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Marijuana Use and Risk of Oral Squamous Cell Carcinoma

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ABSTRACT

Previous laboratory investigations, case reports, and a hospital-based case-control study have suggested that marijuana use may be a risk factor for squamous cell head and neck cancer. We conducted a population-based case-control study to determine whether marijuana use is associated with the development of oral squamous cell carcinoma (OSCC). Case subjects ($n = 407$) were 18–65-year-old residents of three counties in western Washington State who were newly diagnosed with OSCC from 1985 through 1995. Control subjects ($n = 615$), who were similar to the cases with respect to age and sex, were selected from the general population using random-digit telephone dialing. Lifetime histories of marijuana use and exposure to known OSCC risk factors were ascertained using a structured questionnaire. Information on genetic polymorphisms in glutathione *S*-transferase enzymes was obtained from assays on participant DNA. Odds ratios for associations with features of marijuana use were adjusted for sex, education, birth year, alcohol consumption, and cigarette smoking. A similar proportion of case subjects (25.6%) and control subjects (24.4%) reported ever use of marijuana (adjusted odds ratio, 0.9; 95% confidence interval, 0.6–1.3). There were no trends in risk observed with increasing duration or average frequency of use or time since first or last use. No subgroup defined by known or suspected OSCC risk factors (age, cigarette smoking, alcohol consumption, and genetic polymorphisms) showed an increased risk. Marijuana use was not associated with OSCC risk in this large, population-based study.

INTRODUCTION

Marijuana is the most commonly used illegal drug in the United States (1), and new users increased among minors during the 1990s (2). Marijuana smoke contains many known carcinogens (3), and experimental studies show that components of marijuana smoke are mutagenic in bacteria (4, 5) and cause molecular and cellular changes in bronchial tissue comparable with those seen among tobacco smokers and consistent with early steps in cancer development (6–8). Such findings raise the possibility that chronic marijuana use could cause premalignant changes in cells throughout the upper aerodigestive tract.

The possibility that marijuana use might be a risk factor for head and neck squamous cell carcinoma (HNSCC) was initially raised by several case reports (9). A small hospital-based study of HNSCC cases ($n = 173$) and blood donor controls ($n = 176$) found that ever users of marijuana were at >2-fold increased risk of HNSCC [odds ratio (OR), 2.6; 95% confidence interval (CI), 1.1–6.6] and that the risk increased with increasing frequency of marijuana use (10). Because hospital-based studies may be particularly susceptible to biases when lifestyle characteristics are the focus of investigation, we analyzed data from a population-based study to test the hypothesis that marijuana use is a risk factor for oral squamous cell carcinoma (OSCC).

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MATERIALS AND METHODS

Study Population. This report is based on participants, data, and biological specimens assembled during two population-based case-control studies originally designed to examine the association between human papillomavirus infection and OSCC risk (11, 12). All participants were residents of King, Pierce, or Snohomish counties, Washington State. Eligible cases in the first study were all 18–65-year-old men diagnosed with first, incident OSCC between January 1985 and December 1989. Eligible cases in the second study include all 18–65-year-old men and women diagnosed with first, incident OSCC between January 1990 and June 1995. OSCC patients were ascertained through the population-based Cancer Surveillance System, a participant in the Surveillance, Epidemiology, and End Results program (14). In both studies, only individuals who could communicate in English were eligible. OSCC was defined as *in situ* and invasive tumors of the tongue, gums, floor of mouth, tonsils, oropharynx, and other intraoral sites. In the earliest of the two studies (11), the definition of OSCC also included cancers of the lip (exclusive of the vermilion border); these cases of lip cancer were excluded from the present report. To be eligible, OSCC cases also were required to have residential telephones to ensure comparability with controls, who were identified for both previous studies through random-digit telephone dialing and frequency-matched to cases on age (18–19 years, 20–24 years, 25–29 years, . . . , 60–65 years) and sex. The protocols for recruitment of cases and controls in both studies were approved by the institutional review board of the Fred Hutchinson Cancer Research Center.

Combined across the two studies, 407 cases and 615 controls participated in an in-person interview (see below). These figures represented participation rates for cases in the first and second study of 54.4% and 63.3%, respectively, and 59.7% overall. Only eligible cases and controls were included in calculation of participation rates. Among the 275 nonparticipating cases in the previous two studies, 125 had died before they could be contacted for recruitment. The control response rates for the two studies were 63% and 61%, respectively; these rates incorporate both the household screening phase of random-digit telephone dialing and the success at interviewing eligible controls from among screened households.

