Original Article

Association of Marijuana Use and the Incidence of Testicular Germ Cell Tumors

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BACKGROUND: The incidence of testicular germ cell tumors (TGCTs) has been increasing the past 4 to 6 decades; however, exposures that account for this rise have not been identified. Marijuana use also grew during the same period, and it has been established that chronic marijuana use produces adverse effects on the human endocrine and reproductive systems. In this study, the authors tested the hypothesis that marijuana use is a risk factor for TGCT. **METHODS:** A population-based, case-control study of 369 men ages 18 to 44 years who were diagnosed with TGCT from January 1999 through January 2006 was conducted in King, Pierce and Snohomish Counties in Washington State. The responses of these men to questions on their lifetime marijuana use were compared with the responses of 979 age-matched controls who resided in the same 3 counties during the case diagnosis period. **RESULTS:** Men with a TGCT were more likely to be current marijuana smokers at the reference date compared with controls (odds ratio [OR], 1.7; 95% confidence interval [95% CI], 1.1-2.5). In analyses according to histologic type, most of the association between current marijuana use and TGCT was observed in men who had nonseminomas/mixed histology tumors (current use: OR, 2.3; 95% CI, 1.3-4.0). Age at first use among current users (age $<$18 years [OR, 2.8] vs age $\geq$18 years [OR, 1.3]) and frequency of use (daily or weekly [OR, 3.0] vs less than once per week [OR, 1.8]) appeared to modify the risk. **CONCLUSIONS:** An association was observed between marijuana use and the occurrence of nonseminoma TGCTs. Additional studies of TGCTs will be needed to test this hypothesis, including molecular analyses of cannabinoid receptors and endocannabinoid signaling, which may provide clues regarding the biologic mechanisms of TGCTs. **Cancer 2009;115:1215–23. © 2009 American Cancer Society.**

KEY WORDS: marijuana use, seminomas, nonseminomas, testicular tumors.

Testicular germ cell tumors (TGCTs) are the most common type of malignancy in American men between ages 15 and 34 years.[1] These cancers traditionally are classified into 2 broad groups: pure

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seminoma (60% of TGCTs) and nonseminoma (40% of TGCTs). Nonseminomas include tumors that have purely nonseminomatous elements (eg, embyronal carcinomas) as well as tumors that have both seminomatous and nonseminomatous elements.[2] The age-specific incidence of nonseminomas peaks 10 years earlier (ages 20-35 years) compared with seminomas (ages 30-45 years).[3] During the last half of the 20th century, the incidence of TGCTs increased by 3% to 6% per year in the US as well as in Europe, Australia, New Zealand, and Canada.[2,4-6] The rising rates have been evident for both seminoma and nonseminoma. There are few established risk factors for TGCT beyond cryptorchidism, gonadal dysgenesis, age, race, and family history of TGCT[6-8]; most (but not all) studies indicate that risk factors do not vary between the 2 histologic groups.[8,9] The current prevailing paradigm is that the disease is initiated in early fetal life, when some primordial germ cells fail to differentiate, remain susceptible to malignant transformation, and develop into carcinoma in situ. It is believed that these neoplasms progress to invasive cancer under the influence of adult steroid hormones and/or gonadotropins.[10,11]

The increasing incidence of TGCTs over time strongly suggests that young men have been exposed to 1 or more increasingly prevalent causal factors. One exposure that has been increasing in the United States and in Europe over the same period as the rise in the incidence of TGCTs is the use of marijuana.[12-14] Chronic marijuana use has multiple adverse effects on the endocrine and reproductive systems. For example, chronic marijuana use is associated with reduced hypothalamic release of gonadotropin-releasing hormone, decreased plasma levels of gonadotropins (follicle-stimulating hormone, lutenizing hormone, and prolactin) and testosterone, reduced spermatogenesis, and impotency in men.[15-17] In mice, cannabis-like compounds target cannabinoid receptors in Leydig and Sertoli cells, influencing testosterone secretion and Sertoli cell survival.[18-22] Male infertility and poor semen quality also are associated with the risk of TGCT.[6] Therefore, we tested the hypothesis that marijuana use is a risk factor for TGCT using data from the Adult Testicular Cancer Lifestyle and Blood Specimen (ATLAS) Study, a population-based case-control study conducted in the Seattle/Puget Sound region of Washington State.

