Original Contribution

Null Association Between Histology of First and Second Primary Malignancies in Men With Bilateral Testicular Germ Cell Tumors

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Testicular germ cell tumors (TGCTs), the most common neoplasms of young men, are categorized histologically as either seminomas or nonseminomas/mixed germ cell tumors. These subtypes differ by age at diagnosis and clinical course, but little is known about etiological distinctions. To test the hypothesis that histological subtypes have distinct sets of unrecognized etiological factors, we used a recently described approach, estimating the association between histological types of first and second tumors of men with 2 primary TGCTs. The study population of 488 men each with 2 primary TGCTs was ascertained through population-based cancer registries in the United States between 1972 and 2006. Univariate logistic regression analysis revealed that the histology of second primary TGCTs was associated with the histology of first TGCTs (odds ratio = 1.70, 95% confidence interval: 1.14, 2.52); however, the association did not persist in analyses adjusted for age at diagnosis of first TGCT (odds ratio = 1.09, 95% confidence interval: 0.71, 1.70). These results would be expected if the subtypes share etiology but experience different rates of progression to diagnosis or if the histological fate of TGCTs is influenced by age-related processes. Men with 2 primary TGCTs provide novel opportunities to learn whether histological subtypes are likely to share etiology, so results may inform research designed to identify causes.

causality; germ cell neoplasms; metachronous second primary neoplasms; multiple primary neoplasms; second primary neoplasms; seminoma; synchronous multiple primary neoplasms; testicular neoplasms

Abbreviations: CCR, California Cancer Registry; CI, confidence interval; OR, odds ratio; SEER, Surveillance Epidemiology and End Results; TGCT, testicular germ cell tumor.

Testicular germ cell tumors (TGCTs) are the most common neoplasms of men aged 15–34 years in the United States. Survival rates have improved dramatically since the 1970s, but it is now clear that survivors experience elevated risks of important sequelae, including infertility (1, 2), cardiovascular disease (3), and primary cancers at other organ sites (4). Understanding the etiology of TGCTs as a basis of prevention has therefore emerged as a research priority. Consistent epidemiologic results suggest that TGCTs have both genetic and environmental causes. Notably elevated risk among family members (5) and associations with inherited genotypes (6–9) point to genetic causes, whereas dramatic increases in incidence rates—which nearly doubled between 1975 and 2007 (10)—can be explained only by environmental causes. However, the identified genotypic risk variants account for only 4%–6% of familial risk (7), and environmental factors that would explain increasing rates are unknown. Therefore, despite years of epidemiologic investigation, etiological factors responsible for the majority of TGCT risk remain elusive.

One possible obstacle to identifying the causes of TGCTs is the failure to recognize etiologically distinct TGCT subtypes, which is requisite to treating etiological subtypes as separate outcomes in epidemiologic studies. TGCT tumor histologies are broadly categorized as seminomas or nonseminomas/mixed germ cell tumors (“nonseminomas”) on the basis of the degree of differentiation, but the extent to which these

subtypes share risk factors is unknown. These subtypes differ in clinical characteristics (11) and ages of peak incidence (12). However, they share the following commonalities: seminomas and nonseminomas have overlapping age--incidence curves, and the risk of each subtype is associated with personal history of undescended testis (13), family history of TGCTs, and some risk alleles (6–8). Moreover, characteristic cytogenetic changes (gain of material on chromosomes X, 7, 8, 12p, and 21; loss of material on Y, 4, 5, 1p, 11, 13, and 18) are commonly observed in both seminomas and nonseminomas (14).

The conventional epidemiologic strategy for investigating whether cancer subtypes share etiology is to directly examine whether candidate subtypes are associated with 1 or more of the same measured risk factors. Authors of a recent systematic review of studies providing TGCT data of this type (15) concluded that published epidemiologic studies do not support the hypothesis that testicular seminoma and nonseminoma have different etiologies. However, this approach is not well suited to TGCTs because risk factors remain largely unknown.

Fortuitously, a method was recently described for investigating whether subtypes of a malignancy share etiology when risk factors are not measured (16). The approach uses a novel data structure, a series of individuals with a history of 2 primary tumors, and is implemented by examining the association of tumor subtype within participants. For example, in a series of men with 2 primary TGCTs, etiological heterogeneity of seminoma versus nonseminoma subtypes of TGCT can be investigated by estimating the association of a seminomatous second TGCT with a seminomatous first TGCT. When subtypes share a common etiology, the expected value of the resulting odds ratio is unity (1.0), corresponding to independent occurrence of subtypes; values greater than 1.0 are expected when subtypes have different etiologies. To interpret these values intuitively, one may consider that, if subtypes had distinct sufficient causes, there would be a tendency for individual participants to have 2 primary tumors of a single subtype as a consequence of exposure to 1 of the sufficient causes. By this rationale, men with a history of 2 primary TGCTs provide a unique opportunity to investigate whether seminomatous and nonseminomatous tumors are likely to share etiology.

