Prospectively Identified Incident Testicular Cancer Risk in a Familial Testicular Cancer Cohort

Anand Pathak1, Charleen D. Adams1, Jennifer T. Loud1, Kathryn Nichols2, Douglas R. Stewart1, and Mark H. Greene1

Abstract

Background: Human testicular germ cell tumors (TGCT) have a strong genetic component and a high familial relative risk. However, linkage analyses have not identified a rare, highly penetrant familial TGCT (FTGCT) susceptibility locus. Currently, multiple low-penetration genes are hypothesized to underlie the familial multiple-case phenotype. The observation that two is the most common number of affected individuals per family presents an impediment to FTGCT gene discovery. Clinically, the prospective TGCT risk in the multiple-case family context is unknown.

Methods: We performed a prospective analysis of TGCT incidence in a cohort of multiple-affected-person families and sporadic-bilateral-case families; 1,260 men from 140 families (10,207 person-years of follow-up) met our inclusion criteria. Age-, gender-, and calendar time-specific standardized incidence ratios (SIR) for TGCT relative to the general population were calculated using SEERiStat.

Results: Eight incident TGCTs occurred during prospective FTGCT cohort follow-up (versus 0.67 expected; SIR = 11.9; 95% CI, 5.1–23.4; excess absolute risk = 7.2/10,000). We demonstrate that the incidence rate of TGCT is greater among bloodline male relatives from multiple-case testicular cancer families than that expected in the general population, a pattern characteristic of adult-onset Mendelian cancer susceptibility disorders. Two of these incident TGCTs occurred in relatives of sporadic-bilateral cases (0.15 expected; SIR = 13.4; 95% CI, 1.6–48.6).

Conclusions: Our data are the first to indicate that despite relatively low numbers of affected individuals per family, members of both multiple-affected-person FTGCT families and sporadic-bilateral TGCT families comprise high-risk groups for incident testicular cancer.

Impact: Men at high TGCT risk might benefit from tailored risk stratification and surveillance strategies. Cancer Epidemiol Biomarkers Prev; 24(10); 1–8. ©2015 AACR.
<table>
<thead>
<tr>
<th>First author</th>
<th>Country</th>
<th>Year</th>
<th>Study design</th>
<th>Testicular cancer (TC) cases</th>
<th>Controls</th>
<th>TC in 1° or 2° relative</th>
<th>Families reported</th>
<th>Risk estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tollerud, DJ (19)</td>
<td>U.S.</td>
<td>1985</td>
<td>Retrospective multicenter</td>
<td>269</td>
<td>259</td>
<td>Cases = 6</td>
<td>Control = 1</td>
<td>NR</td>
</tr>
<tr>
<td>Forman, D (14)</td>
<td>U.K.</td>
<td>1992</td>
<td>Retrospective multicenter</td>
<td>794</td>
<td>749</td>
<td>Cases = 12</td>
<td>Control = 2</td>
<td>42</td>
</tr>
<tr>
<td>Westergaard, T (20)</td>
<td>Denmark</td>
<td>1996</td>
<td>Retrospective Population-based cohort</td>
<td>Father cohort = 2,113</td>
<td>NA</td>
<td>Fathers = 12</td>
<td>Brothers = 4</td>
<td>NR</td>
</tr>
<tr>
<td>Heimdal, K (15)</td>
<td>Norway and Sweden</td>
<td>1996</td>
<td>Retrospective multicenter hospital-based cohort</td>
<td>Father cohort = 2,113 Brother's sub-cohort = 702</td>
<td>NA</td>
<td>Fathers = 12</td>
<td>Brothers = 4</td>
<td>NR</td>
</tr>
<tr>
<td>Dieckmann, K (12)</td>
<td>Germany</td>
<td>1997</td>
<td>Prospective multicentric cohort</td>
<td>1,692</td>
<td>518</td>
<td>Cases = 13</td>
<td>Control = 3</td>
<td>NR</td>
</tr>
<tr>
<td>Dong, C (13)</td>
<td>Sweden</td>
<td>2001</td>
<td>Retrospective family cancer database</td>
<td>4,640</td>
<td>NA</td>
<td>62</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Hemminki, K (17)</td>
<td>Sweden</td>
<td>2004</td>
<td>Retrospective multigenerational registry</td>
<td>Sons = 4,082 Fathers = 3,878</td>
<td>0</td>
<td>67</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Bremen, K (11)</td>
<td>Germany</td>
<td>2004</td>
<td>Retrospective multi-national population-based case/control</td>
<td>269</td>
<td>797</td>
<td>Cases = 11</td>
<td>Controls = 6</td>
<td>NR</td>
</tr>
<tr>
<td>Hemminki, K (16)</td>
<td>Sweden</td>
<td>2006</td>
<td>Retrospective population registry</td>
<td>Sons = 4,586 Fathers = 4,314</td>
<td>0</td>
<td>43</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Walschaerts, M (10)</td>
<td>France</td>
<td>2007</td>
<td>Retrospective hospital-based case/control</td>
<td>229</td>
<td>800</td>
<td>40,104 in cases and controls</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Nordsborg, RK (8)</td>
<td>Denmark</td>
<td>2011</td>
<td>Retrospective population-based case/control</td>
<td>3,297</td>
<td>6,594</td>
<td>7,524 families with &gt;1 TGCT</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Valberg, M (9)</td>
<td>Norway</td>
<td>2013</td>
<td>Retrospective Hierarchical frailty modeling</td>
<td>1,135,320</td>
<td>NA</td>
<td>7,524 families with &gt;1 TGCT</td>
<td>NR</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: FRR, frailty relative risk; RR, relative risk.
Materials and Methods