MATERIALS AND METHODS

Study Participants

Cases

Cases who were eligible for participation in the ATLAS Study were men ages 18 to 44 years; were residents of King, Pierce, or Snohomish Counties, Washington State; were diagnosed with an invasive TGCT between January 1, 1999 and January 31, 2006; had a landline residential telephone at the time of diagnosis (because controls were ascertained through random-digit dialing of landline residential telephone numbers); and were capable of communicating in English. Potentially eligible cases were identified from the files of the population-based Cancer Surveillance System (CSS), a part of the Surveillance, Epidemiology and End Results Program of the National Cancer Institute,[23] based on the following International Classifications of Diseases for Oncology (ICD-O) topography and histology codes: topography, codes C62.0 through C62.9; histology, codes 9060 through 9091.[24]

To contact each case to determine his final eligibility and recruitment, we asked his follow-up physician to determine whether there was any reason why the man should not be approached for the study. If no such reason was given, then we sent the man an introductory letter and followed up with a telephone call from a study interviewer who assessed final eligibility and attempted to recruit him into the study protocol.

Of the 550 total cases identified with eligible diagnosis dates, we interviewed 371 men (67.5%). The rest of the cases fell into the following categories: individual refusal (n = 112; 62.6% of noninterviewed men), lost to follow-up (n = 50; 27.9%), physician refusal (n = 11; 6.1%), and died (n = 6; 3.4%). Of the 371 cases who were interviewed successfully, we excluded 2 men from our analyses who had tumors classified as choriocarcinoma based on the uniqueness of this histology.

Controls

Mitofsky-Waksberg random digit dialing with a clustering factor ('k') of 5 was used to recruit controls.[25-27] Controls were men without a history of TGCT who resided in the same 3 counties as the cases during the case diagnosis period and were frequency-matched to the cases

on 5-year age groups using 1-step recruitment.[28] Each telephone number was called at least 9 times over $\geq$2 weeks, including weekday, weekend, and evening calls. When a call was answered, the interviewer sought to determine whether the telephone rang in a residence and was a landline telephone, the county of the residence, and whether a man aged 18 to 44 years of age lived in the residence. If the household census identified a man aged 18 to 44 years and he was eligible after age stratification criteria were applied, then the interviewer attempted to obtain the name and address of the man so that a letter of introduction to the study could be sent to him. After mailing of the letter, an interviewer called the man to conduct a final eligibility assessment and attempted to recruit him into the study protocol.

Of the 1875 eligible controls who were identified, we interviewed 979 men (52.2%). The screening proportion was calculated as the number of screened households divided by the number of all confirmed households plus the number of presumed households (answering machine on every call, immediate hang-up, and refusal to answer screening questions). The screening response rate was 82.9%, which, when combined with the interview proportion, yielded an overall response proportion of 43.3%.

Interviews

After providing written informed consent, cases and controls were interviewed in person by trained interviewers in a place of the respondent's choosing (including home, place of work, or research institution office) using a structured questionnaire. All questions referred to the time period before each man's assigned reference date. For each case, the reference date was the month and year of his TGCT diagnosis. Each control was assigned a reference date that was selected at random from among all possible dates given the distribution of diagnosis years for cases identified as of the time the controls were selected through random-digit dialing. Information collected during the interview included 1) demographic characteristics, 2) cigarette smoking and alcohol consumption, 3) recreational drug use, and 4) other known or suspected risk factors for TGCT. Before the in-person interview, each participant was asked to complete a self-administered questionnaire regarding his family history of cancer and ethnic heritage.

Each man was asked whether he had ever used marijuana, hashish, or both. Each man who reported having used marijuana was asked to recall different periods in his life when he used this drug, defined by the ages in which he first and last used it at a given frequency (times per day, week, month, or year), and form (marijuana, hashish, or both).