MATERIALS AND METHODS

Study population

Men contributing to this analysis resided in the United States and had bilateral TGCTs, with both diagnoses occurring between 1972 and 2006 and both tumors meeting each of the following criteria: 1) malignant primary tumor, 2) located in testis, and 3) of germ cell origin. Histological type was assigned on the basis of International Classification of Diseases for Oncology, Third Revision, codes as follows: seminoma (code 9061, 9062, or 9063) or nonseminomou/mixed germ cell tumor (code 9065, 9070, 9071, 9080, 9081, 9082, 9083, 9084, 9085, 9100, or 9101). We included in the seminoma group a small number of tumors described as dysgerminoma (code 9060) or germi-

neoplasia, testicular tumors of non–germ cell origin, and extragondal germ cell tumors were excluded.

Data were obtained from the California Cancer Registry (CCR) and the Surveillance, Epidemiology, and End Results (SEER) program registries outside of California. The CCR is a network of 10 regional registries located throughout California. By record matching, CCR personnel identified 177 men with a history of bilateral TGCTs. SEER is a network of 17 population-based cancer registries located in various sites across the United States. By using record matching with data from the 17 SEER registries (12), SEER personnel identified 443 men with a history of 2 primary TGCTs; we excluded from this series 132 cases identified in SEER registries within California, thereby avoiding duplicate inclusion of cases identified through the CCR, leaving 311 cases for analysis.

For each man included in the analysis, both TGCT diagnoses were documented, with histological type and date of diagnosis obtained from registry records. Information on race/ethnicity and date of birth was extracted from registry records. Dates were coded as month, day, and year for men identified through the CCR. Redacted components of date were imputed for men identified through SEER as follows: the date of each diagnosis was imputed as the first day of the month and year provided by SEER, and the date of birth was imputed as June 1 of the year of birth provided by SEER.

This study was approved by the University of Southern California’s institutional review board.

Statistical methods

Unconditional logistic regression was used to estimate the odds ratio and corresponding 95% confidence interval relating seminoma as the histological type of the first TGCT (“exposure”) to seminoma as the histological type of the second TGCT (“outcome”). The crude odds ratios were estimated first, and the intervals between diagnoses and ages at first and second diagnoses were included as covariates in subsequent analysis models.

Diagnoses were scored as synchronous if the interval between them was less than 60 days and as asynchronous otherwise. When both tumors were diagnosed on the same day, TGCT in the right testicle was coded as “first” for purposes of analysis. Means and ranges of ages at first and second diagnoses were estimated within subgroups defined by ascertainment scheme and histology of both tumors. Analyses were performed by using SAS, version 9.1, software (SAS Institute, Inc., Cary, North Carolina).

RESULTS

As shown in Table 1, 488 men with a history of 2 historically confirmed primary TGCTs were ascertained. The majority (87.3%) were non-Hispanic whites. The mean ages at diagnoses of first and second TGCT were 31.2 years and 35.6 years, respectively. Seminomas comprised 61.1% of first primary and 70.5% of second primary TGCTs. These attributes were similar for cases identified by each registry. As shown in Table 2, men for whom the first primary TGCT was a seminoma tended to be older at the time of first diagnosis than those whose first TGCT was a nonseminoma/
mixed germ cell tumor (mean age at first diagnosis = 33.8 vs. 27.3 years, respectively). Within each of these groups, the age at diagnosis of the second TGCT tended to be higher for those whose second TGCT was a seminoma. These results accord with well-documented patterns of later diagnosis of seminoma compared with nonseminoma/mixed germ cell tumors.

Table 3 provides odds ratio estimates of concordance between the histologies of first and second primary tumors. In univariate analyses addressing only histological type of each tumor, men for whom the initial TGCT was a seminoma were more likely to also have seminoma as the histology of the second TGCT (odds ratio = 1.70, 95% confidence interval: 1.14, 2.52). Subsequent analyses were adjusted for age at each TGCT diagnosis and for the length of interval between diagnoses. Adjusting only for the interval between diagnoses did not substantially alter the estimate (odds ratio = 1.79, 95% confidence interval: 1.20, 2.67). However, adjusting for age at first diagnosis eliminated any apparent association between histological types of first and second TGCTs (odds ratio = 1.09, 95% confidence interval: 0.71, 1.70). Results did not materially differ from this result (<3.6%) after adjustment for age at second diagnosis, after simultaneous adjustment for age at diagnosis and interval between diagnoses, or after the exclusion of men with a spermatocytic seminoma (n = 3) or with a seminoma reported as a germinoma or dysgerminoma (n = 17) (data not shown). Moreover, results changed little when we included only synchronous or asynchronous diagnoses (Table 3).

