Heterogenous effect of androgen receptor CAG tract length on testicular germ cell tumor risk: shorter repeats associated with seminoma but not other histologic types

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Introduction

Testicular germ cell tumors (TGCTs) are the most common malignancies affecting young men. Rates of these tumors have doubled worldwide over the last 40 years (1). Well-established risk factors are family history, a prior TGCT and cryptorchidism (2–4).

Age-specific incidence rates suggest that these tumors are involved in disease etiology, but familial risk and genome-wide association studies implicate genetic factors as well. We investigated whether variation in the functional CAGn polymorphism in the androgen receptor (AR) gene is associated with TGCT risk, using data from a population-based family study. We estimated odds ratios (OR) and 95% confidence intervals (CI) for the association of CAG repeat length and TGCT risk using matched pairs logistic regression. Analyses of 273 TGCT case–mother pairs revealed no association between AR CAG repeat length and overall TGCT risk. However, risk of seminoma was significantly associated with shorter CAG repeat length [CAG 20–21 versus CAG £ 19: OR = 0.82 (95% CI: 0.43–1.58), CAG 22–23 versus CAG £ 19: OR = 0.39 (95% CI: 0.19–0.83) and CAG 24 versus CAG £ 19: OR = 0.42 (95% CI: 0.20–0.86)], with a highly significant trend over these four categories of decreasing CAG repeat length (P trend = 0.0030). This is the first report of a statistically significant association between AR CAG repeat length and seminoma risk, suggesting that increased AR transactivation may be involved in development of seminoma and/or progression of carcinoma in situ/intratubular germ cell neoplasia unclassified to seminoma. This result provides a rationale whereby androgenic environmental compounds could contribute to increases in TGCT incidence, and identifies for the first time a potential biological pathway influencing whether TGCTs achieve seminomatous versus non-seminomatous histology, a clinically and biologically important distinction.

Materials and methods

Study population

A detailed description of the study population appears in supplementary Materials and Methods (available at Carcinogenesis Online). Briefly, the study was conducted at the University of Southern California using data provided by the California Cancer Registry on men diagnosed with TGCT between 1974 and 2006. In all, 15 939 eligible TGCT cases were sent a questionnaire; of those, 5455 (34%) completed the questionnaire as of April 2010. Among cases with completed questionnaires, 978 have been recontacted to request DNA, and 724 cases (74%) have consented. Family members of participating cases were enrolled based on the contribution to genetic analyses. As of April 2010, DNA specimens were provided by 2223 participants, including 627 TGCT cases. The present analysis includes 273 TGCT case–mother pairs from whom DNA specimens had been isolated at the time of laboratory analysis. This study was approved by the Institutional Review Board of the University of Southern California.
Laboratory analysis

Genomic DNA was extracted using standard protocols. Genotyping of AR CAG repeat length was performed using polymerase chain reaction with fluorescently labeled oligonucleotide primers, as described (18).

Statistical analysis

Analyses were conducted using SAS version 9.1 (SAS Institute, Cary, NC). Because the AR is an X-linked gene, males have only one copy, inherited through their mothers. For this analysis, CAG repeat length was compared between 273 TGCT cases and their mothers (546 individuals), assigning the allele transmitted from the mother to the TGCT case as the 'case' allele and the mother’s second, untransmitted allele as the ‘control’ allele, based on the transmission/disequilibrium test for an X-linked marker in males (17). Paired t-tests were used to compare CAG repeat length between case and control alleles. Conditional logistic regression was used to test for associations between CAG repeat length and TGCT risk, measured by odds ratios (ORs) and corresponding 95% confidence intervals (CIs). CAG repeat lengths were categorized into four groups (≤19, 20–21, 22–23 and ≥24) and two groups (≤19 and ≥20) based upon quartile distribution of repeat length among control (i.e. untransmitted) alleles. Wald tests for trend were conducted with CAG repeat length coded as an ordinal variable. Analyses were repeated within subgroups of pairs based on case histology. Case–mother pairs were excluded from the analysis if genoytype data were missing on either individual (6 pairs) or the pair showed genetic incompatibility (15 pairs).

Unconditional logistic regression was used for case–case analyses comparing seminoma cases to mixed GCT cases or nonseminoma cases, in analyses stratified by age at diagnosis (≤25, 26–30, 31–35, 36–40, >40).