Statistical Analysis

Analyses were conducted for all cases combined and for cases classified by histologic subtype: Seminomas included those with ICD-O histologies 9060-9064; and nonseminomas included embryonal (9070), yolk sac (9071), teratoma (9080, 9082-9084), nonseminoma not otherwise specified (9065), and mixed germ cell tumors with (9085) and without (9081, 9101) seminomatous features. By using the data collected on episodes of marijuana use, we created analytic variables for ever use, former versus current use, age at first use, lifetime duration of use, and frequency of use. Frequency of use was calculated in 2 forms, 1 averaged over each man's lifetime and 1 for the current episode of use, if applicable.

Odds ratios (OR) and 95% confidence intervals (CI) were calculated as estimates of relative risk using unconditional logistic regression. Polytomous logistic models were used to compare controls with each of the case groups defined by histologic type. *P* values comparing OR by histology were obtained by using likelihood-ratio tests, and *P* values for trend were evaluated among ever-users of marijuana by fitting a grouped linear version of the variable of interest in that group. To assess the extent of confounding, we included in the logistic regression models terms for age and reference year (because the controls were frequency matched to the cases on these characteristics), history of cryptorchidism, first-degree family history of TGCT, race, and income. We also examined confounding by 2 additional habits that may be expected to be correlated with marijuana use, smoking, and drinking alcohol, and observed that drinking alcohol (frequency of use in the 5 years before reference date) and current smoking were confounders. Final models were adjusted for age, reference year, alcohol use, current smoking, and history of cryptorchidism. Subgroup analyses were performed by age group, excluding men who had a history of cryptorchidism and men who had a first-degree

Table 1. Characteristics of Testicular Cancer Cases and Population Controls: Western Washington State, January 1999 to January 2006

	Controls (N=979)		Cases (N=369)	
Characteristic	No.	%	No.	%
Age at reference date, y				
18-24	121	12.3	55	14.9
25-29	127	13.0	60	16.3
30-34	245	25.0	94	25.5
35-39	247	25.2	83	22.5
40-44	239	24.4	77	20.9
Reference y				
1999-2002	630	64.4	217	58.8
2003-2006	349	35.6	152	41.2
Race and Hispanic ethnicity				
White non-Hispanic	783	80.0	324	87.8
African American (Hispanic or non-Hispanic)	28	2.9	0	0
White Hispanic	15	1.5	10	2.7
Other non-Hispanic	117	12.0	28	7.6
Other Hispanic	36	3.7	7	1.9
Household income at reference date				
<$25,000	110	11.3	55	14.9
$25,000-50,000	236	24.2	108	29.3
$50,000-90,000	400	41.0	126	34.2
≥$90,000	229	23.5	79	21.5
Refused	4		1	
Highest level of education				
≤High school	229	23.4	96	26.0
Trade school	124	12.7	49	13.3
College	473	48.3	183	49.6
>College	153	15.6	41	11.1
History of cryptorchidism				
No	955	97.5	329	90.1
Yes	24	2.5	36	9.9
Do not know	0		4	
First-degree family history of testicular cancer				
No	863	99.0	315	97.2
Yes	9	1.0	9	2.8
Do not know	107		45	

family history of TGCT. All analyses were performed in Stata/SE (Stata Statistical Software, version 9.2; StataCorp, College Station, Tex).

To evaluate the extent to which the reporting of marijuana use among our controls was consistent with other population-based data, we analyzed publicly available data for men ages 18 to 34 years from the National Survey on Drug Use and Health (NSDUH) (formerly known as the National Household Survey on Drug Abuse) that was conducted between 1999 through 2006. We did not include data on men ages 35 to 44 years, because the NSDUH data aggregated them with men ages 45 to 49 years (who were not included in our study). We compared the observed number of controls who reported ever using marijuana, current versus former marijuana use, and current marijuana use $\geq$1 days per week with the expected number based on the age- and race-specific proportions in the NSDUH data. We calculated observed-to-expected (O/E) ratios, and corresponding 95% CIs using the Poisson process and logarithmic transformation.[29]