**DISCUSSION**

Men with a history of 2 primary TGCTs provide an opportunity to investigate etiological heterogeneity of testicular seminoma and nonseminoma. Earlier studies (reviewed elsewhere (17)) reported that men with a history of 1 TGCT are 12–124 times more likely to develop a second primary TGCT.
in the contralateral testicle than are unaffected men of like age and race/ethnicity to develop a first primary TGCT. This remarkably elevated risk indicates that occurrences of the 2 primary TGCTs are rarely independent. Therefore, in most men with bilateral TGCTs, common etiological factors likely predisposed them to both primary tumors. We reasoned that if seminoma and nonseminoma have distinct etiologies, then, among bilateral TGCT patients, a man’s first and second TGCT are likely to be the same in accordance with a history of exposure to either seminoma-related or nonseminoma-related risk factors. Alternatively, if common factors predispose to both seminoma and nonseminoma, the histology of the first TGCT is likely to be unassociated with that of the second TGCT. To learn which of these predictions was realized, we estimated the odds ratio by measuring concordance of histological types among men with a history of bilateral TGCT, an approach recently formalized for investigating risk heterogeneity of tumor subtypes (16).

In this analysis of the largest set of data on bilateral TGCT studied to date, after controlling for age at diagnosis, there was little or no association between the histology of first and second tumors. A null association corresponds to independent occurrence of seminomas and nonseminomas and is expected when risk profiles of the 2 histological types are perfectly correlated (16). This result therefore indicates that, after accounting for age at diagnosis, subsets of TGCT cases classified by tumor histology are unlikely to have distinct etiologies.

The relationship between etiological heterogeneity and estimated concordance of tumor subtypes depends on several assumptions (16) that must be considered in the context of this study. First, both tumors of each pair should be true primary tumors, rather than a single primary tumor followed by a metastatic tumor misdiagnosed as a second primary tumor. This assumption is that second primary tumors are not caused by treatment of the first. Although both chemotherapy and radiotherapy can be used to treat TGCTs, and both are plausible carcinogens, this assumption also seems reasonable. Systemic therapy causes increases in malignancies along well-known lines of carcinogenesis that do not include testicular cancer (4), and the testicle is an extremely rare site for metastasis for any malignancy (21). Moreover, approximately 80% of seminomas and 50–60% of nonseminomas present as clinical stage I disease, and the contralateral testicle is outside the field of radiation for treatment of stage I disease. Further, recognition of excellent cure rates with surgery alone in stage I disease has moved treatment paradigms away from the use of radiation and chemotherapy following initial diagnosis and toward active surveillance, with systemic therapies used only in patients who relapse. In addition, temporal considerations preclude chemotherapy or radiotherapy as causes of a second primary tumor when diagnoses are synchronous, and in this study, odds ratio estimates among men with synchronous diagnoses did not substantially differ from estimates among men with asynchronous diagnoses. Finally, if histological subtypes had distinct etiologies, in order for treatment effects to spuriously create the null association observed, seminoma treatment would have to preferentially predispose to nonseminoma and nonseminoma treatment to seminoma, which seems implausible.

A final assumption is that second primary tumors are sampled as population-based incident cases. Although the design of this study precludes strict sampling of this form—as would any study of paired primary TGCTs—second cases of the participants were ascertained as incident cases from defined source populations. We recognize that the base populations were open and dynamic. However, TGCT survival has been excellent in recent decades (96% during the period 1999–2005 (22)), so most losses would be caused by migration out of the population in which the first diagnosis occurred. For sampling of second cases to bias the odds ratio estimate toward unity, individuals destined to develop second primary TGCTs must have left the source population in a differential fashion, with men who developed a second primary tumor of concordant histology (e.g., seminoma following seminoma) often leaving and those who developed a second TGCT of discordant histology (e.g., nonseminoma following seminoma) preferentially remaining behind to be ascertained. We cannot envision circumstances that would create this pattern.

A final circumstance that could, in theory, lead spuriously to a null estimate of the odds ratio is misclassification of tumor histology. However, histology data for tumors ascertained by registries were scored by procedures routinely used by SEER.