Data and Biological Specimen Collection. Participating cases and controls were interviewed in-person by trained personnel using a structured questionnaire. The same questionnaire was used in both studies. To elicit histories of marijuana use, we initially asked each participant whether he or she had ever smoked marijuana or hashish (a stronger form of marijuana). If a participant had ever smoked these substances, he or she was asked about different episodes of marijuana or hashish use during his or her lifetime, with each episode representing a different frequency of use (elicited in terms of times per day, week, month, or year). For each episode, each participant was asked about the frequency of use, the age (in whole years) he or she started and stopped using marijuana or hashish at that frequency, and whether marijuana, hashish, or both substances were used. The interview also elicited demographic characteristics and extensive histories of tobacco use and alcohol consumption as described previously (13). All questions were directed toward the time period before each participant's reference date. The reference date for a case was the month and year he or she was diagnosed. Reference dates for controls were assigned at random from among the possible case diagnosis dates that had occurred before the selection of a particular control through random-digit telephone dialing.

Genetic Polymorphism Analyses. Because the putative carcinogenicity of marijuana may derive, at least in part, from exposure to polycyclic aromatic hydrocarbons and other tobacco-related carcinogens, we assayed for polymorphisms in several glutathione *S*-transferase (GST) genes (*GSTM1*, *GSTT1*, and *GSTP1*), which are known to be involved in the biotransformation of these compounds. Biological specimens from which genomic DNA could be ex-

tracted had been sought from each participating case and control during the original studies (13). Across the two studies, such specimens were available for 365 of 407 interviewed cases (89.7%) and 576 of 615 interviewed controls (93.7%). The null polymorphisms of *GSTM1* and *GSTT1* were assayed as described previously (15, 16). The *GSTP1* (I105V) polymorphism (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=947894) was assayed as follows: a 176-bp fragment was amplified using primers 5′-ACC-CCA-GGG-CTC-TAT-GGG-AA-3′ and 5′-TGA-GGG-CAC-AAG-AAG-CCC-CT-3′. Each 30-μl reaction contained 15 μl of Qiagen Taq PCR Master Mix, 0.2 μM each primer, and 100 ng of genomic DNA. Thermal cycling conditions were as follows: 1 cycle at 94°C for 5 min; 40 cycles at 94°C for 2 min, 60°C for 1 min, and 72°C for 2 min; and 1 cycle at 72°C for 5 min. *Bsm*A1 (New England Biolabs) restriction fragments were separated on a 4% nusieve gel.

Quality control samples included wells containing known genotype (positive controls), wells with PCR reagent only (negative controls), and paired replicate aliquots. Two reviewers independently read the gels and assigned genotypes without knowledge of the case-control status or other characteristics of each participant. Genetic analyses, which were restricted to whites because of variation in allele frequency across racial groups and because most of our study population was white (92.4% among controls), included the following numbers of subjects (percentage of total interviewed): *GSTM1*, 340 cases (89.0%) and 548 controls (83.5%); *GSTT1*, 339 cases (88.8%) and 547 controls (83.3%); and *GSTP1*, 355 cases (91.5%) and 565 controls (87.2%).

Statistical Analysis. We created variables to characterize various features of marijuana use, including ever use, time since first use, time since last use, years of use, and average frequency of use. The average frequency of use was calculated by first determining, for each episode, the number of times the participant had used marijuana (based on the length of the episode and the reported frequency of use). We then summed the total number of uses over each participant's lifetime and divided that number by the total weeks of use. These quantitative measures of the extent of marijuana use were categorized for analyses. A separate exposure category, with respect to each of these measures, was established for participants whose who had <1 year of use because this exposure level was felt to be minimal and could not be distinguished from years since first use or years since last use (*i.e.*, because their reported age at first and last use of marijuana was the same). The remaining categories used for marijuana use were established so that they represented either 5- or 10-year intervals (years of use, years since first use, years since last use) or could be directly compared with the results of Zhang *et al.* (times used/week; Ref. 10). Participants who reported never using marijuana comprised the referent group for comparisons.