RESULTS

Cases were more likely to be white men, whether Hispanic or non-Hispanic white, and none of the cases were African American (Table 1). Cases tended to be from somewhat

Table 2. Risk of Testicular Cancer Associated With Marijuana Use for All Histologies: Western Washington State, January 1999 to January 2006

	Controls (N = 979)		Cases (N = 369)			
Characteristic	No.	%	No.	%	OR*	95% CI
Ever used marijuana						
No	313	32.0	101	27.4	1.0	Referent
Yes	666	68.0	268	72.6	1.3	1.0-1.8
Marijuana use as of reference date						
Never	313	32.0	101	27.4	1.0	Referent
Former	474	48.4	171	46.3	1.2	0.9-1.7
Current	192	19.6	97	26.3	1.7	1.1-2.5
Age at first use among current marijuana users, y						
<18	143	14.6	76	20.6	1.8	1.2-2.8
≥18	49	5.0	21	5.7	1.4	0.8-2.5
Length of use among current marijuana users, y						
<10	44	4.5	27	7.3	1.8	1.0-3.3†
≥10	148	15.1	70	19.0	1.6	1.1-2.5
Frequency of current marijuana use						
Daily or ≥1 d per wk	101	10.3	57	15.4	2.0	1.3-3.2
Less than once per wk	91	9.3	40	10.8	1.4	0.9-2.3

OR indicates odds ratio; 95% CI, 95% confidence interval.
*Adjusted for age at reference date, reference year, alcohol use, current smoking, and history of cryptorchidism.
†The 95% CI excludes 1.0.

lower income households and were slightly less likely to have more than a college education compared with controls.

Men with TGCT were more likely than controls to have a history of cryptorchidism (9.9% vs 2.5%; age-adjusted OR, 4.4; 95% CI, 2.6-7.5) and a first-degree family history of TGCT (2.8% vs 1.0%; age-adjusted OR, 2.9; 95% CI, 1.1-7.3) (Table 1).

Men with TGCT were slightly more likely to have ever smoked marijuana than controls (72.6% vs 68.0%; OR, 1.3; 95% CI, 1.0-1.8) (Table 2). Twenty-six percent of cases reported being current marijuana smokers at the reference date compared with 20% of controls (OR, 1.7; 95% CI, 1.1-2.5). The ORs for first use at age <18 years among current users was somewhat higher than for first use at age ≥18 years (OR, 1.8 vs 1.4). The ORs did not differ appreciably by total years of use, but the risk associated with daily or weekly use among current users was somewhat higher than less frequent use (OR, 2.0 vs 1.4).

When we conducted similar analyses according to histologic type, the association between current marijuana use and TGCT was limited primarily to nonseminomas (OR, 2.3; 95% CI, 1.3-4.0) compared with pure seminomas (OR, 1.3; 95% CI, 0.8-2.1; $P = .08$ for the difference between the 2 histologic groups) (Table 3). For nonseminomas, the risk was higher only for current users who started using marijuana at age <18 years (OR, 2.8; 95% CI, 1.6-5.1) compared with age ≥18 years (OR, 1.3; 95% CI, 0.6-3.2; $P = .08$ for the difference in OR). There appeared to be increasing risk with years of use (ie, the OR was 1.8 for <10 years of use vs 2.7 for >10 years of use). However, the difference in those estimates was not statistically significant ($P = .32$). Risk did not vary according to whether use was daily or weekly, so we combined these frequencies (OR, 3.0; 95%CI, 1.5-5.6); the OR associated with use on a less than weekly basis was 1.8 (95% CI, 0.9-3.5). Subanalyses by age or after excluding men who had a family history or who had undescended testes did not change the results substantially.

