Original Article

Population-Based Case-Control Study of Recreational Drug Use and Testis Cancer Risk Confirms an Association Between Marijuana Use and Nonseminoma Risk

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BACKGROUND: Testicular germ cell tumor (TGCT) incidence increased steadily in recent decades, but causes remain elusive. Germ cell function may be influenced by cannabinoids, and 2 prior epidemiologic studies reported that the use of marijuana may be associated with nonseminomatous TGCT. Here, the authors evaluate the relation between TGCTs and exposure to marijuana and other recreational drugs using a population-based case-control study. **METHODS:** In total, 163 patients who were diagnosed with TGCT in Los Angeles County from December 1986 to April 1991 were enrolled, and 292 controls were matched on age, race/ethnicity, and neighborhood. Participants were asked about drug use by a structured, in-person interview. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using conditional logistic regression analysis adjusted for history of cryptorchidism; education; religiosity; and reported use of marijuana, cocaine, and amyl nitrite. **RESULTS:** Compared with never use, ever use of marijuana had a 2-fold increased risk (OR, 1.94; 95% CI, 1.02-3.68), whereas ever use of cocaine had a negative association with TGCT (OR, 0.54; 95% CI, 0.32-0.91). Stratification on tumor histology revealed a specific association of marijuana use with nonseminoma and mixed histology tumors (OR, 2.42; 95% CI, 1.08-5.42). **CONCLUSIONS:** A specific association was observed between marijuana use and the risk of nonseminoma and mixed tumors. To the authors' knowledge, this is the first report of a negative association between cocaine use and TGCT risk. The current results warrant mechanistic studies of marijuana's effect on the endocannabinoid system and TGCT risk and caution that recreational and therapeutic use of cannabinoids by young men may confer malignant potential to testicular germ cells. ***Cancer*** **2012;000:000–000.** © *2012 American Cancer Society.*

KEYWORDS: testicular germ cell tumors, nonseminoma, marijuana, cannabis, Δ^9-tetrahydrocannabinol, cocaine, amyl nitrite, recreational drug use, tobacco, alcohol.

INTRODUCTION

Testicular germ cell tumors (TGCTs) are the most common neoplasm of adolescent and adult men ages 15 to 45 years,[1] and young survivors face significant sequelae, including elevated rates of cardiovascular disease and second primary malignancies.[2] Therefore, understanding the etiology of TGCT as the basis of prevention is an important research priority. TGCT incidence has been rising for decades,[3] which implies a change in exposure to 1 or more nongenetic risk or protective factors. However, no such characteristics have been identified despite years of investigation. An established risk factor for TGCT is cryptorchidism, or undescended testicles, which, in rodent models, can be caused by exogenous estrogens during key periods of development.[4,5] These findings, along with epidemiologic data indicating the onset of greatest TGCT risk around the time of puberty, implicate aberrant effects of steroid hormones in the perinatal and peripubertal periods. Potential exposures in the perinatal period include elevated maternal estrogens and exposure to exogenous hormones, such as diethylstilbestrol. However, studies of these exposures are limited by the need to retrospectively measure relevant exposures after TGCT diagnosis, 15 or more years later. Additional epidemiologic data are consistent with the possibility that risk may be related to variation in endogenous hormone levels during puberty, because proxies such as severe acne and male pattern baldness were associated with an increased likelihood of testis cancer.[6]

More recently, exogenous compounds with postulated or demonstrated endocrine function have been proposed as possible testicular carcinogens, with polychlorinated biphenyls, organochlorines, and other persistent organic pollutants implicated by recent epidemiologic studies.[7-10] Constituents of marijuana smoke also may act on the endocrine system,

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and marijuana use has increased in recent decades[11,12] and is highest among males ages 15 to 20 years in the United States.[12] Thus, Daling et al postulated that marijuana use may increase TGCT risk and, in a population-based case-control study,[13] observed that patients with TGCT were more likely to have smoked marijuana and that the risk increased with frequency and duration of use, as well as earlier initiation of use and use ongoing until diagnosis. The association was limited to nonseminoma histologic types. Subsequent results from a smaller, hospital-based, friend-control study also reported an association of marijuana use with the risk of nonseminoma.[14] Results from those studies and the biologic plausibility of endocrine action of marijuana constituents indicate that marijuana smoke may contain a testicular carcinogen. Alternatively, the reported marijuana-testis cancer association may arise at least in part from a tendency of men who smoke marijuana to more frequently experience unrecognized testis cancer risk factors, such as psychosocial correlates of marijuana use, which may include exposure to other recreational drugs. In the current population-based case-control study, we estimated associations between testis cancer risk and exposure to marijuana, accounting for several demographic factors and detailed histories of other recreational drug use.