Study population

Multiple-case families with (i) ≥2 confirmed TGCT subjects, (ii) a combination of TGCT and extra-gonadal germ cell tumor (both designated “multiple-affected-person” families), and (iii) families containing only a single individual with bilateral TGCT (designated “sporadic-bilateral-subject” families) were enrolled in the "Multidisciplinary Etiologic Study of Familial Testicular Cancer" (NCI Protocol 02-C-0178; NCI-00039598). In the aggregate, these 3 subsets of families were designated “multiple-case” families, because a subject with sporadic bilateral testicular cancer by definition had two cases of TGCT. Kindreds with a female germ cell tumor patient were excluded from the current analysis. The study protocol explicitly included sporadic-bilateral TGCT subjects (i.e., men with bilateral testicular cancer and a negative family history of TGCT) because bilateral affection of paired organs has long been regarded as one of clinical features, suggesting the presence of an underlying cancer susceptibility disorder. Our original analytic plan was to seek candidate gene germline mutations identified in multiple-affected-person families, within our sporadic-bilateral subjects. It was our a priori hypothesis that at least a subset of sporadic-bilateral TGCT patients would be found to have germline mutations in the same susceptibility gene(s) identified in multiple-affected-person families, that is, that they would have the same genetic cause of their cancer.

Participants completed family, medical, epidemiologic, and psychosocial questionnaires and donated blood samples. All subjects provided written informed consent. Families with two or more affected males or a sporadic bilateral case were eligible for travel to the NIH Clinical Center for a protocol-based etiologic evaluation, including detailed history and physical examination, semen, and laboratory analyses, ultrasound imaging of the testes or ovaries, and ultrasound imaging or computed tomography of the kidneys (41). This study was approved by the NCI Institutional Review Board. Ninety-three percent of all participants reported their racial category as white. Twelve hundred and sixty enrolled individuals from 140 families were included in this study; females and non-bloodline relatives were excluded from the current analysis.

Statistical analysis

Referent age-adjusted population cancer rates for white males were computed by 5-year age group and 5-year calendar periods using the NCI SEER9 database (1973–2010). The at-risk interval was defined from the family enrollment date (the date on which the first subject from each family signed the study-related informed consent document) to date of cancer diagnosis, death or end of study. Accrued person-years were calculated, and an observed-to-expected SIR for incident TGCT was calculated using SEER’Stat, as previously described (42). All TGCT (n = 224) diagnosed prior to each family’s date of study enrollment were excluded from the incident TGCT calculation.

Results

Twelve hundred and sixty men from 140 families with 10,207 person-years of follow-up were included in this study. Eight of the...
1,260 subjects developed TGCT during follow-up; six incident cases had no prior testicular cancer history, while two were metachronous TGCTs. Six incident cases occurred in multiple-affected-person families and two incident cases occurred among the relatives of men with sporadic-bilateral TGCT. Table 2 summarizes the demographic and clinical characteristics of the individuals with TGCT prior to enrollment and characteristics of incident TGCTs, including number of individuals affected in the family, presence of microlithiasis, personal history of UDT, familial pattern of affection and TGCT morphology. Prior TGCT cases and incident cases had similar distributions of these variables. Table 3 summarizes the clinical characteristics of study participants with an incident cancer.

