy ETS exposure, respectively, among ever-smokers (p for trend = 0.0016).

Table 4 shows the combined effects of ETS exposure (ever *versus* never) and each of four potential effect modifiers: tobacco cigarette smoking, alcohol use, marijuana use, and mutagen sensitivity. For these analyses, we used >1.0 breaks/cell as the cutoff value to define mutagen hypersensitivity. We categorized subjects as heavy alcohol drinkers if they had 100 or more drinks/per month and tobacco cigarette smoking into never smokers/ever smokers. The effects of ETS and mutagen hypersensitivity were more than multiplicative; the adjusted OR for the joint category of ETS exposure and mutagen sensitivity was greater than the product of the two component effects for those two factors, *i.e.*, 17.5 > 2.6 × 2.0 = 5.2. The effects of ETS and alcohol consumption appeared more than additive but less than multiplicative, *i.e.*, 4.9 (alcohol only) + 2.5 (ETS only) minus 1 = 6.4 < 10.2 (both exposures) < 4.9 × 2.5 = 12.25. The effects of ETS and marijuana use appeared more than additive but less than multiplicative, *i.e.*, 3.5 (mar-

Table 1 The prevalence of ETS in cases and controls, by category of selected demographic factors, smoking, alcohol, and mutagen sensitivity

	Controls			Cases		
	ETS Yes (*n*)	%	Total	ETS Yes (*n*)	%	Total
Total	139	83.7	166	145	93.6	155
Age						
<60	68	84.0	81	64	92.8	69
60–69	44	83.0	53	43	95.6	45
≥70	27	84.3	30	38	92.7	41
Gender						
Male	90	84.9	106	92	94.9	97
Female	49	81.7	60	53	91.4	58
Race						
White	127	84.7	150	129	94.9	136
Non-white	12	75.0	16	16	84.2	19
Education						
≤High school	43	97.7	44	89	93.7	95
College	65	75.6	86	41	97.6	42
Postgraduate	31	86.1	36	14	87.5	16
Cigarette smoking						
Never	46	78.0	59	23	88.5	26
Ever	92	86.8	106	121	95.3	127
Quit	60	85.7	70	36	97.3	37
Current[a]	32	88.9	36	85	94.4	90
Pack-Years						
0	46	78.0	59	23	88.5	26
1–22.4	40	83.3	48	20	95.2	21
22.5–44.9	26	92.9	28	36	97.3	37
≥45	17	94.4	18	57	93.4	61
Marijuana use						
No	126	84.6	149	126	96.2	131
Yes	13	76.5	17	19	79.2	24
Alcohol use (drinks/months)						
<100	118	83.1	142	89	93.7	95
≥100	14	93.3	15	51	92.7	55
Mutagen sensitivity (breaks/cell)						
<1	78	82.1	95	29	90.6	32
≥1	25	86.2	29	46	93.9	49
Tumor sites (ICD9)						
Lip (140)				1	50.0	2
Tongue (141)				43	91.5	47
Gum (143)				10	90.9	11
Floor of mouth (144)				14	100.0	14
Other parts of mouth (145)				9	90.0	10
Oropharynx (146)				10	90.9	11
Nasopharynx (147)				2	100.0	2
Hypopharynx (148)				10	100.0	10
Other oral cavity (149)				1	100.0	1
Esophagus (150)				1	100.0	1
Larynx (161)				44	95.7	46

[a] Current smoking category included these who were still smoking and people who had quit smoking for <5 years.

Table 2 Estimated effects of ETS (OR and 95% CI) on the risk of head and neck cancer, by covariates selected for adjustment