MATERIALS AND METHODS

Study Participants and Data

A population-based case-control study was conducted in Los Angeles County, California to investigate associations of testis cancer risk with exposures of the perinatal period, childhood, adolescence, and early adulthood. Eligible cases were men diagnosed with TGCT between December 20, 1986 and April 4, 1991 in Los Angeles County; who were ages 18 to 35 years at diagnosis; spoke English; and were born either in the United States, Europe, Canada, or the Middle East. The Los Angeles County Cancer Surveillance Program identified 201 men who met these criteria. Of these, 8 had either died before being contacted or were too ill to participate, 6 had cognitive difficulties precluding participation, 14 could not be located, 2 were not invited to participate because health care providers denied permission to contact, and 8 chose not to participate. The remaining 163 eligible cases (81%) participated in the study. For each participating case, we attempted to enroll up to 4 unaffected control men who were matched to the case on date of birth (within 3 years), race, ethnicity, and neighborhood of residence at the time of diagnosis. An established procedure was used to identify potential controls by defining a sequence of housing units on a specified block; attempting to identify the sex, age, race, and ethnicity of the inhabitants of each unit; and thereby determining whether 1 or more appropriately matched potential controls were identified. If no potential control was identified after canvassing 150 units, then race was excluded from the matching criteria; and, if a matched control based on the relaxed criteria was not identified within 150 subsequent units, then the case was not included in matched case-control analyses. The procedure described above identified 371 individuals as potential controls for the 163 eligible cases. Of these, 292 men (78.7%) chose to participate. We enrolled controls that were not matched on race/ethnicity for 24 cases, and we were unable to enroll any matched control for 24 additional cases. From 1 to 4 matched controls were enrolled for each remaining case: We identified 1 control for 16 cases, 2 controls for 95 cases, 3 controls for 26 cases, and 4 controls for 2 cases.

Interviews were conducted in person at participants' homes and were administered by trained interviewers using structured questionnaires. All participants were interviewed between October 16, 1987 and December 15, 1994. Information was requested for a reference period up to 1 year before the diagnosis of TGCT for cases and the same date for each case's matched controls. Participants were asked to provide demographic information; family history of specific cancers and urogenital conditions; personal history of some infectious diseases; and personal use of tobacco, alcohol, and numerous recreational drugs. Participants were asked whether they had ever used each drug, and ever users were asked the first and last years of use and the average number of times per week of use.

Statistical Methods

Relevant variables from the questionnaire were tested individually using univariate conditional logistic regression that estimated odds ratios (ORs) and 95% confidence intervals (CIs). The current analyses were focused on use of recreational drugs in relation to TGCT risk. We selected for model-building variables that either were notable for the magnitude of their disease association or statistical significance or were demonstrated TGCT risk factors.

All drugs were treated as dichotomous variables indicating ever use versus never use, and ever use of most drugs was divided further into current use at reference date or former use. Frequency of use was categorized as never, less than once per week, or more than once per week. Duration of use was categorized as never, less than

10 years of use, and 10 or more years of use. Sensitivity analyses were conducted to explore the effect of using different cutoff points for categories of marijuana frequency and duration. Duration of alcohol consumption was not queried. Because of sparse numbers, duration of mushroom use was categorized as never, less than 2 years, or more than 2 years (the median duration of mushroom use), and frequency and duration of use were not examined for mushrooms; barbiturates; heroin; lisergic acid diethylamide (LSD); Quaaludes; phencyclidine (PCP); ethyl chloride; butyl nitrate; poppers other than amyl nitrite, ethyl chloride, or butyl nitrate; or recreational drugs other than those queried in the interview.

The covariates that were used in the multivariate models were selected by adding variables 1 at a time to the model and assessing whether the point estimate of the OR changed by >10%, and covariates with this level of influence were retained in the model. The following covariates were identified as confounders of associations between use of marijuana or cocaine and TGCT: cryptorchidism (yes vs no), religiosity (any religious affiliation vs none), and education level (college degree or higher, some college, high school diploma, or less than a high school diploma). In addition, estimates of association for each other drug were adjusted for ever use of marijuana, cocaine, and amyl nitrite.

By using information from original pathology reports, histologic type was coded as either seminoma (International Classification of Diseases for Oncology histology codes 9061, 9062, and 9063), nonseminoma (codes 9065, 9070, 9071, 9080, 9082, 9083, 9084, and 9100), or mixed germ cell tumors (GCTs) (codes 9081, 9085, and 9101). For statistical analyses, we distinguished between pure seminoma of any type (*seminoma*) and nonseminoma or mixed GCT of any type (*nonseminoma/mixed GCT*). These definitions follow the convention of grouping histologic types according to the degree of differentiation while constituting subgroups likely to be uniformly scored between clinical centers and from the study enrollment period forward in time. Moreover, they are comparable to the definitions used in published studies that address marijuana use in relation to TGCT risk.[13,14] Heterogeneity of disease associations of selected drugs across histologic subtypes of TGCT (seminoma vs nonseminoma/mixed GCT) was tested by including an interaction term between histology and the respective main variable into the conditional logistic model together with confounders. Controls were coded with the same histology as their matched case. Histology-specific ORs were computed using the full model with the interaction term.

RESULTS

Cases were more likely than controls to have a history of cryptorchidism (10.4% vs 2.7%) and to have a higher education level, and they were less likely than controls to report a religious affiliation (Table 1). Ever use of marijuana was associated with a nearly 2-fold increased risk of TGCT of any histologic type (OR, 1.94; 95% CI, 1.02-3.68), after adjusting for education, religiosity, history of cryptorchidism, ever use of cocaine, and ever use of amyl nitrite. Compared with never users, current marijuana users had a nonsignificant increase in risk (OR, 1.38; 95% CI, 0.67-2.87), whereas former users had greater than 2-fold risk (OR, 2.28; 95% CI, 1.17-4.43). These associations did not follow simple dose-response patterns over categories defined by duration or frequency of use. For example, compared with never users, those who reported using marijuana less often than once per week were more than twice as likely to develop TGCT (OR, 2.10; 95% CI, 1.09-4.03), whereas those who reported more frequent use had a lesser and nonsignificant increase in risk (OR, 1.53; 95% CI, 0.73-3.24). Men who reported <10 years of marijuana use were more than twice as likely to develop TGCT (OR, 2.09; 95% CI, 1.09-3.98), whereas a lesser and nonsignificant increase was observed for those reporting ≥10 years of use (OR, 1.51; 95% CI, 0.66-3.47) (Table 2).