Table I. Characteristics of all TGCT cases; all cases, seminoma cases, mixed germ cell tumor cases and nonseminoma cases

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All casesa (N = 273, N (%)</th>
<th>Seminoma (N = 140, N (%))</th>
<th>Mixed germ cell tumors (N = 53, N (%))</th>
<th>Pure nonseminoma (N = 68, N (%))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosisb ≤25</td>
<td>48 (17.6)</td>
<td>9 (6.4)</td>
<td>9 (17.0)</td>
<td>28 (41.2)</td>
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<tr>
<td>26–30</td>
<td>52 (19.1)</td>
<td>18 (12.9)</td>
<td>14 (26.4)</td>
<td>16 (23.5)</td>
</tr>
<tr>
<td>31–35</td>
<td>67 (24.5)</td>
<td>39 (27.9)</td>
<td>13 (24.5)</td>
<td>13 (19.1)</td>
</tr>
<tr>
<td>36–40</td>
<td>57 (20.9)</td>
<td>35 (25.0)</td>
<td>13 (24.5)</td>
<td>7 (10.3)</td>
</tr>
<tr>
<td>&gt;40</td>
<td>48 (17.6)</td>
<td>39 (27.5)</td>
<td>4 (7.6)</td>
<td>4 (5.9)</td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (0.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diagnosis date</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1974–1984</td>
<td>24 (8.8)</td>
<td></td>
<td></td>
<td>8 (11.8)</td>
</tr>
<tr>
<td>1985–1989</td>
<td>35 (12.3)</td>
<td>17 (12.1)</td>
<td>3 (5.6)</td>
<td>14 (20.6)</td>
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<td>1990–1994</td>
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<td>27 (19.3)</td>
<td>14 (26.4)</td>
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<td>1995–2000</td>
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<td>51 (36.4)</td>
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<td>2000–2006</td>
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<td>34 (24.3)</td>
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<td>1 (0.4)</td>
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<tr>
<td>Race/ethnicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic white</td>
<td>244 (89.1)</td>
<td>125 (89.3)</td>
<td>48 (90.6)</td>
<td>62 (88.2)</td>
</tr>
<tr>
<td>Hispanic white</td>
<td>16 (6.2)</td>
<td>8 (5.7)</td>
<td>3 (5.7)</td>
<td>5 (7.4)</td>
</tr>
<tr>
<td>African-American</td>
<td>4 (1.5)</td>
<td>1 (0.7)</td>
<td>1 (1.9)</td>
<td>2 (2.9)</td>
</tr>
<tr>
<td>Asian</td>
<td>5 (1.8)</td>
<td>3 (2.1)</td>
<td>1 (1.9)</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>Unknown</td>
<td>4 (1.5)</td>
<td>3 (2.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGCT laterality</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unilateral</td>
<td>244 (89.4)</td>
<td>126 (90.0)</td>
<td>41 (77.4)</td>
<td>65 (95.6)</td>
</tr>
<tr>
<td>Bilateral</td>
<td>29 (10.6)</td>
<td>14 (10.0)</td>
<td>12 (22.6)</td>
<td>3 (4.4)</td>
</tr>
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<td>Personal history of cryptorchidm</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>219 (80.2)</td>
<td>112 (80.0)</td>
<td>44 (83.0)</td>
<td>53 (77.9)</td>
</tr>
<tr>
<td>Unilateral</td>
<td>40 (14.7)</td>
<td>19 (13.6)</td>
<td>6 (11.3)</td>
<td>13 (19.1)</td>
</tr>
<tr>
<td>Bilateral</td>
<td>6 (2.2)</td>
<td>4 (2.9)</td>
<td>2 (3.8)</td>
<td>0b</td>
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<tr>
<td>Laterality unknown</td>
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<td>5 (3.6)</td>
<td>1 (1.9)</td>
<td>2 (3.0)</td>
</tr>
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<td>Family history</td>
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</tr>
<tr>
<td>None</td>
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<td>40 (28.6)</td>
<td>26 (49.0)</td>
<td>31 (45.6)</td>
</tr>
<tr>
<td>TGCT only</td>
<td>76 (27.8)</td>
<td>45 (32.1)</td>
<td>11 (20.8)</td>
<td>12 (17.7)</td>
</tr>
<tr>
<td>CO onlyc</td>
<td>80 (29.3)</td>
<td>47 (33.6)</td>
<td>14 (26.4)</td>
<td>19 (27.9)</td>
</tr>
<tr>
<td>TGCT and COd</td>
<td>19 (7.0)</td>
<td>8 (5.7)</td>
<td>2 (3.8)</td>
<td>6 (8.8)</td>
</tr>
<tr>
<td>Histologyd</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seminoma</td>
<td>140 (51.3)</td>
<td>140 (100)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Pure seminoma</td>
<td>134 (95.7)</td>
<td>134 (95.7)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Anaplastic seminoma</td>
<td>4 (2.9)</td>
<td>4 (2.9)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Spermatocytic seminoma</td>
<td>2 (1.4)</td>
<td>2 (1.4)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Mixed germ cell tumor</td>
<td>53 (19.4)</td>
<td>—</td>
<td>53 (100)</td>
<td>—</td>
</tr>
<tr>
<td>Pure Nonseminoma</td>
<td>68 (24.9)</td>
<td>—</td>
<td>—</td>
<td>68 (100)</td>
</tr>
<tr>
<td>Embryonal carcinoma</td>
<td>36 (13.2)</td>
<td>—</td>
<td>—</td>
<td>36 (52.9)</td>
</tr>
<tr>
<td>Teratocarcinoma</td>
<td>18 (6.5)</td>
<td>—</td>
<td>—</td>
<td>18 (26.5)</td>
</tr>
<tr>
<td>Teratoma</td>
<td>6 (8.8)</td>
<td>—</td>
<td>—</td>
<td>6 (8.8)</td>
</tr>
<tr>
<td>Yolk sac</td>
<td>3 (1.1)</td>
<td>—</td>
<td>—</td>
<td>3 (4.4)</td>
</tr>
<tr>
<td>Choriocarcinoma</td>
<td>5 (1.8)</td>
<td>—</td>
<td>—</td>
<td>5 (7.4)</td>
</tr>
<tr>
<td>Unknown</td>
<td>12 (4.4)</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