	No. of cases	No. of controls	No covariates (crude)	Pack-years of smoking	Pack-years of smoking plus[a]
ETS					
Never[b]	10	27	1.0	1.0	1.0
Ever	145	139	2.8 (1.3–6.0)	2.1 (0.9–5.0)	2.4 (0.9–6.8)
Degree of ETS exposure					
Never[b]	10	27	1.0	1.0	1.0
Moderate	98	115	2.3 (1.1–5.0)	1.8 (0.7–4.2)	2.1 (0.7–6.1)
Heavy[c]	47	24	5.3 (2.2–12.7)	3.7 (1.4–9.7)	3.6 (1.1–11.5)
P for trend			0.0001	0.0039	0.0249
ETS at home					
Never	37	60	1.0	1.0	1.0
Occasionally	41	46	1.4 (0.8–2.6)	1.4 (0.7–2.7)	1.6 (0.8–3.3)
Regularly	77	60	2.1 (1.2–3.5)	1.5 (0.9–2.8)	1.7 (0.8–3.3)
P for trend			0.0065	0.1596	0.1574
ETS at work					
Never	36	55	1.0	1.0	1.0
Occasionally	46	50	1.4 (0.8–2.5)	1.2 (0.6–2.3)	1.0 (0.5–2.1)
Regularly	73	61	1.8 (1.1–3.1)	1.4 (0.8–2.7)	1.0 (0.5–2.1)
P for trend			0.0288	0.2380	0.9240
Spouse smoking					
No	80	104	1.0	1.0	1.0
Yes	33	22	2.0 (1.1–3.6)	1.6 (0.8–3.1)	1.7 (0.8–3.7)

[a] Also adjusted for age (continuous variable); gender (male, 0; female, 1); race (white, 0; non-white, 1); education (≤high school, 0; college, 2; >college, 2); heavy alcohol use (<100/month, 0; ≥100/month, 1); and marijuana use (no, 0; yes, 1).
[b] Never exposed to ETS at both home and work.
[c] Regularly exposed to ETS at both home and work.

Table 3 Estimated crude effects (OR and 95% CI) of ETS on the risk of head and neck cancer, by active cigarette smoking

	Never smoking			Ever smoking		
	Cases	Controls	OR_{crude} (95% CI)	Cases	Controls	OR_{crude} (95% CI)
ETS						
Never	3	13	1.0	6	14	1.0
Ever	23	46	2.2 (0.6–8.4)	121	92	3.1 (1.1–8.3)
Degree of ETS						
Never	3	13	1.0	6	14	1.0
Moderate	17	40	1.8 (0.5–7.3)	80	74	2.5 (0.9–6.9)
Heavy	6	6	4.3 (0.8–23.5)	41	18	5.3 (1.8–16.1)
P for trend			0.0082			0.0016
ETS at home						
Never	8	28	1.0	28	32	1.0
Occasionally	9	10	3.2 (1.0–10.4)	32	35	1.1 (0.5–2.1)
Regularly	9	21	1.5 (0.5–4.5)	67	39	2.0 (1.0–3.7)
P for trend			0.4483			0.0264
ETS at work						
Never	7	24	1.0	27	30	1.0
Occasionally	11	17	2.2 (0.7–6.9)	35	33	1.2 (0.6–2.4)
Regularly	8	18	1.5 (0.5–5.0)	65	43	1.7 (0.9–3.2)
P for trend			0.4670			0.0997
Spouse smoking						
No	11	36	1.0	68	67	1.0
Yes	2	7	0.9 (0.2–5.2)	31	15	2.0 (1.0–4.0)

ijuana only) + 2.6 (ETS only) − 1 = 5.1 < 7.1 (both exposures) < 3.5 × 2.6 = 9.1. In each case, however, the power for testing each null hypothesis (effects are additive or multiplicative) and for comparing the fits of additive *versus* multiplicative models was low.

Discussion

We report here an effect of exposure to ETS on the risk of head and neck cancer. Not only did we find an elevated cancer risk among individuals exposed to ETS, but we also observed dose-response associations for the degree of ETS exposure, adjusting for several potential confounders. These associations and the dose-response relationships were still present when the analysis was restricted to non-active-smoking cases and controls.

This study has several possible limitations. One limitation is potential selection bias, which might have resulted in an overestimate or underestimate of the ETS effect (bias away from or toward null). Using controls from the blood bank may result in potential selection bias because blood donors might be

Table 4 Estimated combined effects (OR and 95% CI) of ETS (ever *versus* never) and each of four potential modifiers (cigarette smoking, heavy alcohol use, marijuana smoking, and mutagen hypersensitivity) on the risk of head and neck cancers, by covariates selected for adjustment