Men who reported ever using cocaine had a notably reduced risk of TGCT in analyses that were adjusted for the above-mentioned covariates and marijuana use (OR, 0.54; 95% CI, 0.32-0.91). There was no indication that the risk differed further across categories of frequency or duration.

Although the estimate of an association between amyl nitrite use and TGCT did not achieve statistical significance (OR, 0.50; 95% CI, 0.25-1.02), ever use of this drug did confound estimates of disease associations with ever use of both marijuana and cocaine and, thus, was included as a covariate in final analytic models. Neither pronounced disease associations nor confounding effects were noted for reported use of inhaled ethyl chloride, butyl nitrate, or other poppers; hallucinogenic mushrooms; amphetamines; barbiturates; LSD; Quaaludes; PCP; heroin; reported use of any recreational drug not specifically queried in the interview; or a derived variable combining reported use of any of the aforementioned drugs, alcohol, or tobacco smoked in cigarettes.

Ever use of marijuana appeared to be unassociated with risk of seminoma (OR, 1.07; 95% CI, 0.37-3.07) (Table 3) but was associated with a greater than 2-fold risk of either nonseminoma or mixed GCT (OR, 2.42; 95% CI, 1.08-5.42). Associations with nonseminoma or mixed

Table 1. Characteristics of Patients With Testicular Germ Cell Tumor (Cases) and Matched Population-Based Controls: Los Angeles County, California, 1986-1991

Variable	No. (%)		
	All Cases, N = 163	Cases With Matched Controls, N = 139	Controls, N = 292
Age: Median±SD, y	27±12.7	27±3.7	26±3.6
Race/ethnicity			
Non-Hispanic white	127 (77.9)	109 (78.4)	247 (84.6)
Hispanic white	36 (22.1)	30 (21.6)	44 (15.1)
Native American			1 (0.34)
Cryptorchidism	17 (10.4)	14 (10.1)	8 (2.7)
Education			
≥College degree	27 (16.6)	25 (18)	30 (10.3)
Some college	44 (27)	35 (25.2)	72 (24.7)
High school diploma	55 (33.7)	46 (33.1)	86 (29.5)
<High school diploma	37 (22.7)	33 (23.7)	104 (35.6)
Religiosity			
No religious affiliation	48 (29.6)	41 (29.7)	65 (22.3)
Any religious affiliation	114 (70.4)	97 (70.3)	226 (77.7)
TGCT history, first-degree relative[a]	1 (1.3)	1 (0.7)	1 (0.34)
Tumor histology			
Seminoma	56 (34.4)	47 (33.8)	
Nonseminoma/mixed GCT	95 (58.3)	80 (57.6)	
Unknown	12 (7.36)	12 (8.6)	

Abbreviations: GCT, germ cell tumor; SD, standard deviation; TGCT, testicular germ cell tumor.
[a] A history of TGCT was reported for a brother of 1 case and the father of 1 control.

GCT were more pronounced for former marijuana use (OR, 3.04; 95% CI, 1.29-7.19) than for current marijuana use (OR, 1.61; 95% CI, 0.64-4.01). However, the latter association did not achieve statistical significance, in part because there were fewer current users. Further stratification of current users' data on frequency or duration of use yielded estimates that also were not statistically significant. Among former users, there was a 3-fold association of marijuana use among those who reported use less frequent than once per week (OR, 3.30; 95% CI, 1.34-8.09); although the association estimated among more frequent users was somewhat lower, it did not achieve statistical significance (OR, 2.21; 95% CI, 0.74-6.61). Among former users, estimates within both substrata of duration achieved significance, with the association corresponding to longer duration of use (OR, 6.67; 95% CI, 1.37-32.5) exceeding that for shorter duration (OR, 2.81; 95% CI, 1.15-6.86). By contrast, the data suggest that any inverse association of ever using cocaine varied little between seminoma (OR, 0.46; 95%CI, 0.20-1.03) and nonseminoma or mixed GCT (OR, 0.63; 95%CI, 0.33-1.21).

DISCUSSION

We report a 2-fold increased risk of TGCT among ever users of marijuana compared with never users. Furthermore, we observed that ever users of marijuana had a greater than 2-fold risk of nonseminoma/mixed germ cell tumors compared with never users. In contrast, marijuana use was not associated with the risk of seminoma. The moderate but nonsignificant elevation in the risk of mixed GCTs among marijuana users may represent a mixture of effects on seminoma and nonseminoma. These results are in accord with the general findings of both previous epidemiologic studies that explored marijuana use and TGCT risk.[13,14] However, the greater TGCT risk we observed among former users of marijuana compared with current users and the greater risk among infrequent users compared with frequent users was not observed in previous studies. We also observed greater risk among men who had <10 years of use than among those who reported use of longer duration, a pattern that also was reported by 1 previous study[13] when all tumor histologies were combined. Various results may have arisen from the combined influence of differing measures of frequency and duration of marijuana exposure used in the 3 studies,[13,14] from the differing analysis models used in each, from the imperfect measurement of covariates, or simply from sampling variation, because relatively small numbers of patients with nonseminoma participated in each study. Differences also may have arisen in part from nonsynchronous assessment