Eight TGCTs were observed among the 1,260 familial multiple-case TGCT cohort members during prospective follow-up versus 0.67 cases expected (O/E = 29.3; 95% CI, 10.7–63.7) or UDT (O/E = 31.1; 95% CI, 8.5–79.7) in the family suggested higher risks. However, the 95% CI associated with these point estimates overlapped with those from the respective “no” categories, indicating that these differences were not statistically significant. Of note, seven of the eight incident TGCTs occurred among the 119 families with ≥2 affected individuals (O/E = 6.2; 95% CI, 0.2–35.2) in the 21 families with ≥3 affected. Thus, the handful of heavily loaded families did not drive the occurrence of incident TGCT in this cohort.

Table 2. Demographic and clinical characteristics of the FTGCT cohort

<table>
<thead>
<tr>
<th>Age at entry (mean, SD)</th>
<th>Prior personal history of TGCT (n = 224)</th>
<th>Incident cases* (n = 8)</th>
<th>No incident or prior TGCT (n = 1,030)</th>
</tr>
</thead>
<tbody>
<tr>
<td>38.9 (12.2)</td>
<td></td>
<td>33.5 (10.4)</td>
<td></td>
</tr>
<tr>
<td>Incident history of TGCT prior to family enrollment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>224 (17.8%)</td>
<td>34.7 (27.4)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1,036 (82.2%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Numbers of individuals affected in family

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>56 (42.4%)</td>
<td>56 (42.4%)</td>
<td>18 (13.6%)</td>
<td>1 (0.8%)</td>
<td>1 (1.8%)</td>
</tr>
</tbody>
</table>

For those with prior personal history of TGCT (excluding incident cases), n = 222

<table>
<thead>
<tr>
<th>Microlithiasis</th>
<th>Classical testicular microlithiasis (CTM)</th>
<th>CTM/LTM</th>
<th>Limited testicular microlithiasis (LTM)</th>
<th>No microlithiasis</th>
<th>Microlithiasis status unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>17 (7.7%)</td>
<td>2 (0.9%)</td>
<td>25 (11.3%)</td>
<td>25 (11.3%)</td>
<td>153 (68.9%)</td>
</tr>
<tr>
<td></td>
<td>2 (0.9%)</td>
<td>1 (12.5%)</td>
<td>2 (25.0%)</td>
<td>1 (12.5%)</td>
<td>4 (50.0%)</td>
</tr>
</tbody>
</table>

Personal history of undescended testicle

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>208</td>
</tr>
</tbody>
</table>

Family pattern of affection at enrollment

<table>
<thead>
<tr>
<th>Bilateral affected case</th>
<th>Complex</th>
<th>Cousins</th>
<th>Father/son</th>
<th>One of a set of identical twins</th>
<th>Siblings</th>
<th>Uncle/nephew</th>
</tr>
</thead>
<tbody>
<tr>
<td>56 (25.2%)</td>
<td>25 (11.3%)</td>
<td>16 (7.2%)</td>
<td>34 (15.3%)</td>
<td>2 (9.9%)</td>
<td>82 (37.4%)</td>
<td>6 (2.7%)</td>
</tr>
</tbody>
</table>

TGCT morphology

<table>
<thead>
<tr>
<th>Carcinoma, NOS</th>
<th>Mixed germ cell tumor</th>
<th>Seminoma, NOS</th>
<th>Nonseminoma, NOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 (3.6%)</td>
<td>36 (16.2%)</td>
<td>102 (46.0%)</td>
<td>76 (34.2%)</td>
</tr>
</tbody>
</table>

Abbreviation: NOS, not otherwise specified.

*Two of 8 incident cases also had a prior history of TGCT; the remaining 6 did not.
Discussion

In 2002, the National Cancer Institute’s Clinical Genetics Branch initiated an observational, etiologic study of FTGCT (41). During the course of prospective follow-up, 8 persons (6 without, and 2 with, a personal history of TGCT at the time of enrollment) developed TGCT, a nearly 12-fold increase in TGCT risk compared with the number expected from gender-, age- and calendar-time-specific rates from the U.S. white population. These are the first cases of FTGCT to be documented prospectively, and their occurrence permitted us to generate the first quantitative estimates of TGCT risk in the setting of multiple-case families.