In the 1953 episodes of marijuana use reported in our study population (268 cases and 666 controls who 'ever' used), 20 episodes (1%) were hashish use. An

Table 3. Risk of Testicular Cancer Associated With Marijuana Use According to Histology: Western Washington State, January 1999 to January 2006

	Controls (N = 979)		Pure Seminoma (N = 230)				Nonseminoma/Mixed (N = 139)			
Characteristic	No.	%	No.	%	OR*	95% CI	No.	%	OR*	95% CI
Ever used marijuana										
No	313	32.0	65	28.3	1.0	Referent	36	25.9	1.0	Referent
Yes	666	68.0	165	71.7	1.2	0.9-1.8	103	74.1	1.5	0.9-2.4
Marijuana use as of reference date										
Never	313	32.0	65	28.3	1.0	Referent	36	25.9	1.0	Referent
Former	474	48.4	121	52.6	1.2	0.8-1.8	50	36.0	1.2	0.7-2.0
Current	192	19.6	44	19.1	1.3	0.8-2.1	53	38.1	2.3	1.3-4.0
Age at first use among current marijuana users, y										
<18	143	14.6	31	13.5	1.2	0.7-2.0	45	32.4	2.8	1.6-5.1
≥18	49	5.0	13	5.7	1.5	0.7-2.9	8	5.8	1.3	0.6-3.2
Length of use among current marijuana users, y										
<10	44	4.5	9	3.9	1.5	0.7-3.5	18	12.9	1.8	0.8-3.8
≥10	148	15.1	35	15.2	1.2	0.7-2.1	35	25.2	2.7	1.5-5.0
Frequency of current marijuana use										
Daily or ≥1 d per wk	101	10.3	23	10.0	1.3	0.7-2.4	34	24.5	3.0	1.5-5.6
Less than once per wk	91	9.3	21	9.1	1.2	0.7-2.2	19	13.7	1.8	0.9-3.5

OR indicates odds ratio; 95% CI, 95% confidence interval.
*Adjusted for age at reference, reference year, alcohol use, current smoking, and history of cryptorchidism.

additional 247 episodes (12.7%) were both hashish and marijuana use, and the remaining 1683 episodes (86.3%) were marijuana use only. In the episodes in which both were used, there was no way to determine the proportion of each, When we eliminated those respondents who had used hashish, the results did not change.

Among 493 men ages 18 to 34 years in our control group, 295 men were ever marijuana users compared with 276.1 expected based on NSDUH data (O/E ratio, 1.1; 95% CI, 0.90-1.26). Among the 102 current marijuana users (compared with 103 expected; O/E ratio, 1.0; 95% CI, 0.75-1.32), 56 men reported using this drug weekly compared with 76.5 expected (O/E ratio, 0.7; 95% CI, 0.51-1.05.

DISCUSSION

We observed a 70% increased risk of TGCT associated with current marijuana use, and the risk was particularly elevated for current use that was at least weekly or that began in adolescence. These associations were independent of known TGCT risk factors. In addition, all of the associations we observed appeared to be limited to nonseminoma/mixed histologies.

The current results must be interpreted in light of several limitations of our study. First, we only interviewed 67.5% and 52.2% of apparently eligible cases and controls, respectively. Our results may be biased if, among the cases and controls we were unable to interview, the association between marijuana use and TGCT was different from that among those men who we did interview. To have produced a spurious positive association, there would need to be an inverse association among the nonparticipating men. Second, we had to rely on self-report of the use of marijuana: an illicit drug. Patients with cancer may be expected to more accurately admit to the use of an illegal substance than individuals in a control group. However, our finding of an increased risk of TGCT associated with marijuana use that was confined to nonseminoma or mixed histologies indicates that it is unlikely that

over-reporting occurred, because there would be no reason to expect that recall bias would occur preferentially according to tumor type. Furthermore, after adjusting for age and race, the marijuana use characteristics (ever, current, and frequency of use) of our controls were essentially the same as would be predicted from national data. Finally, we did not conduct a centralized pathologic review but relied on the histologic description provided by community pathologists and coded by the CSS. Any resulting misclassification, however, would be expected to obscure differences in associations between pure seminomas and nonseminomas/mixed seminomas.