Table 2. Associations of the Risk of Testicular Germ Cell Tumor With Recreational Drug Use, Alcohol Consumption, and Cigarette Smoking: Los Angeles County, 1986-1991

Exposure	No. of Cases	No. of Controls	Crude OR (95% CI)[a]	Adjusted OR (95% CI)[b]
Marijuana use				
Never	26	70	1.00 (Ref)	1.00 (Ref)
Ever	113	222	1.32 (0.79-2.22)	1.94 (1.02-3.68)
Recency				
Former use	68	112	1.58 (0.91-2.76)	2.28 (1.17-4.43)
Current use	45	110	1.06 (0.59-1.89)	1.38 (0.67-2.87)
Frequency, no. per wk				
<1	58	105	1.41 (0.83-2.41)	2.10 (1.09-4.03)
≥1	55	117	1.14 (0.60-2.17)	1.53 (0.73 -3.24)
Duration, y				
<10	78	142	1.42 (0.82-2.45)	2.09 (1.09-3.98)
≥10	35	79	1.20 (0.67-2.15)	1.51 (0.66-3.47)
Cocaine use				
Never	81	150	1.00 (Ref)	1.00 (Ref)
Ever	58	142	0.73 (0.48-1.11)	0.54 (0.32-0.91)
Frequency, no. per wk				
<1	42	108	0.69 (0.44-1.10)	0.68 (0.42-1.11)
≥1	16	34	0.83 (0.41-1.67)	0.72 (0.32-1.61)
Duration, y				
<10	51	121	0.74 (0.48-1.15)	0.69 (0.43-1.10)
≥10	7	21	0.61 (0.23-1.61)	0.68 (0.22-2.14)
Amyl nitrite use				
Never	124	238	1.00 (Ref)	1.00 (Ref)
Ever	15	54	0.53 (0.28-1.00)	0.50 (0.25-1.02)
Frequency, no. per wk				
<1	12	51	0.46 (0.21-0.92)	0.49 (0.23-1.03)
≥1	3	3	1.98 (0.30-13.26)	1.12 (0.12-10.09)
Duration, y				
<10	14	53	0.50 (0.26-0.97)	0.51 (0.25-1.05)
≥10	1	1	1.58 (0.09-27.23)	1.14 (0.05-26.73)
Mushroom use				
Never	99	190	1.00 (Ref)	1.00 (Ref)
Ever	40	102	0.71 (0.45-1.13)	0.80 (0.45-1.41)
Duration, y				
≤2, median	36	79	0.83 (0.51-1.34)	0.96 (0.54-1.72)
>2	4	23	0.32 (0.11-0.95)	0.28 (0.07-1.06)
Amphetamine use				
Never	104	208	1.00 (Ref)	1.00 (Ref)
Ever	35	84	0.80 (0.49-1.30)	0.90 (0.59-1.67)
Frequency, no. per wk				
<1	23	67	0.66 (0.38-1.14)	0.74 (0.38-1.46)
≥1	12	17	1.43 (0.61-3.31)	1.74 (0.61-4.90)
Duration, y				
<10	32	79	0.76 (0.46-1.26)	0.84 (0.45-1.60)
≥10	2	5	0.80 (0.15-4.27)	1.10 (0.17-7.00)
Barbiturate use				
Never	126	257	1.00 (Ref)	1.00 (Ref)
Ever	13	35	0.70 (0.35-1.39)	0.90 (0.39-2.11)
Frequency, no. per wk				
<1	8	28	0.50 (0.21-1.17)	0.58 (0.20-1.63)
≥1	5	7	1.53 (0.48-4.84)	2.41 (0.63-9.15)
Heroin use				
Never	135	284	1.00 (Ref)	1.00 (Ref)
Ever	4	8	0.92 (0.24-3.59)	1.46 (0.31-6.83)
LSD use				
Never	104	221	1.00 (Ref)	1.00 (Ref)
Ever	35	71	1.02 (0.63-1.67)	1.35 (0.73-2.50)
Frequency, no. per wk				
<1	33	63	1.09 (0.66-1.80)	1.40 (0.75-2.61)
≥1	2	8	0.55 (0.12-2.60)	0.77 (0.13-6.68)

(Continued)

Table 2. (Continued)