aHistology is unknown for 12 TGCT cases.
bAge at first diagnosis is used for cases with two primary TGCTs (bilateral cases).
cCO, cryptorchidm.
dHistology details on 29 bilateral cases (first TGCT/second TGCT = number of cases): seminoma: seminoma/seminoma = 8, seminoma/spermatocytic seminoma = 1, seminoma/unknown = 5; Mixed germ cell tumor: mixed GCT/mixed GCT = 1, seminoma/mixed GCT = 5, seminoma/teratoma = 4, mixed GCT/embryonal = 1, mixed GCT/unknown = 1; pure nonseminoma: teratoma/teratoma = 1, teratoma/unknown = 2.
Results

Characteristics of TGCT cases in the present analysis are shown in Table I; characteristics of all eligible cases and those participating in various phases of the parent study are provided in supplementary Table 1 (available at Carcinogenesis Online), for comparison. The distribution of diagnosis dates and histiotype types is similar between cases analyzed and all other cases. There are slightly more non-Hispanic white men among cases analyzed than among all eligible cases. Among cases analyzed, 64% had a family history of TGCT and/or cryptorchidism, 17% had a history of cryptorchidism and 10% had bilateral TGCT, which represent larger proportions than among all cases because these factors were oversampled by design.

Among all TGCT cases, analyses revealed that mean CAG repeat length does not significantly differ between case and control alleles (P = 0.45) (Table II). Accordingly, logistic regression analyses (Table III) indicate no association between CAG repeat length and overall TGCT risk.

Among nonseminoma cases, mean CAG repeat length was marginally significantly longer in case versus control alleles (P = 0.060) (Table II), consistent with our initial hypothesis. However, the association between longer CAG repeat length and nonseminoma risk did not achieve statistical significance [CAG ≥ 20 versus CAG < 19, OR = 1.80 (95% CI: 0.83–3.90)] (Table III). Unexpectedly, among seminoma cases, we observed significantly shorter mean CAG repeat length in case versus control alleles (P = 0.014) (Table II). Accordingly, reduced risk of seminoma was significantly associated with longer CAG repeat length [CAG 20–21 versus CAG < 19: OR = 0.82 (95% CI: 0.43–1.58), CAG 22–23 versus CAG < 19: OR = 0.39 (95% CI: 0.19–0.83) and CAG ≥ 24 versus CAG < 19: OR = 0.42 (95% CI: 0.20–0.86)], with a highly significant trend over four ordered categories of CAG repeat length (P trend = 0.0030). Moreover, trends observed for seminoma differed significantly from those for nonseminoma (P heterogeneity = 0.012) (Table III). Among mixed GCT tumor cases, risk estimates were in between nonseminoma (shorter CAG repeat length in seminoma cases compared with those with shorter repeat length in seminoma cells compared with those with nonseminoma (P trend = 0.0096) and mixed GCT (P trend = 0.021).