We used standard methods for statistical analysis of case-control studies (17). ORs and 95% CIs were calculated using unconditional logistic regression. Analyses were adjusted for sex, education, birth year (continuous), average drinks of alcohol/week (continuous), pack-years of cigarette smoking (continuous), and whether the data came from the first (11) or second (12) of the previous studies. We assessed whether the data were consistent with effect measure modification between marijuana use and other characteristics (age, sex, cigarette smoking, alcohol consumption, and genetic polymorphisms) by estimating stratum-specific ORs associated with marijuana use and by estimating ORs jointly for marijuana use and each putative modifier relative to a common baseline consisting of individuals who never used marijuana and were in the *a priori* "low-risk" category of the modifier. We performed likelihood ratio tests of the fit of respective models with multiplicative and additive interaction terms compared with the fit of models without such terms. For multiplicative models, all terms were log-linear, whereas for additive models, confounders were log-linear, and the interaction terms were linear. Possible differences in the association with marijuana use according to tumor site (tongue, gum, floor of the mouth, tonsils and hypopharynx, and other sites) were assessed using polytomous logistic regression (18).

To evaluate the extent to which the reporting of marijuana use among our controls was consistent with other population-based studies, we analyzed publicly available data from the National Household Survey of Drug Abuse (NHSDA) conducted in 1988 and 1990–1994 [the NHSDA was not conducted in 1989) (19)]. These years largely include the reference dates we used for eliciting risk factor histories. We compared the observed number of controls who reported ever use of marijuana with the expected number, based on the birth cohort (or in some analyses, age) and sex-specific prevalences of "ever marijuana use" from the NHSDA and the birth cohort and sex-specific distribution of our controls. We calculated the observed:expected ratio of ever marijuana users and corresponding 95% CIs using the logarithmic transformation.

RESULTS

Cases had lower annual incomes and a lower educational level than controls (Table 1). The risk of OSCC was strongly related to cigarette smoking and alcohol consumption, as well as the combination of these characteristics (results not shown).

Table 2 shows the characteristics of ever and never users of marijuana among controls. Ever users of marijuana were more likely than never users to have been born more recently and to be under 50 years of age, to be male, to have a low income, and to have attended graduate school. Marijuana users more often smoked tobacco, but they were less likely than never marijuana users to smoke tobacco at the higher levels (≥30 pack-years). Marijuana users drank alcohol more frequently than nonusers of marijuana.

Twenty percent of cases and 16% of controls only used marijuana,

Table 1 *Characteristics of OSCC[a] cases and controls*

Characteristic	Case % (N = 407)	Control % (N = 615)	OR (95% CI)
Age at reference date (yrs)			
18–39	6.9	10.1	
40–49	20.4	23.2	
50–59	37.6	35.0	
60–65	35.1	31.7	
Sex			
Male	70.8	71.5	
Female	29.2	28.5	
Birth year			
1919–1929	30.0	26.8	
1930–1934	21.4	20.3	
1935–1939	17.0	16.4	
1940–1944	15.2	14.5	
1945–1949	7.4	9.4	
1950–1959	7.4	9.3	
1960–1971	1.7	3.2	
Race[b]			
White	93.9	94.1	1.0
Black	3.7	2.8	1.2 (0.6–3.2)
Other	2.5	3.1	1.6 (0.5–2.6)
Income[b,c]			
<$15,000	23.2	5.8	1.0
$15,000 to $29,999	26.2	20.8	0.4 (0.2–0.7)
$30,000 to $44,999	24.7	25.6	0.3 (0.2–0.5)
≥$45,000	25.9	47.8	0.2 (0.1–0.4)
Education[b]			
High school or less	44.7	29.0	1.0
Technical school	7.9	5.9	1.1 (0.6–1.9)
College	37.8	49.1	0.7 (0.5–1.0)
Graduate school	9.6	16.0	0.7 (0.5–1.2)
Cigarette smoking (pack-years)[d]			
<1	14.6	38.3	1.0
1–9	6.4	15.7	1.0 (0.6–1.8)
10–19	7.6	11.7	1.6 (0.9–2.7)
20–29	10.9	12.2	2.0 (1.2–3.3)
≥30	60.5	22.0	6.1 (4.1–9.3)
Alcohol consumption (drinks/week)[e]			
<1	13.5	26.7	1.0
1–7	26.8	43.1	1.1 (0.7–1.6)
8–14	15.5	14.5	1.6 (1.0–2.7)
15–28	17.2	9.8	2.2 (1.2–3.8)
≥29	27.0	6.0	4.4 (2.0–9.6)

[a] OSCC, oral squamous cell carcinoma; OR, odds ratio; CI, confidence interval.