Passive smoking	Potential modifier	No. of cases	No. of controls	No covariates (crude)	Pack-years of smoking	Pack-years smoking plus[a]
Cigarette smoking						
No	Never	3	13	1.0	1.0	1.0
No	Ever	6	14	1.9 (0.4–9.0)	0.9 (0.2–4.9)	0.4 (0.1–2.7)
Yes	Never	23	46	2.2 (0.6–8.4)	2.2 (0.6–8.4)	1.5 (0.3–6.5)
Yes	Ever	121	92	5.7 (1.6–20.6)	1.9 (0.5–7.4)	1.4 (0.3–6.1)
Alcohol (drinks/month)						
No	<100	6	24	1.0	1.0	1.0
No	≥100	4	1	16.0 (1.5–170)	6.4 (0.5–83.5)	4.9 (0.3–75.8)
Yes	<100	89	118	3.0 (1.2–7.7)	2.0 (0.8–5.4)	2.5 (0.8–7.6)
Yes	≥100	51	14	14.6 (5.0–42.6)	7.8 (2.5–24.1)	10.2 (2.7–37.8)
Mutagen sensitivity (breaks/cell)						
No	<1	3	17	1.0	1.0	1.0
No	≥1	3	4	4.3 (0.6–29.5)	2.9 (0.3–30.3)	2.6 (0.1–71.1)
Yes	<1	29	78	2.1 (0.6–7.7)	1.6 (0.3–8.8)	2.0 (0.2–17.7)
Yes	≥1	46	25	10.4 (2.8–39.1)	11.6 (2.1–63.0)	17.5 (1.9–162)
Marijuana use						
No	No	5	23	1.0	1.0	1.0
No	Yes	5	4	5.8 (1.1–29.4)	3.6 (0.6–21.2)	3.5 (0.4–28.4)
Yes	No	126	126	4.6 (1.7–12.5)	3.1 (1.0–9.8)	2.6 (0.7–9.0)
Yes	Yes	19	13	6.7 (2.0–22.3)	5.8 (1.5–22.8)	7.1 (1.5–34.5)

[a] Also adjusted for age (continuous variable); gender (male, 0; female, 1); race (white, 0; non-white, 1); education (≤high school, 0; college, 2; >college, 2); heavy alcohol use (<100/month, 0; ≥100/month, 1); and marijuana use (no, 0; yes, 1).

more health oriented. To assess the potential selection bias, we have compared selected demographic and potential risk factors between blood donor controls and non-cancer controls from the Surgical Day Hospital during the same study period at Memorial Sloan-Kettering Cancer Center. Generally, the two groups of controls were similar in terms of those selected factors; contrary to expectation, however, blood donor controls had a slightly higher proportion of cigarette smokers and a markedly higher proportion of alcohol drinkers than the other control group, potentially producing an underestimation of the association between cigarette smoking, alcohol drinking, and head and neck cancer and may lead to incomplete control of these potential confounders. Because we did not collect information on passive smoking in non-cancer controls from the Surgical day Hospital, we did not know whether the prevalence of passive smoking in blood donors would also be higher than non-cancer hospital controls so that the selection bias could not be clearly ruled out. On the other hand, because the majority of blood donors were relatives or friends of cancer patients hospitalized at Memorial Sloan-Kettering Cancer Center, they might have a slightly higher chance of exposure to ETS. We believe that the selection of blood donors as controls would probably underestimate the association between ETS and head and neck cancer under study.

When we evaluated the interaction between passive smoking and mutagen sensitivity (Table 4), a possible selection bias might exist because those with blood samples for mutagen assay may be different from individuals without blood samples. A total of 26.1% of controls and 46.8% of cases refused to provide a blood sample for the bleomycin sensitivity test in this study. We have compared the differences between those with and without blood samples on selected variables. This attempt is crucial to show whether there is selection bias because of missing samples that may threaten the validity of the interaction between passive smoking and mutagen sensitivity. The proportion of passive smoking was comparable in those with and without blood specimens for both cases and controls. No obvious difference was found between those with and without blood samples in terms of age, gender, education, and alcohol drinking in both cases and controls. Significant differences were found for cigarette smoking and race in cases; cases with blood specimens had a higher proportion of smokers and non-white than cases without blood specimens. Those differences indicate that the subjects with blood samples might not be a representative group for smoking habits and non-whites from the original study population, which might lead to a stronger confounder effects on the association between passive smoking and head and neck cancer.