Discussion

This study was undertaken based on a longstanding etiologic model (5) proposing two hormone-dependent phases of TGCT development: the first occurring early in development when inappropriate hormonal stimulation interferes with normal maturation of gonocytes leading to formation of CIS/ITGCNU, the presumed TGCT precursor (7), and the second following puberty, when endocrine mechanisms drive CIS/ITGCNU cells that were dormant throughout childhood to progress into invasive TGCT. Features of the model, together with elevated risk of TGCT reported among men with androgen insensitivity, led us to hypothesize that longer CAG repeats and thus lesser AR transactivation would be associated with TGCT risk.

Although we found no association between CAG repeat length and overall TGCT risk, a result in agreement with four previously published hospital-based case-control studies (8,19–21), longer CAG repeats were associated with risk of nonseminoma, though non-significantly. Unexpectedly, we also found risk of seminoma to be associated with shorter CAG repeats, a highly significant result further supported by a significant trend of increasing seminoma risk with shorter repeat length and significant heterogeneity between trends for seminoma and nonseminoma. Published data on AR CAG repeat length among TGCTs of specific histologic types are limited. Consistent with our results, a Swedish study found a significantly greater percentage of CAG >25 in nonseminoma cases than seminoma cases (19), although no such difference was reported in two other studies (8,21). The most recent of these (21) used different cutoffs to categorize CAG repeat length, so results could not be compared directly to those reported here.

Results for the individual histologic types may suggest biological distinctions between seminomas and nonseminomas, which differ in histologic appearance, age at presentation (supplementary Figure 1 is available at Carcinogenesis Online) and clinical prognosis. Seminomas are similar histologically to CIS/ITGCNU cells, and a postulated default pathway of testicular carcinogenesis is for seminoma to arise from CIS/ITGCNU, with activation of pluripotency additionally required for development of nonseminoma (7). Factors-driving progression of CIS/ITGCNU to invasive TGCT remains unclear, but AR expression has been demonstrated in CIS/ITGCNU cells (11). Genome-wide association studies (22–24) recently identified other loci associated with risk of both seminoma and nonseminoma, but the present study is the first to identify a genetic polymorphism for which TGCT risk associations vary significantly by histologic type. This is an important distinction because mechanisms explaining progression of CIS/ITGCNU to seminoma versus nonseminoma have not yet been identified.

The unanticipated finding of association of seminoma risk with shorter CAG repeat length, and thus presumably with greater AR transactivation, indicates that the role of the AR in testicular carcinogenesis may be more complex than simply mediating classical effects of androgens in gonocytes, CIS/ITGCNU and adult Sertoli cells. For example, differences in AR methylation status of seminomas versus nonseminomas may provide an explanation for our discordant findings between these histologic types. In vitro research has linked methylation of sites in the AR minimal promoter with loss of AR expression in hormone-independent prostate cancer cell lines (25). A separate study of TGCT tissue identified two Hha1 sites in the AR that were methylated in differentiated nonseminomas but unmethylated in all seminomas (26). If AR methylation is associated with AR silencing in TGCTs, this suggests that AR activity would be more common in seminomas. It would then be plausible that during the adult phase of TGCT development, shorter CAG repeat length, and thus greater AR transactivation, could drive progression of CIS/ITGCNU to seminoma but be irrelevant in nonseminomas with silenced AR. A second possible role for the polymorphic AR in testicular carcinogenesis may be indirect influence through steroid hormone levels. Observational studies demonstrate lower levels of serum testosterone and estradiol among adult men with shorter CAG repeat length (16). This phenomenon, postulated to reflect a feedback loop-modulating androgen action through the classical AR-mediated pathway (16), would on expectation make CAG repeat length a proxy measure for circulating levels of these hormones, which may have unrecognized effects on malignant potential of the

Table II. AR CAG repeat lengths comparing TGCT case alleles to individually matched control alleles