[b] ORs and 95% CIs adjusted for sex, birth year (continuous), cigarette smoking (continuous pack-years), alcohol consumption (continuous average drinks/week), and study (first or second). Excludes two cases and two controls with missing data on pack-years of cigarette smoking.

[c] Excludes 9 cases and 11 controls with missing data on income.

[d] ORs and 95% CIs adjusted for sex, birth year (continuous), alcohol consumption (continuous average drinks/week), and study (first or second). Excludes two cases and two controls with missing data on pack-years of cigarette smoking.

[e] ORs and 95% CIs adjusted for sex, birth year (continuous), cigarette smoking (continuous pack-years), and study (first or second). Excludes two cases and two controls with missing data on pack-years cigarette smoking.

Table 2 *Characteristics of ever users and never users of marijuana among controls*

Characteristic	Ever user % (N = 150)	Never user % (N = 465)
Birth year		
1919–1929	4.0	34.2
1930–1934	9.3	23.9
1935–1939	11.3	18.1
1940–1944	18.7	13.1
1945–1949	18.7	6.4
1950–1959	28.7	3.0
1960–1971	9.3	1.3
Age (yrs)		
18–39	31.3	3.2
40–49	38.7	18.3
50–59	20.7	39.6
60–65	9.3	38.9
Sex		
Male	78.7	69.2
Female	21.3	30.8
Race		
White	93.3	94.4
Black	4.0	2.4
Other	2.7	3.2
Income[a]		
<$15,000	11.5	3.9
$15,000 to $29,999	16.2	22.1
$30,000 to $44,999	24.3	26.1
≥$45,000	48.0	47.8
Education		
High school or less	22.7	31.2
Technical school	4.7	6.2
College	50.7	48.6
Graduate school	22.0	14.0
Cigarette smoking (pack-years)[b]		
<1	31.3	40.6
1–9	18.0	14.9
10–19	14.7	10.8
20–29	17.3	10.6
≥30	18.7	23.1
Alcohol consumption (drinks/week)		
<1	13.3	31.0
1–7	36.7	45.2
8–14	17.3	13.6
15–28	20.0	6.3
≥29	12.7	3.9
Cigarette smoking & alcohol consumption[b]		
<20 pack-years/<15 drinks/week	48.0	63.5
<20 pack-years/≥15 drinks/week	16.0	2.8
≥20 pack-years/<15 drinks/week	19.3	26.1
≥20 pack-years/≥15 drinks/week	16.7	7.6

[a] Excludes two users and seven never users who had missing information on income.
[b] Excludes two never users who had missing information on pack-years of smoking.

5% of cases and 6% of controls used both marijuana and hashish, and <1% of cases and of controls only used hashish. Table 3 displays the association between marijuana use and oral cancer, adjusted for sex, education, birth year, average number of alcoholic drinks/week, and pack-years of cigarette smoking. There was no association with ever having used marijuana (OR, 0.9; 95% CI, 0.6–1.3), total years of marijuana use, average frequency of marijuana use, years since first use of marijuana, or years since last use of marijuana. There was no discernable difference across oral tumor sites in the association with ever having used marijuana, nor did the association vary by stage at diagnosis (data not shown).

The magnitude of the association between ever marijuana use and oral cancer varied little among subgroups defined by known or suspected demographic, lifestyle, or genetic risk factors, whether such assessments were made against the null hypothesis of multiplicative or additive joint effects (Table 4). Individuals with at least one copy of *GSTM1* had a borderline statistically significant reduced risk of OSCC associated with marijuana use (OR, 0.5; 95% CI, 0.3–1.0), whereas those with the null *GSTM1* genotype showed no association with marijuana use (OR, 1.1; 95% CI, 0.7–1.9; $P < 0.05$ for test of heterogeneity between strata). Evaluated under an additive model of combined effects, the association between marijuana use and OSCC varied in a statistically significant manner across categories of cigarette smoking status (current, former, and never), with an inverse association among current and never smokers, but no association among former smokers. ORs for marijuana use, combined with either alcohol use or alcohol and pack-years of cigarette smoking together, suggested stronger risks for these established OSCC risk factors among never marijuana users.

We observed 150 ever users of marijuana among our controls, and we would have expected 146.8 controls to have reported ever use of marijuana based on the sex- and birth cohort-specific ever marijuana use estimates from the NHSDA (observed:expected ratio, 1.0; 95% CI, 0.8–1.3). A similar expected number of ever marijuana users among controls (n = 141.8) was obtained when the calculations were based on the sex- and age-specific NHSDA data (observed:expected ratio, 1.1; 95% CI, 0.8–1.3).