The second limitation is differential misclassification of ETS, which may bias the estimated effect under study (15, 16). The degree of overreporting may be greater for cases than controls because cases might want to rationalize their disease. Thus, the estimates of ETS effects could be positively biased. Self-reported ETS in the recent past has been validated in several studies and believed to be apparently valid (17–19). The confirmation that dose-response relationships exist between urinary cotinine concentrations and self-reported passive smoking partially validates questionnaire measures of the degree of environmental smoke exposure (19). The results of the analysis of self-reported recent exposure to ETS from any source in relation to urinary concentrations of cotinine indicated that duration of exposure and number of cigarettes to which the subject reported being exposed were strongly related to urinary cotinine (17). However, questionnaire-based information on long-term exposure to ETS is difficult to integrate over time and almost impossible to validate. Nevertheless, the validity of self-reported exposure to ETS in the recent past supports the validity of self-reported of long-term exposure to ETS (20). Thus, we believe that differential misclassification of past exposure to ETS is probably not sufficient to explain the positive findings in this study. The possible differential misclassification of using mutagen sensitivity assays in case-control study was discussed by Caporaso (21). Cultured cells obtained from patients with cancer or control subjects in a hospital setting can differ for abnormal nutrition, secondary metabolic alterations of neoplastic disease, and effect of treatment, hospitalization, in-

activity, or stress, which will allow bias because of differential misclassification. However, a recent paper by Cloos *et al.* (22) reported a high heritability estimate of the susceptibility to bleomycin-induced chromatid breaks, which indicates that a clear genetic basis for mutagen sensitivity-related cancer susceptibility may exist in the general population. If the mutagen sensitivity is highly inherited, the differential misclassification bias for this assay might be minimal.

The third limitation is the small sample size. We only have 10 cases and 27 controls who had no ETS exposure. If we further stratified by cigarette smoking or mutation sensitivity, the number becomes much smaller. The relatively small sample size may lead to the low power of the study and a poor precision of the measurement, which would limit our ability to estimate the ETS effect effectively and precisely.

Confounding by active cigarette smoking and alcohol consumption on the association between ETS and head and neck cancer was apparent. Although we attempted to adjust for active cigarette smoking and alcohol drinking in our analyses, residual confounding might still exist because ETS may be closely related to active cigarette smoking and alcohol consumption.

The observed association between exposures to ETS and head and neck cancer is relatively weak, similar to the observed association between ETS exposure and lung cancer. In comparison with the ETS effect in lung cancer, the OR of ETS for head and neck cancer is slightly higher. It may be caused by the small sample size of this study. On the other hand, considering the upper aerodigestive tracts as a first entrance for the ETS exposure, the degree of exposure might be higher than that in lung. In addition, the mechanism of the ETS carcinogenic action may be different in upper aerodigestive tract cancers from that in lung cancer.

Our results are supported by compelling biological evidence. This evidence includes the higher concentration of carcinogens in SS than MS, the strong causal link between active smoking and both lung and head and neck cancers, and the convincing evidence of the association between ETS exposure and lung cancer (1).

Although non-cigarette smokers are major potential victims of the health consequences of ETS, active smokers might also have a greater opportunity to be exposed to SS, in addition to MS. Therefore, if ETS is associated with certain cancers in nonsmokers, it would be reasonable to assume that ETS would have a similar or even stronger impact on the risk of tobacco-related cancers in smokers when active cigarette smoking and other potential confounding effects are controlled for. The hypothesis is supported by the following: (*a*) cigarette smokers are exposed not only to MS but also to SS from their own cigarettes; (*b*) smokers tend to socialize with other smokers, thereby increasing their exposure to other smokers' SS; (*c*) smokers are more likely than nonsmokers to have a smoking spouse or partner, thus further increasing their exposure to ETS (23). Because most published studies of ETS and lung cancer are limited to nonsmoking women, we may not know the full impact of ETS on the risk of lung cancer. The effects of ETS need to be further studied in nonsmoking men, as well as in active smokers. In the present study, we found slightly different effects of ETS on the risk of head and neck cancer between smokers and nonsmokers. In never-smokers with 26 cases and 59 controls, the crude OR was 2.2 for ETS exposure. The dose-response relationship was apparent with ORs of 1.8 for intermediate ETS exposure and 4.3 for heavy ETS exposure (P for trend = 0.0082). Interestingly, the adjusted ORs for ETS exposure in the whole study population, including both active smokers and never-active smokers, were very similar to those in the subanalysis with never-active smokers: 2.4 for ETS exposure, 2.1 for moderate ETS exposure, and 3.6 for heavy ETS exposure, respectively (P for trend = 0.0249). These observations are consistent with our assumption.