<table>
<thead>
<tr>
<th>Type</th>
<th>Range</th>
<th>Mean ± SD</th>
<th>Median</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All TGCT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case alleles (N = 273)</td>
<td>12–30</td>
<td>21.12 ± 3.01</td>
<td>21.0</td>
<td>0.45</td>
</tr>
<tr>
<td>Control alleles (N = 273)</td>
<td>9–33</td>
<td>21.33 ± 3.30</td>
<td>21.0</td>
<td></td>
</tr>
<tr>
<td>Semicoma only</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case alleles (N = 140)</td>
<td>12–29</td>
<td>20.71 ± 2.92</td>
<td>20.0</td>
<td>0.014</td>
</tr>
<tr>
<td>Control alleles (N = 140)</td>
<td>12–30</td>
<td>21.61 ± 3.19</td>
<td>22.0</td>
<td></td>
</tr>
<tr>
<td>Mixed germ cell tumor only</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case alleles (N = 53)</td>
<td>17–30</td>
<td>21.92 ± 3.00</td>
<td>22.0</td>
<td>0.69</td>
</tr>
<tr>
<td>Control alleles (N = 53)</td>
<td>13–33</td>
<td>21.66 ± 3.66</td>
<td>22.0</td>
<td></td>
</tr>
<tr>
<td>Nonseminoma only</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case alleles (N = 68)</td>
<td>14–29</td>
<td>21.51 ± 3.00</td>
<td>21.0</td>
<td>0.060</td>
</tr>
<tr>
<td>Control alleles (N = 68)</td>
<td>9–29</td>
<td>20.51 ± 3.15</td>
<td>20.0</td>
<td></td>
</tr>
</tbody>
</table>

*P value from paired t-test comparing mean length of case (transmitted) alleles and control (untransmitted) alleles.
male germ cell lineage. Such effects could plausibly arise, for example, if men with shorter CAG repeats and thus lower testosterone levels experienced suboptimal signaling through a nonclassical androgen pathway. In contrast to the classic genomic pathway, in which the androgen–AR complex modulates gene transcription by binding specific DNA sequence elements, more recently described nonclassical actions do not depend on AR–DNA interactions and proceed on a far more rapid time frame through additional effectors (27). Understanding of this pathway’s signaling requirements for normal male development and spermatogenesis is just beginning to emerge (28).

### Table III. ORs and 95% CIs for the association of AR CAG repeat length and risk of TGCT among all cases and subgroups defined by histology, comparing case and control alleles

<table>
<thead>
<tr>
<th></th>
<th>All TGCT case/control</th>
<th>Seminoma cases/mixed GCT cases</th>
<th>Mixed GCT cases</th>
<th>Nonseminoma cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. All races/ethnicities</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. CAG repeats</td>
<td>(N = 546)</td>
<td>(N = 280)</td>
<td>(N = 106)</td>
<td>(N = 136)</td>
</tr>
<tr>
<td>≤19</td>
<td>83/73</td>
<td>48/31</td>
<td>12/13</td>
<td>17/25</td>
</tr>
<tr>
<td>&gt;20</td>
<td>190/200</td>
<td>92/109</td>
<td>41/40</td>
<td>51/43</td>
</tr>
<tr>
<td>20–21</td>
<td>80/73</td>
<td>45/36</td>
<td>14/13</td>
<td>19/19</td>
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<tr>
<td>22–23</td>
<td>56/61</td>
<td>24/37</td>
<td>13/12</td>
<td>17/11</td>
</tr>
<tr>
<td>≥24</td>
<td>54/66</td>
<td>23/36</td>
<td>14/15</td>
<td>15/13</td>
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<tr>
<td>OR (95% CI)</td>
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<tr>
<td>≤19</td>
<td>1.0 (ref)</td>
<td>1.0 (ref)</td>
<td>1.0 (ref)</td>
<td>1.0 (ref)</td>
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<tr>
<td>&gt;20</td>
<td>0.84 (0.58–1.21)</td>
<td>0.54 (0.31–0.93)</td>
<td>1.10 (0.47–2.59)</td>
<td>1.80 (0.83–3.90)</td>
</tr>
<tr>
<td>20–21</td>
<td>0.96 (0.61–1.49)</td>
<td>0.82 (0.43–1.58)</td>
<td>1.13 (0.40–3.20)</td>
<td>1.53 (0.61–3.87)</td>
</tr>
<tr>
<td>22–23</td>
<td>0.81 (0.50–1.29)</td>
<td>0.39 (0.19–0.83)</td>
<td>1.13 (0.40–3.20)</td>
<td>2.11 (0.83–5.34)</td>
</tr>
<tr>
<td>≥24</td>
<td>0.72 (0.44–1.17)</td>
<td>0.42 (0.20–0.86)</td>
<td>1.01 (0.35–2.94)</td>
<td>1.79 (0.66–4.85)</td>
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<td>0.0030</td>
<td>0.98</td>
<td>0.017</td>
</tr>
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<td>P heterogeneity</td>
<td>0.17</td>
<td>0.012</td>
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</tr>
</tbody>
</table>