DISCUSSION

In this large, population-based study, we did not find any association between marijuana use and OSCC risk. The absence of an increased risk was largely consistent across analyses using different measures of marijuana use (*e.g.*, ever use, frequent *versus* infrequent use, and long-term *versus* short-term use) and among subgroups representing different levels of underlying risk.

The possibility that marijuana use might increase the risk of cancer was initially raised more than 20 years ago when marijuana smoke components yielded positive results in Ames salmonella/microsome mutagenesis assays (4, 5). However, in other non-human model systems, Δ^9-tetrahydrocannabinol, the psychoactive component of marijuana, both induced expression, and inhibited the activity, of cytochrome p450 1A1 (3); the combination of tobacco extracts and Δ^9-tetrahydrocannabinol also led to reduced cytochrome p450 1A1 activity. In studies focusing directly on tumor development and growth, cannabinoids have been shown to have both tumorigenic (20,

Table 3 *Risk of OSCC[a] associated with use of marijuana*

Marijuana use	Case % (N = 407)	Control % (N = 615)	OR (95% CI)
Ever use			
Never	74.4	75.6	1.0[b]
Ever	25.6	24.4	0.9 (0.6–1.3)
Years of use[c]			
<1 yr	7.9	6.5	0.8 (0.4–1.2)
1 yr	1.0	3.1	0.2 (0.1–0.7)
2–5 yrs	4.7	3.9	1.3 (0.6–2.6)
6–15 yrs	5.9	6.5	0.7 (0.4–1.4)
>15 yrs	6.1	4.4	1.2 (0.6–2.2)
Times used/week[c]			
<1 year use	7.9	6.5	1.0 (0.6–1.8)
<1 times/week	10.1	9.3	0.8 (0.5–1.4)
1–7 times/week	6.1	6.7	0.8 (0.4–1.6)
>7 times/week	1.5	2.0	0.5 (0.2–1.6)
Years since first use			
<1 yr total use	7.9	6.5	1.0 (0.6–1.8)
<15 yrs	3.7	3.1	0.7 (0.3–1.6)
16–20 yrs	4.2	5.4	0.7 (0.3–1.4)
21–25 yrs	5.6	6.3	0.9 (0.5–1.7)
>25 yrs	4.2	3.1	0.9 (0.4–2.0)
Years since last use[c]			
<1 yr total use	7.9	6.5	1.0 (0.6–1.8)
Current use	8.1	5.4	1.1 (0.6–2.0)
<10 yrs	3.0	3.6	0.7 (0.3–1.7)
11–20 yrs	4.9	7.3	0.7 (0.4–1.3)
>20 yrs	1.7	1.6	0.7 (0.3–2.1)

[a] OSCC, oral squamous cell carcinoma; OR, odds ratio; CI, confidence interval.
[b] Reference group for calculation of ORs. All ORs are adjusted for sex, education, birth year (continuous), alcohol consumption (continuous average drinks/week), cigarette smoking (continuous pack-years), and study (first or second). Excludes two cases and two controls with missing data on pack-years of cigarette smoking.
[c] Among individuals who used marijuana for at least 1 year and compared with persons who had never used marijuana.

Table 4 *Risk of OSCC[a] associated with use of marijuana in subgroups of the study population*