Possible interaction effects were suggested between ETS and mutagen sensitivity and other risk factors for head and neck cancer. The interplay between carcinogens and intrinsic host susceptibility is an important factor in environmental carcinogenesis. Mutagen hypersensitivity, an indirect marker for DNA repair, interacts with tobacco smoking in head and neck cancer risk (24–27). Synergy between mutagen hypersensitivity and ETS was suggested in this study because the effects were much more than multiplicative, which suggests that the development of the upper aerodigestive cancers may be affected by gene-environment interaction. Because of the low power for testing these interactions, however, the present findings will need to be replicated in future large studies.

In summary, we found that ETS is associated with a dose-dependent increased risk of head and neck cancer. This association is supported by other evidence that provides a biologically plausible basis for the hypothesis that ETS is a risk factor for human head and neck cancer. Our results need to be examined with caution because of potential residual confounding effects of active tobacco smoking and small sample size. Further large-scale epidemiological studies are needed to replicate our results, to examine the relationships between ETS and increased risk of cancer, and to assess potential interactions between ETS and other risk factors.

References

1. Dockery, D. W., and Trichopoulos, D. Risk of lung cancer from environmental exposures to tobacco smoke. Cancer Causes Control, *8:* 333–345, 1997.

2. United States Environmental Protection Agency. Respiratory health effects of passive smoking: lung cancer and other disorders. Washington, DC: Environmental Protection Agency, 1992.

3. Boffetta, P., Agudo, A., Ahrens, W., Benhamou, E., Benhamou, S., Darby, S. C., Ferro, G., Fortes, C., Gonzalez, C. A., Jockel, K. H., Krauss, M., Kreienbrock, L., Kreuzer, M., Mendes, A., Merletti, F., Nyberg, F., Pershagen, G., Pohlabeln, H., Riboli, E., Schmid, G., Simonato, L., Tredaniel, J., Whitley, E., Wichmann, H. E., and Saracci, R. Multicenter case-control study of exposure to environmental tobacco smoke and lung cancer in Europe. J. Natl. Cancer Inst., *90:* 1440–1450, 1998.

4. Blot, W. J., and McLaughlin, J. K. Passive smoking and lung cancer risk: what is the story now? J. Natl. Cancer Inst., *90:* 1416–1417, 1998.

5. Strome, M., Ward, P., Johnson, J., and Goepfert, H. Carcinoma of the tonsil. Head Neck, *15:* 465–468, 1993.

6. Tan, E. H., Adelstein, D. J., Droughton, M. L., Van Kirk, M. A., and Lavertu, P. Squamous cell head and neck cancer in nonsmokers. Am. J. Clin. Oncol., *20:* 146–150, 1997.

7. Spitz, M. R., and Hsu, T. C. Mutagen sensitivity as a marker of cancer risk. Cancer Detect. Prev., *18:* 299–303, 1994.

8. Olden, K. Mutagen hypersensitivity as a biomarker of genetic predisposition to carcinogenesis. J. Natl. Cancer Inst., *86:* 1660–1661, 1994.

9. Hsu, T. C., Johnston, D. A., Cherry, L. M., Ramkissoon, D., Schantz, S. P., Jessup, J. M., Winn, R. J., Shirley, L., and Furlong, C. Sensitivity to genotoxic effects of bleomycin in humans: possible relationship to environmental carcinogenesis. Int. J. Cancer, *43:* 403–409, 1989.

10. Cherry, L. M., and Hsu, T. C. Bleomycin-induced chromosome damage in lymphocytes of medullary thyroid carcinoma patients and their family members. Anticancer Res., *3:* 367–372, 1983.

11. Hsu, T. C., Cherry, L. M., and Samaan, N. A. Differential mutagen susceptibility in cultured lymphocytes of normal individuals and cancer patients. Cancer Genet. Cytogenet., *17:* 307–313, 1985.