### Table 3. Odds Ratio Associations of a Seminomatous Second TGCT Following a Seminomatous First TGCT in the United States, Estimated by Unconditional Logistic Regression, 1972–2006

<table>
<thead>
<tr>
<th>Set of Bilateral Cases</th>
<th>No.</th>
<th>Unadjusted OR (95% CI)</th>
<th>Adjusted for Interval Between Diagnoses OR (95% CI)</th>
<th>Adjusted for Age at First Diagnosis OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>488</td>
<td>1.70 (1.14, 2.52)</td>
<td>1.79 (1.20, 2.67)</td>
<td>1.09 (0.71, 1.70)</td>
</tr>
<tr>
<td>With synchronous diagnoses</td>
<td>144</td>
<td>1.46 (0.67, 3.18)</td>
<td>1.42 (0.65, 3.11)</td>
<td>1.15 (0.51, 2.61)</td>
</tr>
<tr>
<td>With asynchronous diagnoses</td>
<td>344</td>
<td>1.77 (1.11, 2.80)</td>
<td>2.05 (1.26, 3.33)</td>
<td>1.04 (0.61, 1.76)</td>
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Abbreviations: CI, confidence interval; OR, odds ratio; TGCT, testicular germ cell tumor.

a Relative to odds of a second seminoma following a first nonseminoma/mixed TGCT.
Histology data in the present study can therefore be assumed to capture the same 2 subtypes as population-based registry data, which have revealed distinct ages of peak occurrence of seminomatous versus nonseminomatous TGCTs.

Interpreting the odds ratio estimate as an indicator of minimal risk heterogeneity therefore seems reasonable. The possibility that a common set of environmental and genetic factors underlie the occurrence of both seminomas and nonseminomas of the testis accords well with a proposed model of TGCT development based on tumor biology. Histological subtypes are postulated to arise from the same precursor lesion, intratubular germ cell neoplasia, with seminoma emerging by default and nonseminoma developing if additional cellular reprogramming activates pluripotency (14). As a corollary, indolent behavior of seminoma is proposed as an explanation for the later peak age at diagnosis of this subtype.

In the full set of data and in all subsets examined, crude estimates of the odds ratios were greater than estimates adjusted for age at first TGCT diagnosis, indicating that age confounds the association between histological types of first and second tumors. Older age at first diagnosis appears to satisfy criteria for a positive confounder, because it is associated with seminomatous histology of both first and second primary tumors but is not a consequence of either (23). Rothman and Greenland described age as a “surrogate confounder,” measuring time since birth but often associated with unmeasured factors that influence disease risk (23). Intriguingly, marijuana use—an environmental exposure common in the peripubertal and early adult period—has been reported to be specifically associated with nonseminoma risk (24–26). Although speculative, the possibility that age may serve as a proxy confounder due at least in part to age-related exposures or processes that may influence TGCT histology may warrant exploration. If identified, such factors might suggest innovative strategies of risk abatement aimed at influencing tumor behavior by favoring the development of seminoma, which tends to be less aggressive than nonseminoma. However, this distinction in clinical behavior provides a more conventional explanation for the apparently confounding effect of age at first TGCT, with an indolent course of seminoma resulting in delayed discovery and thereby greater age at diagnosis of this subtype.

In summary, in a large, high-quality set of data on men with bilateral TGCTs, we found no association between the histology of first and second TGCTs in analyses adjusted for age at first diagnosis. It therefore seems unlikely that important environmental causes of TGCTs have eluded epidemiologic investigation simply because these subtypes have not been treated as adequately distinct. This result therefore lends credence to the alternate possibility that exposures underlying both subtypes occur early in development and have consequently been difficult to measure following TGCT diagnosis, which may occur many years later. The possibility that early events may predispose to both seminoma and nonseminoma has long been postulated (reviewed elsewhere (27)) and is illustrated by recent genome-wide association studies identifying genotypic variants associated with the risk of both subtypes in and near the gene-encoding KIT ligand (6, 8). This molecule has a key role in early germ cell development, so risk variants may represent a driver of both seminoma and nonseminoma that acts very early. Because genotypic variants are unlikely to change over time, they are more likely than early exposures to be measured accurately and thereby identified as risk factors. However, environmental causes generally provide more direct routes of risk abatement, making their discovery crucial. The possibility that environmental causes responsible for increasing TGCT incidence act early in development also accords with age-period-cohort analyses (reviewed elsewhere (27)) showing that the year of a man’s birth is a better predictor of risk than the year of TGCT diagnosis. Together with these considerations, results of the present study indicate that research aimed at identifying environmental causes of TGCTs should address both seminoma and nonseminoma by using methods suited to measuring environmental exposures beginning at early developmental stages.

The apparently confounding effect of age at first TGCT diagnosis likely arises from the association of seminomatous histology with age in the general population, which may in turn reflect the indolent course of this histological type, although age-related influences on tumor histology are theoretically possible and, if discovered, could suggest innovative strategies of risk abatement. It therefore seems advisable that future etiological studies be designed not only to investigate factors that may influence the risk of all TGCTs, but also to examine the effects of age-related factors, such as marijuana exposure in adolescence (28), that may be specifically associated with the risk of individual histological subtypes.

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