Subgroup	Marijuana use	Case % (N = 407)	Control % (N = 615)	OR[b] (95% CI)	OR[c] (95% CI)
Age[d]					
<55	No	26.0	28.6	1.0	1.0
	Yes	18.4	20.6	0.8 (0.5–1.2)	0.8 (0.5–1.2)
≥55	No	48.4	47.0	1.0	0.6 (0.4–0.9)
	Yes	7.1	3.7	1.5 (0.8–2.9)	1.0 (0.5–1.9)
				$LRT_{mult:}$ P = 0.11[e]	
				$LRT_{add:}$ P = 0.11[f]	
Sex[g]					
Male	No	50.4	52.4	1.0	1.0
	Yes	20.4	19.2	0.9 (0.6–1.4)	0.9 (0.6–1.4)
Female	No	24.1	23.2	1.0	2.3 (1.6–3.5)
	Yes	5.2	5.2	0.7 (0.4–1.4)	1.7 (0.9–3.3)
				$LRT_{mult:}$ P = 0.516[e]	
				$LRT_{add:}$ P = 0.396[f]	
Cigarette smoking status at reference date[h]					
Never smoker	No	12.0	28.5	1.0	1.0
	Yes	2.0	7.0	0.6 (0.2–1.4)	0.6 (0.2–1.4)
Former smoker	No	17.0	32.4	1.0	1.2 (0.7–1.8)
	Yes	7.4	8.8	1.3 (0.7–2.3)	1.5 (0.8–2.7)
Current smoker	No	45.5	14.8	1.0	5.1 (3.3–7.8)
	Yes	16.2	8.6	0.6 (0.3–0.9)	2.9 (1.7–5.0)
				$LRT_{mult:}$ P = 0.07[e]	
				$LRT_{add:}$ P = 0.029[f]	
Py of cigarette smoking[h]					
<1	No	12.3	30.7	1.0	1.0
	Yes	2.2	7.7	0.5 (0.2–1.2)	0.5 (0.2–1.2)
1–19	No	7.9	19.4	1.0	1.0 (0.5–1.9)
	Yes	6.2	8.0	1.2 (0.6–2.4)	1.3 (0.7–2.4)
20–29	No	5.4	8.0	1.0	1.5 (0.8–2.7)
	Yes	5.4	4.2	1.6 (0.7–3.6)	2.3 (1.2–4.6)
≥30	No	48.9	17.4	1.0	6.0 (3.8–9.3)
	Yes	11.6	4.6	0.7 (0.4–1.2)	4.0 (2.2–7.5)
				$LRT_{mult:}$ P = 0.117[e]	
				$LRT_{add:}$ P = 0.112[f]	
Alcohol consumption (dwk)[i]					
<1	No	12.0	23.4	1.0	1.0
	Yes	1.5	3.2	0.7 (0.2–1.9)	0.7 (0.2–1.9)
1–14	No	31.9	44.3	1.0	1.0 (0.6–1.5)
	Yes	10.3	13.2	1.2 (0.7–1.9)	1.3 (0.8–2.3)
15–28	No	13.0	4.9	1.0	3.1 (1.6–6.0)
	Yes	4.7	5.0	0.5 (0.2–1.0)	1.4 (0.7–2.9)
≥29	No	17.4	2.9	1.0	6.4 (3.2–12.7)
	Yes	9.1	2.9	0.6 (0.3–1.4)	3.9 (1.9–8.0)
				$LRT_{mult:}$ P = 0.73[e]	
				$LRT_{add:}$ P = 0.096[f]	
Smoking and drinking[j]					
<20 p-y & <15 d/wk	No	18.0	48.0	1.0	1.0
	Yes	5.4	11.7	1.1 (0.6–2.0)	1.1 (0.6–2.0)
<20 p-y & ≥15 d/wk	No	2.2	2.1	1.0	3.2 (1.3–8.1)
	Yes	3.0	3.9	0.7 (0.2–2.1)	2.2 (1.0–4.7)
≥20 p-y & <15 d/wk	No	25.9	19.7	1.0	3.7 (2.5–5.5)
	Yes	6.2	4.7	1.0 (0.6–2.0)	3.9 (2.1–7.2)
≥20 p-y & ≥15 d/wk	No	28.4	5.7	1.0	15.3 (9.3–25.1)
	Yes	10.8	4.1	0.5 (0.3–1.0)	8.3 (4.6–14.8)
				$LRT_{mult:}$ P = 0.326[e]	
				$LRT_{add:}$ P = 0.254[f]	
GSTM1[k]					
Non-null	No	38.5	36.9	1.0	1.0
	Yes	9.1	13.1	0.5 (0.3–1.0)	0.5 (0.3–1.0)
Null	No	35.9	37.8	1.0	1.0 (0.7–1.4)
	Yes	16.4	12.2	1.2 (0.7–2.0)	1.1 (0.7–1.9)
				$LRT_{mult:}$ P = 0.036[e]	
				$LRT_{add:}$ P = 0.053[f]	
GSTT1[k]					
Non-null	No	58.1	60.3	1.0	1.0
	Yes	19.1	20.6	0.7 (0.5–1.2)	0.7 (0.5–1.2)
Null	No	16.2	14.3	1.0	1.0 (0.7–1.7)
	Yes	6.5	4.8	1.2 (0.6–2.7)	1.3 (0.6–2.6)
				$LRT_{mult:}$ P = 0.222[e]	
				$LRT_{add:}$ P = 0.349[f]	
GSTP1[k]					
^{105}Ile/Ile	No	32.1	35.0	1.0	1.0
	Yes	10.1	11.5	0.8 (0.4–1.4)	0.8 (0.4–1.4)
^{105}Val/Ile or ^{105}Val/Val	No	42.0	39.8	1.0	1.1 (0.8–1.6)
	Yes	15.8	13.6	1.0 (0.6–1.7)	1.1 (0.6–1.8)
				$LRT_{mult:}$ P = 0.424[e]	
				$LRT_{add:}$ P = 0.419[f]	