Exposure	No. of Cases	No. of Controls	Crude OR (95% CI)[a]	Adjusted OR (95% CI)[b]
Quaalude use				
Never	118	243	1.00 (Ref)	1.00 (Ref)
Ever	21	49	0.86 (0.48-1.55)	0.93 (0.45-1.90)
Frequency, no. per wk				
<1	18	43	0.84 (0.45-1.57)	0.83 (0.39-1.77)
≥1	3	6	1.03 (0.23-4.49)	2.05 (0.37-11.47)
PCP use				
Never	125	259	1.00 (Ref)	1.00 (Ref)
Ever	14	33	0.83 (0.40-1.69)	0.77 (0.33-1.79)
Frequency, no. per wk				
<1	12	31	0.75 (0.35-1.59)	0.67 (0.27-1.63)
≥1	2	2	2.41 (0.33-17.79)	3.85 (0.42-35.64)
Inhaled ethyl chloride use				
Never	138	288	1.00 (Ref)	1.00 (Ref)
Ever	1	4	0.53 (0.06-4.83)	0.72 (0.06-8.92)
Butyl nitrate use				
Never	137	288	1.00 (Ref)	1.00 (Ref)
Ever	2	4	0.88 (0.15-5.00)	1.18 (0.17-8.34)
Use of other poppers				
Never	136	290	1.00 (Ref)	1.00 (Ref)
Ever	3	2	3.76 (0.62-22.93)	4.66 (0.73-29.79)
Use of any poppers				
Never	119	236	1.00 (Ref)	1.00 (Ref)
Ever	20	56	0.70 (0.42-1.15)	0.70 (0.39-1.23)
Use of other recreational drugs[c]				
Never	126	266	1.00 (Ref)	1.00 (Ref)
Ever	13	26	0.99 (0.51-1.95)	1.43 (0.64-3.16)
Frequency, no. per wk				
<1	9	16	1.12 (0.49-2.56)	1.61 (0.62-4.15)
≥1	4	9	0.87 (0.26-2.89)	1.31 (0.33-5.26)
Any recreational drug use[d]				
Never	26	67	1.00 (Ref)	1.00 (Ref)
Ever	113	225	1.24 (0.74-2.08)	1.16 (0.66-2.04)[e]
Alcohol consumption				
Never	44	94	1.00 (Ref)	1.00 (Ref)
≥1/wk	95	198	0.95 (0.60-1.51)	0.98 (0.57-1.67)
Cigarette smoking				
Never	81	187	1.00 (Ref)	1.00 (Ref)
Ever	58	105	1.19 (0.78-1.81)	0.98 (0.58-1.67)
Recency				
Current	29	62	1.01 (0.59-1.72)	0.89 (0.46-1.69)
Former	29	43	1.58 (0.91-2.76)	1.15 (0.58-2.26)
Frequency, no. of cigarettes/day				
<20	30	48	1.49 (0.86-2.58)	1.11 (0.57-2.13)
≥20	28	57	1.03 (0.60-1.77)	0.91 (0.48-1.74)
Duration, y				
<10	32	63	1.15 (0.68-1.95)	0.91 (0.48-1.74)
≥10	26	39	1.54 (0.83-2.86)	1.26 (0.61-2.59)

Abbreviations: CI, confidence interval; LSD, lisergic acid diethylamide; OR, odds ratio; PCP, phencyclidine; Ref, referent category.

[a] Analyses were matched on age, race, and neighborhood.

[b] Unless otherwise noted, adjusted for marijuana use (except when estimating marijuana variables), cocaine use (except when estimating cocaine variables), amyl nitrite use (except when estimating amyl nitrite variables), cryptorchidism, religiosity, education.

[c] Street drugs other than marijuana, cocaine, amyl nitrite, butyl nitrate, ethyl chloride, hallucinogenic mushrooms, amphetamines, barbiturates, LSD, Quaaludes, PCP, or heroin.

[d] Any of the street drugs listed in the table, excluding cigarette smoking and alcohol use.

[e] Adjusted for cryptorchidism, religiosity, and education.

Table 3. Associations Between Marijuana Use and Cocaine Use and Histologic Subtypes of Testicular Germ Cell Tumors: Los Angeles County, 1986-1991

Variable	Seminoma		Nonseminoma/Mixed GCT	
	No. of Cases/Controls	OR[a] (95% CI)	No. of Cases/Controls	OR[a] (95%CI)
Marijuana use				
Never	11/15	1.00 (Ref)	12/43	1.00 (Ref)
Ever	36/79	1.07 (0.37-3.07)	68/127	2.42 (1.08-5.42)
Current	18/42	0.99 (0.30-3.31)	24/59	1.61 (0.64-4.01)
Former	18/37	1.07 (0.35-3.22)	44/68	3.04 (1.29-7.19)
Frequency among current users, no. per wk				
<1	5/17	0.68 (0.14-3.29)	8/24	1.46 (0.47-4.56)
≥1	13/25	1.03 (0.28-3.82)	16/35	1.47 (0.52-4.08)
Duration among current users, y				
<10	4/12	1.06 (0.19-5.76)	11/23	1.72 (0.57-5.20)
≥10	14/30	1.00 (0.26-3.81)	13/36	1.59 (0.51-4.92)
Frequency among former users, no. per wk				
<1	10/20	1.11 (0.32-3.81)	29/38	3.30 (1.34-8.09)
≥1	8/17	0.95 (0.24-3.75)	15/30	2.21 (0.74-6.61)
Duration among former users, y				
<10	17/33	1.30 (0.42-4.05)	38/62	2.81 (1.15-6.86)
≥10	1/3	0.37 (0.02-7.37)	6/6	6.67 (1.37-32.5)
Cocaine use				
Never	27/38	1.00 (Ref)	46/95	1.00 (Ref)
Ever	20/56	0.46 (0.20-1.03)	34/75	0.63 (0.33-1.21)
Frequency, no. per wk				
<1	15/41	0.50 (0.21-1.15)	24/57	0.57 (0.27-1.17)
≥1	5/15	0.31 (0.07-1.28)	10/18	0.82 (0.31-2.19)
Duration, y				
<10	18/45	0.47 (0.20-1.07)	30/65	0.64 (0.33-1.25)
≥10	2/11	0.35 (0.05-2.42)	4/10	0.52 (0.11-2.51)

Abbreviations: CI, confidence interval; GCT, germ cell tumor; OR, odds ratio; Ref, referent category.
[a] Adjusted for age, race, neighborhood, cryptorchidism, education (college graduate or more, some college, high school graduate, some high school, or less than high school), religiosity (any vs none), and amyl nitrite use (ever vs never). Marijuana and cocaine were mutually adjusted for the effect of the other.

of exposure, because trends of marijuana use have been changing,[11,12] and enrollment into the current study tended to occur earlier than enrollment into the others. Nonetheless, the results presented here confirm both the epidemiologic association of marijuana use with TGCT risk and the specificity of this risk factor for nonseminomatous tumors.