12. Hsu, T. C. Genetic predisposition to cancer with special reference to mutagen sensitivity. In Vitro Cell. Dev. Biol., *23:* 591–603, 1987.

13. Breslow, N. E., and Day, N. E. Unconditional logistic regression for large strata. *In:* N. E. Breslow and N. E. Day (eds.), Statistical Methods in Cancer

Research. I. The Analysis of Case-control Studies, pp. 192–244. Lyon, France: IARC, 1980.

14. Zhang, Z. F., Morgenstern, H., Spitz, M. R., Yu, G. P., Marshall, J. M., Tashkin, D., Hsu, T. C., and Schantz, S. P. Marijuana smoking and increased risk of squamous cell carcinoma of the head and neck. Cancer Epidemiol. Biomark. Prev., *8:* 1071–1078, 1999.

15. Levois, M., and Switzer, P. Differential exposure misclassification in case-control studies of environmental tobacco smoke and lung cancer. J. Clin. Epidemiol., *51:* 37–54, 1998.

16. Nilsson, R. Environmental tobacco smoke and lung cancer: a reappraisal. Ecotoxicol. Environ. Saf., *34:* 2–17, 1996.

17. Riboli, E., Preston-Martin, S., Saracci, R., Haley, N. J., Trichopoulos, D., Becher, H., Burch, J. D., Fontham, E. T., Gao, Y. T., and Jindal, S. K. Exposure of nonsmoking women to environmental tobacco smoke: a 10-country collaborative study. Cancer Causes Control, *1:* 243–252, 1990.

18. Cummings, K. M., Markello, S. J., Mahoney, M., Bhargava, A. K., McElroy, P. D., and Marshall, J. R. Measurement of current exposure to environmental tobacco smoke. Arch. Environ. Health, *45:* 74–79, 1990.

19. Jarvis, M. J. Uptake of environmental tobacco smoke. IARC Sci. Publ., *81:* 43–58, 1987.

20. Trichopoulos, D. Risk of lung cancer and passive smoking. Important Adv. Oncol., *77–85:* 1995.

21. Caporaso, N. Genetics of smoking-related cancer and mutagen sensitivity. J. Natl. Cancer Inst., *91:* 1097–1098, 1999.

22. Cloos, J., Nieuwenhuis, E. J., Boomsma, D. I., Kuik, D. J., van der Sterre, M. L., Arwert, F., Snow, G. B., and Braakhuis, B. J. Inherited susceptibility to bleomycin-induced chromatid breaks in cultured peripheral blood lymphocytes. J. Natl. Cancer Inst., *91:* 1125–1130, 1999.

23. Inaba, S., Kurisu, Y., Nagata, C., Takatsuka, N., Kawakami, N., and Shimizu, H. Associations of individuals' health-related behavior with their own or their spouses' smoking status. J. Epidemiol., *8:* 42–46, 1998.

24. Schantz, S. P., Zhang, Z. F., Spitz, M. S., Sun, M., and Hsu, T. C. Genetic susceptibility to head and neck cancer: interaction between nutrition and mutagen sensitivity. Laryngoscope, *107:* 765–781, 1997.

25. Spitz, M. R., Fueger, J. J., Halabi, S., Schantz, S. P., Sample, D., and Hsu, T. C. Mutagen sensitivity in upper aerodigestive tract cancer: a case-control analysis. Cancer Epidemiol. Biomark. Prev., *2:* 329–333, 1993.

26. Spitz, M. R., Hsu, T. C., and Schantz, S. P. Genetic and environmental interactions as risks for aerodigestive cancers. Adv. Exp. Med. Biol., *320:* 31–34, 1992.

27. Cloos, J., Spitz, M. R., Schantz, S. P., Hsu, T. C., Zhang, Z. F., Tobi, H., Braakhuis, B. J., and Snow, G. B. Genetic susceptibility to head and neck squamous cell carcinoma. J. Natl. Cancer Inst., *88:* 530–535, 1996.

Environmental Tobacco Smoking, Mutagen Sensitivity, and Head and Neck Squamous Cell Carcinoma

Zuo-Feng Zhang, Hal Morgenstern, Margaret R. Spitz, et al.

Cancer Epidemiol Biomarkers Prev 2000;9:1043-1049.