[a] OSCC, oral squamous cell carcinoma; OR, odds ratio; CI, confidence interval; LRT, likelihood ratio test; p-y, pack-years; d/wk, average drinks/week; mult, multiplicative; add, additive.
[b] OR for ever marijuana use *versus* never marijuana use, within indicated subgroups. Excludes two cases and two controls with missing data on pack-years cigarette smoking.
[c] Odds ratio for joint association of ever marijuana use and each characteristic, relative to common reference group. Excludes two cases and two controls with missing data on pack-years cigarette smoking.
[d] Adjusted for sex, education, alcohol use (continuous average drinks/week), cigarette smoking (continuous pack-years), and study (first or second).
[e] P values from likelihood ratio test of hypothesis that the joint association does not depart from multiplicative model.
[f] P values from likelihood ratio test of hypothesis that the joint association does not depart from additive model.
[g] Adjusted for age, education, birth year (continuous), alcohol use (continuous average drinks/week), cigarette smoking (continuous pack-years), and study (first or second).
[h] Adjusted for sex, education, birth year (continuous), alcohol use (continuous average drinks/week), and study (first or second).
[i] Adjusted for sex, birth year (continuous), cigarette smoking (continuous pack-years), and study (first or second).
[j] Adjusted for sex, education, birth year (continuous), and study.
[k] Adjusted for sex, education, birth year (continuous), alcohol use (continuous average drinks/week), cigarette smoking (continuous pack-years), and study (first or second), restricted to white participants. Analyses stratified by *GSTM1*, *GSTT1*, and *GSTP1* polymorphisms are based on 340 cases and 548 controls, 339 cases and 547 controls, and 355 cases and 565 controls, respectively. *GSTP1* genotypes were in Hardy-Weinberg equilibrium among controls (P = 0.06).

21) and antitumor (22, 23) properties. These findings suggest that the ultimate effect of marijuana use on OSCC development, if any, results from opposing physiological pathways. Nonetheless, human *in vivo* studies of chronic marijuana smokers have found increased premalignant changes in bronchial tissue (8, 24) akin to those observed in lung tissue from tobacco smokers.

Reports of young patients with OSCC and other respiratory tract cancers raised the question of whether marijuana use contributed to these malignancies (9). These reports lacked comparison groups and control for established risk factors and thus provide little evidence for or against the existence of an association. A cohort study did not provide results specific to OSCC or HNSCC, but for lung cancer and other smoking-related and/or alcohol-related cancers in general, no association was found (25). The only epidemiological study of marijuana use and HSNCC found a 2–3-fold increased risk associated with ever marijuana use, a dose-response relationship with frequency and duration of use, and evidence of particularly strong associations with ever use among several subgroups [*e.g.,* younger individuals, smokers, and those exhibiting mutagen sensitivity (10)]. Although our study had a greater proportion of participants who had used marijuana for >5 years [10.9% *versus* 3.5% in Zhang *et al.* (10)], we did not confirm their findings.

Differences in the extent to which control groups represent the population from which the cases arise potentially explain the discrepancy between our null findings and the increased risks observed by Zhang *et al.* (10). Blood donors comprise a highly self-selected population likely to be depleted of individuals with high-risk lifestyle behaviors; thus, the prevalence of marijuana use by blood donor controls in Zhang *et al.* (10) may have been spuriously low in comparison with what would have been observed in a group of controls that more closely reflected the source population for their cases. Alternatively, as Zhang *et al.* (10) hypothesized, controls may in general underreport marijuana use to a greater extent than cases. Zhang *et al.* (10) used published NHSDA estimates (26) to show that the observed prevalence of ever marijuana use among their controls was similar to the prevalence expected in the general population (10). The published data from which Zhang *et al.* (10) calculated expected numbers, however, excluded persons who had initiated marijuana use after 20 years of age (Table 5 in Ref. 10; Table 3 in Ref. 26), whereas the observed number of users among their controls included individuals who had used marijuana regardless of the age at initiation. We recalculated the sex- and birth cohort-adjusted expected prevalence of ever marijuana use among controls in Zhang *et al.* (10) without the exclusion based on age at initiation (using publicly available NHSDA data for individuals who were ≥18 years old in 1992–1994). The expected number of ever marijuana users was 40.6, whereas only 17 users were observed. If a similar deficit was not present among the HNSCC cases in Zhang *et al.* (10), some or all of the 2.6-fold association with marijuana use they observed would be due to a spuriously low exposure prevalence among their controls. The results of similar calculations performed for our control group showed no difference in the observed and expected number of ever marijuana users.