The main psychoactive compound in marijuana, Δ^9-tetrahydrocannabinol (THC), binds and partially agonizes the human cannabinoid (CB) receptors, CB1 and CB2. These receptors are activated by endocannabinoids (ECs), mainly N-arachidonoylethanolamide (AEA) and 2-arachidonoylglycerol (2-AG). AEA also binds and stimulates the transient potential vanilloid receptor type 1 (TRPV1), which has been proposed to confer heat resistance to germ cells and maturing sperm.[15] AEA is degraded in the cell by fatty acid amid hydrolase (FAAH).[16] CB receptors and their endocannabinoid ligands are expressed in the pituitary and hypothalamus, and it has been demonstrated that they are involved in the regulation of reproduction at the hypothalamic and pituitary levels.[16] In males, as reviewed by Pagotto et al,[17] it has been demonstrated that cannabinoids decrease luteinizing hormone (LH), decrease testosterone production and secretion, and suppress spermatogenesis, in accordance with reports indicating reduced testosterone levels after marijuana use. Both CB1 and CB2 also are expressed in the male reproductive system, with distinct patterns of cellular and developmental specificity. In mice, messenger RNA and protein encoded by the CB2 ortholog are expressed in all stages of spermatogenesis, whereas 2-AG levels are highest in spermatogonia (germline stem cells in the sexually mature male) and gradually diminish in subsequent stages of spermatogenesis, whereas AEA levels are relatively unchanged.[18] These precise expression patterns may reflect the regulation of crucial events in spermatogenesis

by endogenous cannabinoid signaling, because mice that were engineered to be unable to express the CB2 ortholog had pronounced reproductive deficits, and endocannibinoid system (ECS) signaling in the male reproductive system is conserved across several species.[18] Therefore, it is plausible that unscheduled signaling by exogenous THC may impair testicular health by disrupting ECS signaling either in the pituitary and hypothalamus or in the gonads.

Cannabinoid compounds, including THC, in various model systems have demonstrated proapoptotic, antiproliferative, antiangiogenic, antimigrative, and antiadhesive properties.[19] Limited mechanistic investigation has indicated that these effects are mediated by numerous pathways, some of which involve signaling through CB1, CB2, and TRPV1.[19] Therefore, the cannabinoid system is investigated increasingly as a potential source of novel chemotherapeutics. Although these observations may appear to be at odds with the carcinogenic effects of marijuana on the testis, the directions of some biologic effects of cannabinoids appear to be tissue-specific and cell type-specific,[19] and we are unaware of any pharmacologic research addressing cannabinoid signaling in the testis. Thus, it is too early to speculate whether this avenue of research will provide biologic insight into the plausibility of a causal role for marijuana use in the development of nonseminomatous and mixed histology tumors of the testis.

The specific association between marijuana use and the risk of nonseminomatous and mixed histology TGCT has yet to be explained. However, a mechanism involving androgen signaling or response may be plausible, because THC recently was linked to androgen levels; and, based on recent results presented elsewhere, we postulate that variation in androgen receptor (AR) biology may distinguish TGCT histology. In a separate population-based epidemiologic study, we observed shorter CAG repeat lengths in exon 1 of the *AR* gene, and it is predicted that these result in greater AR transactivation[20] and are associated with the risk of seminoma, whereas the opposite association was observed for nonseminoma.[21] However, data on the association of TGCT histology with genotypes defined by this polymorphism are not entirely consistent. Although 4 hospital-based case-control studies[22-25] also reported an association between longer *AR* CAG repeats and nonseminoma, the results were not statistically significant, and 1 of those studies revealed significantly greater frequency of shorter repeats in patients with seminomas than in patients with nonseminomas.[23] No such difference was reported in 2 of those studies[22,24]; although the fourth study reported no association between repeat length and nonseminoma alone or all TGCTs, it did not report on comparisons of repeat length between patients with seminoma alone versus either unaffected controls or men with nonseminoma alone.[25] Apart from age at diagnosis, few other factors have been associated differentially with seminomas and nonseminomas. Therefore, to provide further insight regarding the histology-specific association of marijuana use observed here and reported in the earlier studies,[13,14] it seems advisable for future TGCT research to address marijuana jointly with indicators of androgen action.