Furthermore, stratified analysis revealed that the risk was similarly increased in multiple-affected-person families (O/E = 11.6) and sporadic-bilateral-subject families (O/E = 13.4). Our results confirm that men from both multiple-affected-person TGCT families and sporadic-bilateral subject TGCT families truly do comprise two subsets of the general population that are at substantially increased TGCT risk. Although the number of cancer events in each group is small, and the excess absolute risks are low, each O/E ratio is statistically significantly elevated relative to general population expectation. Nonetheless, our observations in the relatives of men with sporadic-bilateral TGCT warrant replication, a task that may be approachable using the Scandinavian population-based registry system.

Our results are somewhat surprising given that a combination of low penetrance genes is thought to underlie the etiology of familial testicular cancer, and that about 75% of families contain only two cases, because it is generally believed that polygenic susceptibility does not produce familial aggregations of disease (31). To the best of our knowledge, ours is the only existing longitudinal cohort study targeting men from extended multiple-case TGCT families that could be used to address this fundamental question. In particular, the prospective occurrence of incident TGCT in the relatives of men from sporadic-bilateral-subject families further supports the broader notion that there is a genetic component to this pattern of affection. This unexpected result is consistent with the recognition that men who are homozygous for KITLG TGCT-associated risk alleles have a TGCT odds ratio that is greater than 6 (25, 26), the strongest SNP/cancer association yet reported. FTGCT may be the first well-documented example of a disease presentation that will become more common now that our ability to identify polygenic disorders has become more tractable. Potential mechanisms for this phenomenon include (i) the existence of intermediate-risk variants, like KITLG; (ii) the presence of common, low-penetration variants acting as modifiers of the risks associated with as yet undiscovered rare, high-penetrance variants; and (iii) common variants proving to be highly active functionally.

We attempted to determine whether specific clinical features might permit identification of a subset of family members that was at particular risk of developing incident TGCT. Within the constraints imposed by the small number of prospective cancer events, none of the characteristics we examined (Table 4) were significantly correlated with cancer risk above and beyond the level seen in the entire set of family members. The SIRs associated with a family history of either microlithiasis (O/E = 29.3) or undescended testes (O/E = 31.1) trended toward greater risks, but these differences were not statistically significant. We are continuing to enroll and follow additional FTGCT kindred, and hope to eventually achieve sufficient statistical power to answer these questions definitively. We should note that our prior report linking microlithiasis to the risk of FTGCT included many of the same families analyzed here (35); thus, these findings do not comprise independent confirmation of that provocative observation, which does merit corroboration in the context of elucidating the pathogenesis of testicular cancer. The microlithiasis association question is one of the major foci of our ongoing research.

This is the first study to demonstrate quantitatively that the incidence of testicular cancer is substantially increased relative to the general population in a cohort of multiple-case families, including both multiple-affected-person and sporadic-bilater-subject kindreds. Although there is a substantial epidemiologic literature aimed at estimating familial risks of TGCT, all prior reports targeted sporadic/unselected TGCT, and used retrospective, cross-sectional or record linkage designs (Table 1). In contrast, our study was family-based, prospective, excluded prevalent cases from the risk assessment, had clinical details on a significant fraction of study participants, included a relatively large number of individuals at risk, had central pathology review of TGCT cases performed (87.5% of incident cases), and was based on an average follow-up of more than 8 years. Nonetheless, the number of cancer events was small, limiting our ability to more precisely define subsets of family members that might be at particularly high risk. In addition, individual level information relative to testicular microlithiasis was available only for the 132 individuals who had undergone testicular ultrasound, either as part of our study or during the

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**Table 3. Summary of study participants who developed incident TGCT during prospective follow-up**