Marijuana cigarettes do not contain filters, as tobacco cigarettes do, and are typically smoked well into the proximal end. Furthermore, marijuana smokers may inhale more deeply and hold the smoke in their lungs longer than tobacco smokers (27, 28). These latter characteristics of marijuana smoking may explain why lung tar levels are higher for marijuana cigarettes compared with tobacco cigarettes (29). If marijuana is an oral cavity carcinogen, we nonetheless may not have observed an association because the amount of marijuana consumed by a typical user is substantially less than the amount of tobacco consumed by a typical tobacco smoker (1), and a substantial proportion of our population were not chronic, long-term users of this drug. We also did not ask about the number of marijuana cigarettes or bowls of hashish smoked or the depth of inhalation.

Our study included only OSCC, whereas laryngeal carcinomas comprised a large proportion (27%) of the cases in Zhang *et al.* (10). Marijuana use was reported similarly by their laryngeal cancer cases (22%) and tongue carcinoma cases (approximately 19%), and we found no evidence that the marijuana use association varied by OSCC site. Potential etiologic heterogeneity among HNSCC of different organs thus does not seem to explain the differences between our results and those of Zhang *et al.* (10).

Our study was not without important limitations. We had relatively low participation, and any association between marijuana use and participation status that differed between cases and controls could have biased our results. If an association between marijuana use and OSCC truly exists, but the prevalence of marijuana use among our controls is close to that expected (as discussed above), then under-recruitment of cases who had used marijuana or under-reporting of marijuana use by cases due to the drug's illegality could have led to our null associations. Our study observed well-established associations with tobacco smoking and alcohol drinking, however, providing some reassurance that low participation among our cases has not noticeably affected our study. Our study also did not have data on mutagen sensitivity status, which Zhang *et al.* (10) found to be a strong modifier of the risk associated with marijuana use. Because Zhang *et al.* (10) observed an association between HNSCC and marijuana use even without considering mutagen sensitivity status, we also should have observed an association if the prevalence of mutagen sensitivity among our participants was similar to that in Zhang *et al.* (10).

Studies of HNSCC have not observed consistent associations with the *GSTM1*-null, *GSTT1*-null, or *GSTP1* I105V polymorphisms (30–34), but few have examined whether these polymorphisms modify the risk associated with exposure to sources of carcinogens that the enzymes metabolize. We did not find that carriers of the "high-risk" genotypes of *GSTM1*, *GSTT1*, or *GSTP1* who also smoked marijuana were at greater risk than predicted on a multiplicative or additive scale. Marijuana use was associated with a borderline statistically significant 50% reduced risk of OSCC among individuals carrying at least one copy of the *GSTM1* gene, statistically distinguishable from the absence of an association among *GSTM1*-null homozygotes. The reduced risk of OSCC among carriers of *GSTM1* could represent a chance finding or could be consistent with induction of detoxifying *GSTM1* activity by marijuana constituents. Although we also observed statistically distinguishable heterogeneity in the association with marijuana use among current, former, and never cigarette smokers, the pattern of ORs did not fit any obvious biologically based model, nor was similar effect modification seen with pack-years of cigarette smoking. In general, our study was not sufficiently large to allow us to reliably assess the potential modifying effects of known or suspected OSCC risk factors on risks associated with marijuana use.

Although the evidence from nonepidemiological investigations suggests that marijuana smoking could cause upper respiratory tract cancer, we did not observe an association with OSCC in this study. Nonetheless, because our data included relatively few individuals who had used marijuana for many years, we cannot discount the possibility that long-term use of this drug is related to OSCC risk. As individuals born since the 1940s age into their sixth decade of life (when the baseline rates of OSCC start to rise dramatically), the prevalence of long-term use marijuana use in the population will increase. This demographic change will permit future studies to assess more definitively the role of marijuana in OSCC development.

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