To our knowledge, this is the first epidemiologic study to investigate the use of cocaine and additional recreational drugs in relation to TGCT risk. We observed an association between reported use of cocaine and lesser risk of TGCT, which appeared strongest for mixed GCTs. A mechanism whereby cocaine may reduce TGCT risk has not been proposed, but a parsimonious explanation may be loss of germ cells through a cocaine-mediated process. The effects of cocaine on the murine testis suggest several means whereby exposure plausibly may result in germ cell death. Experimental administration of cocaine causes several morphologic changes of the rat testis: seminiferous tubule diameter and germinal epithelium are reduced,[26,27] and spermatogenesis is rapidly disrupted accompanied by cell sloughing and reduction in testicular volume.[27] Ultrastructural changes were observed in Sertoli cells and germ cells but not in Leydig cells, suggesting germinal lines as primary site of cocaine's testicular toxicity.[27] Although the mechanism underlying these changes has not been identified, Li et al[28] identified a receptor protein in rat testes that saturably and specifically binds cocaine. If this receptor is involved in testicular maintenance, then the endogenous ligand may be unable to fulfill its normal function in the presence of cocaine. An equally plausible explanation, because cocaine causes severe vasoconstriction, may simply be hypoxic cell death within the testis.[28] Finally, a role for cocaine in opposing effects of THC was suggested by metabolic studies that assessed the effects of both drugs in the rat,[29] in which a general trend of reduced glucose use was identified after administration of THC and was counteracted when cocaine was administered with THC. Without suggesting a specific metabolic mechanism whereby cocaine may protect against TGCT, these results indicate interplay between the effects of these 2 drugs, which may be germane to the opposing associations with TGCT risk reported here.

Cocaine use was identified as an important confounder in our analyses of marijuana effects, as may be anticipated, because marijuana use is associated with a

tendency to use other recreational drugs. It appears that other studies estimating associations between marijuana use and TGCT risk did not address potential confounding effects of cocaine. True effects of marijuana use in these source populations may be somewhat greater than reported if cocaine also is associated with lesser risk in these settings.

Strengths of our study include the population-based design and the extensive in-person interviews that included queries about demographic variables, risk factors, and detailed history of use of numerous recreational drugs. Statistical power of the study was adequate to examine main disease associations with respect to dichotomized levels of strong risk factors, even within subgroups of tumor histology. However, the study size was not adequate to address marijuana dose by jointly examining associations with respect to recency, frequency, and duration of use. Neither were we able to examine statistical interactions between exposures or to statistically test the heterogeneity of single exposure associations across histologic subtypes.

Several cautions should attend the interpretation of case-control data on self-reported drug use. Previous authors[13,14] noted that controls may be less motivated than cases to report use of illicit drugs, which may create spurious associations between disease risk and drug use. However, such reporting bias appears to be a less likely explanation for the specific association of marijuana use with nonseminoma or mixed GCT consistently observed in the current study and in previous studies.[13,14] Moreover, such bias would not lead to the association of cocaine use with *lesser* TGCT risk observed in the current study. Therefore, observed associations are unlikely to have arisen from reporting bias alone.

However, use of each of the specific drugs investigated in this study may be associated with other unmeasured exposures, behaviors, or sociodemographic factors that may have confounded the association between marijuana use and the risk of nonseminomatous TGCT. Recognized correlates of marijuana use were not completely captured by our questionnaire, because these include complex psychosocial variables, including sensation-seeking behavior, peer-group integration, and parental or adult supervision.[30] Our study did query religion, a negative correlate of marijuana use that was identified as a confounder of the marijuana-TGCT association. For confounding by unmeasured variables to account for reported marijuana-TGCT associations, such factors would need to be associated with marijuana use in source populations for all 3 studies and would have to be associated specifically with the risk of nonseminoma. Similar forms of unrecognized bias conceivably may be present in all 3 studies, which are similar in numerous respects; however, it seems far less likely that the specific association with a single histologic subtype would be spurious.

We conclude that marijuana use is associated with an elevated risk of TGCT, especially nonseminoma or mixed histology tumors. This consistent finding across 3 epidemiologic studies now warrants mechanistic research investigating biologic processes whereby constituents of marijuana smoke may influence testicular carcinogenesis. Moreover, the possibility that any effect of marijuana on TGCT risk may involve perturbation of the endocannabinoid system should be considered if cannabinoid agonists or antagonists are to be used as therapeutic agents, as has been proposed for numerous conditions, including endocrine-related cancers and infertility.[18,31,32]

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CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

REFERENCES

1. Linger R, Dudakia D, Huddart R, et al. Analysis of the DND1 gene in men with sporadic and familial testicular germ cell tumors. *Genes Chromosomes Cancer*. 2008;47:247-252.
2. Fung C, Vaughn DJ. Complications associated with chemotherapy in testicular cancer management. *Nat Rev Urol*. 2011;8:213-222.
3. Cortessis V. Epidemiologic insights into the occurrence and causes of testicular cancer. In: Raghavan D, ed. American Cancer Society Atlas of Clinical Oncology—Germ Cell Tumors. Hamilton, Ontario, Canada: BC Decker Inc.; 2003.
4. Grocock CA, Charlton HM, Pike MC. Role of the fetal pituitary in cryptorchidism induced by exogenous maternal oestrogen during pregnancy in mice. *J Reprod Fertil*. 1988;83:295-300.
5. Emmen J, McLuskey A, Adham IM, et al. Involvement of insulin-like factor 3 (Insl3) in Diethylstilbestrol-induced cryptorchidism. *Endocrinology*. 2000;141:846-849.
6. Trabert B, Sigurdson AJ, Sweeney AM, Amato RJ, Strom SS, McGlynn KA. Baldness, acne and testicular germ cell tumours. *Int J Androl*. 2011;34(4 pt 2):e59-e67.
7. Biggs ML, Davis MD, Eaton DL, et al. Serum organochlorine pesticide residues and risk of testicular germ cell carcinoma: a population-based case-control study. *Cancer Epidemiol Biomarkers Prev*. 2008;17:2012-2018.
8. McGlynn KA, Quraishi SM, Graubard BI, Weber JP, Rubertone MV, Erickson RL. Persistent organochlorine pesticides and risk of testicular germ cell tumors. *Natl Cancer Inst*. 2008;100:663-671.
9. McGlynn KA, Quraishi SM, Graubard BI, Weber JP, Rubertone MV, Erickson RL. Polychlorinated biphenyls and risk of testicular germ cell tumors. *Cancer Res*. 2009;69:1901-1909.