<table>
<thead>
<tr>
<th>Case</th>
<th>Study subset</th>
<th>UDT</th>
<th>Testicular microlithiasis</th>
<th>TGCT in family</th>
<th>Prior TGCT histology (laterality)</th>
<th>Age at Dx</th>
<th>Incident TGCT histology (laterality)</th>
<th>Age at Dx</th>
<th>Vital status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MAP</td>
<td>No</td>
<td>Unknown</td>
<td>2</td>
<td>Nonseminoma (L)</td>
<td>14</td>
<td>Nonseminoma (R)</td>
<td>25</td>
<td>Alive</td>
</tr>
<tr>
<td>2</td>
<td>MAP</td>
<td>No</td>
<td>Yes</td>
<td>2</td>
<td>Seminoma (L)</td>
<td>34</td>
<td>Seminoma (R)</td>
<td>40</td>
<td>Alive</td>
</tr>
<tr>
<td>3</td>
<td>MAP</td>
<td>No</td>
<td>Unknown</td>
<td>2</td>
<td>None</td>
<td>—</td>
<td>Nonseminoma (R)</td>
<td>17</td>
<td>Alive</td>
</tr>
<tr>
<td>4</td>
<td>MAP</td>
<td>No</td>
<td>Unknown</td>
<td>2</td>
<td>None</td>
<td>—</td>
<td>Unknown</td>
<td>32</td>
<td>Alive</td>
</tr>
<tr>
<td>5</td>
<td>MAP</td>
<td>No</td>
<td>Yes</td>
<td>2</td>
<td>None</td>
<td>—</td>
<td>Seminoma (R)</td>
<td>35</td>
<td>Alive</td>
</tr>
<tr>
<td>6</td>
<td>MAP</td>
<td>No</td>
<td>Yes</td>
<td>3</td>
<td>None</td>
<td>—</td>
<td>Mixed germ cell (R)</td>
<td>39</td>
<td>Alive</td>
</tr>
<tr>
<td>7</td>
<td>SB</td>
<td>No</td>
<td>No</td>
<td>1</td>
<td>None</td>
<td>—</td>
<td>Seminoma (L)</td>
<td>47</td>
<td>Alive</td>
</tr>
<tr>
<td>8</td>
<td>SB</td>
<td>Yes</td>
<td>Unknown</td>
<td>1</td>
<td>None</td>
<td>—</td>
<td>Seminoma (R)</td>
<td>41</td>
<td>Alive</td>
</tr>
</tbody>
</table>

Abbreviations: Dx, diagnosis; MAP, multiple-affected-person family; SB, sporadic-bilateral-case family.

*a*Number of individuals with TGCT in family at the time of enrollment.

*b*For the two subjects who had a unilateral TGCT at the time of study entry, and then developed an incident TGCT during prospective follow-up.

= 6; expected = 0.52; O/E = 11.6; 95% CI, 4.2–25.1) and the latter (observed = 2; expected = 0.15; O/E = 13.4; 95% CI, 1.6–48.6).
course of their routine clinical care. This restricted our stratified
SIR analysis of microlithiasis to families rather than individuals.
Critical risk factor information, such as history of unde-
cended testicle, was based largely on unconfirmed subject self-
report. Medical record documentation of UDT was exceedingly
difficult to obtain. The study was not designed to disentangle
the relative contributions of genetic, developmental and envi-
ronmental factors to the etiology of TGCT. Rather, its primary
focus was on susceptibility gene discovery, toward which end
analyses that have shaped our current understanding of TGCT
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importance in that we have documented high TGCT risk in a
family context where the presence of a rare, highly penetrant,
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and risk-reducing capabilities. First, the results are of etiologic
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clinically actionable, despite the absence of an effective screening program.

Finally, modeling exercises have suggested that combining data from GWAS risk loci and strong clinical risk factors (e.g., family history, UDIT, infertility) might permit the development of risk stratification models that could identify specific subsets of men with even more dramatic elevations in risk, upon whom more aggressive education and surveillance activities might be appropriately focused (50, 51), especially if it could be demonstrated that the GWAS risk SNPs were not also associated with the clinical risk factors, a question for which limited data are contradictory (52, 53). Thus, for example, men aged 30–34 in our study who were in the top 1% of genetic risk and who also had a personal history of cryptorchidism were estimated to be at a 50-fold increase in TGCT risk relative to average population risk, assuming that the TGCT risk SNPs were not also associated with undescended testicle risk (50). We are continuing to develop this line of research in the hope that clinically actionable levels of risk can be defined.

This study presents the first prospectively collected data on incident testicular cancer in a multiple-case familial testicular cancer cohort, providing strong evidence that TGCT incidence is substantially higher in this group than in the general population. These findings support the notion that the combined effect of common, low-penetrance mutations can confer a significant risk of cancer, and provide a rationale for developing more sophisticated risk stratification strategies that might unambiguously identify subsets of men that warrant enhanced education and TGCT surveillance.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: M.H. Greene
Development of methodology: M.H. Greene
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J.T. Loud, K. Nichols, M.H. Greene
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A. Pathak, C.D. Adams, D.R. Stewart, M.H. Greene
Writing, review, and/or revision of the manuscript: A. Pathak, C.D. Adams, J.T. Loud, D.R. Stewart, M.H. Greene
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J.T. Loud, K. Nichols, M.H. Greene
Study supervision: J.T. Loud, M.H. Greene

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