Our original hypothesis sought an increasing exposure that would be associated with the risk of all histologic types of TGCT, because the incidence of seminomas, nonseminomas, and mixed histologies has been increasing. We observed, however, that the excess risk of TGCT associated with marijuana use was essentially confined to the nonseminomas and mixed histology tumors. In fact, the increase in the incidence of seminoma from 1973 to 1998 in the US was 64% compared with an increase of only 24% for nonseminoma.[2] However, the opposite was true in the Netherlands and Norway, where the largest increase occurred in the nonseminoma histologic groups.[30] If the increase in nonseminomas was caused in part by an increase in the use of marijuana, then some other increasing exposures must account for the higher incidence of seminomas over time. Akre et al.[8] have suggested that increased maternal age, increased placental weight, and decreased parity are factors associated more closely with seminoma than with nonseminoma. These exposures also gave been increasing over the past decades[31-33] and, thus, could explain differential increases in incidence according to histology.

We can only speculate why marijuana use may be associated with TGCT. Moller and Skakkebaek[34] reported a significant association between subfertility in men and the subsequent risk of TGCT, and it has been suggested that both TGCT and subfertility in men may be caused by 1 or more common exposures. Could 1 of these common exposures be the use of marijuana? It has been established that marijuana use adversely affects male fertility, including sperm output, motility, and fertilizing capacity, in various species, including humans.[17,35] In addition, chronic marijuana exposure adversely affects both the endocrine and reproductive systems in humans.[17,36] It has been suggested that puberty is a 'window of vulnerability' during which environmental factors increase the risk of TGCT.[37] This is consistent with our finding that the elevated risk of nonseminomatous TGCTs in particular was associated with the use of marijuana starting at age <18 years. It also is speculated that primitive germ cells persisting into the pubertal period multiply under the stimulation of gonadotropins and other hormones.[38] Thus, it is possible that altered levels of gonadotropins and other hormones during this 'window of vulnerability' because of exposure to marijuana increase the risk of TGCTs. However, none of these explanations likely would be specific to nonseminomas. Indeed, if the association is true, then new avenues of research will be needed to address the specificity of the association to nonseminomas.

The mechanism by which marijuana exerts its effects on various biologic processes remained unknown until cannabinoid receptors were identified in the 1990s. Cannabinoid receptors are part of the G-protein–coupled receptor family and comprise 2 major subtypes, brain-type receptors (CB1) and spleen-type receptors (CB2).[20,21,39] They are G-protein–coupled, 7 transmembrane spanning receptors and influence a variety of biologic responses. CB1 and CB2 are expressed in the testes and sperm as well as in the brain, heart, uterus, embryo, spleen, and immune cells.[17]

There are 2 major endogenous cannabinoid-like (endocannabinoid) lipid mediators, N-arachidonoylethanolamine (anandamide) and 2-arachidonoylglycerol, that are produced from arachidonic acid. They mimic many of the effects of tetrahydrocannabinol (THC) and activate both CB1 and CB2.[18,40-43] The endocannabinoid system is operative in the male reproductive organs.[14] Endocannabinoid signaling is associated with antitumor effects on a variety of human tumor cells in vitro and in xenograft models in vivo,[44,45] findings that appear to be inconsistent with our observation of an association between marijuana use and TGCTs. Endocannabinoids are degraded rapidly by fatty acid amid hydrolase and monoacylglycerol lipase, whereas marijuana derivatives are metabolized mainly by cytochrome P_{450} enzymes with a half-life of approximately 4 days in chronic marijuana users.[46] Thus, relatively prolonged activation of CB1 and CB2 in marijuana users may disrupt normal antitumorigenic endocannabinoid signaling. Alternatively, the effects of

cannabinoid/endocannabinoid signaling on tumorigenesis may be organ specific and age dependent. Although both the main psychoactive component of marijuana, THC, and anandamide have higher affinity for CB1, cannabinol, an oxidation product of THC, has 10-fold higher affinity for CB2 compared with CB1.[21] Therefore, the activation of CB receptors coupled to different effectors may lead to distinct biologic functions. In addition, other biologically active components of marijuana may function through pathways other than the endocannabinoid system. Future epidemiologic and model system studies are needed to confirm or refute our findings. Such studies should include assessment of the role of CB receptors and endocannabinoid signaling in TGCTs.

Conflict of Interest Disclosures

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