10. Chia VM, Li Y, Quraishi SM, et al. Effect modification of endocrine disruptors and testicular germ cell tumor risk by hormone-metabolizing genes. *Int J Androl*. 2010;33:588-596.
11. Gfroerer JC, Wu LT, Penne MA. Initiation of Marijuana Use: Trends, Patterns, and Implications. DHHS Publication SMA 02–3711, Analytic Series A-17. Rockville, MD: Substance Abuse and Mental Health Services Administration, Office of Applied Studies, U.S. Department of Health and Human Services; 2003.
12. U.S. Department of Health and Human Services. Trends in Marijuana Use, 1979-2008. National Survey on Drug Use and Health and National Household Survey on Drug Abuse. Washington, DC: Substance Abuse and Mental Health Services Administration, Office of Applied Studies, U.S. Department of Health and Human Services; 2008.
13. Daling JR, Doody DR, Sun X, et al. Association of marijuana use and the incidence of testicular germ cell tumors. *Cancer*. 2009;115: 1215-1223.
14. Trabert B, Sigurdson AJ, Sweeney AM, Strom SS, McGlynn KA. Marijuana use and testicular germ cell tumors. *Cancer*. 2011;117: 848-853.
15. Grimaldi P, Orlando P, Di Siena S, et al. The endocannabinoid system and pivotal role of the CB2 receptor in mouse spermatogenesis. *Proc Natl Acad Sci U S A*. 2009;106:11131-11136.
16. Rettori V, Laurentiis A De, Fernandez-Solari J. Alcohol and endocannabinoids: neuroendocrine interactions in the reproductive axis. *Exp Neurol*. 2010;224:15-22.
17. Pagotto U, Marsicano G, Cota D, Lutz B, Pasquali R. The emerging role of the endocannabinoid system in endocrine regulation and energy balance. *Endocr Rev*. 2006;27:73-100.
18. Bari M, Battista N, Pirazzi V, Maccarrone M. The manifold actions of endocannabinoids on female and male reproductive events. *Front Biosci*. 2011;16:498-516.
19. Freimuth N, Ramer R, Hinz B. Antitumorigenic effects of cannabinoids beyond apoptosis. *J Pharmacol Exp Ther*. 2010;332:336-344.
20. Buchanan G, Yang M, Cheong A, et al. Structural and functional consequences of glutamine tract variation in the androgen receptor. *Hum Mol Gen*. 2004;13:1677-1692.
21. Davis-Dao CA, Siegmund KD, Vandenberg DJ, et al. Heterogenous effect of androgen receptor CAG tract length on testicular germ cell tumor risk: shorter repeats associated with seminoma but not other histologic types. *Carcinogenesis*. 2011;32:1238-1243.
22. Rajpert-De Meyts E, Leffers H, Daugaard G, et al. Analysis of the polymorphic CAG repeat length in the androgen receptor gene in patients with testicular germ cell cancer. *Int J Cancer*. 2002;102: 201-204.
23. Giwercman A, Lundin KB, Eberhard J, et al. Linkage between androgen receptor gene CAG trinucleotide repeat length and testicular germ call cancer histological type and clinical stage. *Eur J Cancer*. 2004;40:2152-2158.
24. Garolla A, Ferlin A, Vinanzi C, et al. Molecular analysis of the androgen receptor gene in testicular cancer. *Endocr Relat Cancer*. 2005;12:645-655.
25. Vastermark A, Giwercman YL, Hagstromer O, et al. Polymorphic variation in the androgen receptor gene: association with risk of testicular germ cell cancer and metastatic disease. *Eur J Cancer*. 2011; 47:413-419.
26. George VK, Li H, Teloken C, Grignon DJ, Lawrence WD, Dhabuwala CB. Effects of long-term cocaine exposure on spermatogenesis and fertility in peripubertal male rats. *J Urol*. 1996;155:327-331.
27. Rodriguez MC, Sanchez-Yague J, Paniagua R. Effects of cocaine on testicular structure in the rat. *Reprod Toxicol*. 1992;6:51-55.
28. Li H, George VK, Crossland WJ, Anderson GF, Dhabuwala CB. Characterization of cocaine binding sites in the rat testes. *J Urol.* 1997;158(3 pt 1):962-965.
29. Husain S, Anwer J. Characteristics of cocaine interaction with delta-9-tetrahydrocannabinol on glucose metabolism in the rat testis. *Pharmacol Biochem Behav*. 1991;40:625-628.
30. Sharma M. Determinants of marijuana use, abuse and dependence. *J Alcohol Drug Educ*. 2009;1:3-6.
31. Bifulco M, Malfitano AM, Pisanti S, Laezza C. Endocannabinoids in endocrine and related tumours. *Endocr Relat Cancer*. 2008;15: 391-408.
32. Guindon J, Hohmann AG. The endocannabinoid system and cancer: therapeutic implication. *Br J Pharmacol*. 2011;163:1447-1463.