| Non-Hispanic whites | | | | |
| No. CAG repeats | (N = 488) | (N = 250) | (N = 96) | (N = 120) |
| ≤19 | 75/63 | 44/24 | 11/13 | 14/22 |
| >20 | 169/181 | 81/101 | 37/35 | 46/38 |
| 20–21 | 73/66 | 40/33 | 14/11 | 17/18 |
| 22–23 | 49/54 | 22/33 | 11/11 | 15/9 |
| ≥24 | 47/61 | 19/35 | 12/13 | 14/11 |
| OR (95% CI) | | | | |
| ≤19 | 1.0 (ref) | 1.0 (ref) | 1.0 (ref) | 1.0 (ref) |
| >20 | 0.79 (0.53–1.17) | 0.43 (0.23–0.79) | 1.22 (0.51–2.95) | 1.89 (0.84–4.24) |
| 20–21 | 0.92 (0.57–1.49) | 0.65 (0.32–1.34) | 1.47 (0.49–4.44) | 1.49 (0.56–3.95) |
| 22–23 | 0.77 (0.47–1.26) | 0.36 (0.17–0.78) | 1.10 (0.38–3.26) | 2.30 (0.85–6.22) |
| ≥24 | 0.65 (0.39–1.09) | 0.29 (0.13–0.65) | 1.11 (0.37–3.55) | 1.97 (0.70–5.53) |
| P_trend | 0.10 | <0.001 | 0.98 | 0.12 |
| P heterogeneity | 0.055 | 0.0040 |

A| Histologic subgroups include all TGCT cases (unilateral and bilateral) without cryptorchidism.  
B| Logistic regression [comparing case (transmitted) allele to control (untransmitted) allele].  
C| P value from heterogeneity test comparing the trend in seminoma cases with the trend in mixed germ cell tumor or nonseminoma cases.

### Table IV. Case–case analyses displaying ORs and 95% CIs for the association of AR CAG repeat length with TGCT histology comparing seminoma cases with mixed germ cell tumor and nonseminoma cases

<table>
<thead>
<tr>
<th></th>
<th>Seminoma cases/mixed GCT cases</th>
<th>OR (95% CI)</th>
<th>Seminoma cases/ nonseminoma cases</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. All races/ethnicities</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. CAG repeats</td>
<td>(N = 188)</td>
<td>(N = 203)</td>
<td>(N = 183)</td>
<td>(N = 183)</td>
</tr>
<tr>
<td>≤19</td>
<td>46/12</td>
<td>1.0 (ref)</td>
<td>46/17</td>
<td>1.0 (ref)</td>
</tr>
<tr>
<td>&gt;20</td>
<td>90/40</td>
<td>0.55 (0.26–1.17)</td>
<td>90/50</td>
<td>0.56 (0.27–1.17)</td>
</tr>
<tr>
<td>20–21</td>
<td>45/14</td>
<td>0.81 (0.33–1.98)</td>
<td>45/18</td>
<td>1.00 (0.41–2.45)</td>
</tr>
<tr>
<td>22–23</td>
<td>24/13</td>
<td>0.47 (0.18–1.24)</td>
<td>24/16</td>
<td>0.36 (0.14–0.96)</td>
</tr>
<tr>
<td>≥24</td>
<td>21/13</td>
<td>0.36 (0.13–0.95)</td>
<td>21/16</td>
<td>0.34 (0.13–0.92)</td>
</tr>
<tr>
<td>P_trend</td>
<td>0.021</td>
<td>0.12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| B. Non-Hispanic whites | | | | |
| No. CAG repeats | (N = 171) | (N = 183) | (N = 183) | (N = 183) |
| ≤19 | 43/11 | 1.0 (ref) | 43/14 | 1.0 (ref) |
| >20 | 81/36 | 0.52 (0.24–1.16) | 81/45 | 0.47 (0.22–1.02) |
| 20–21 | 40/14 | 0.69 (0.28–1.77) | 40/16 | 0.87 (0.34–2.22) |
| 22–23 | 22/11 | 0.46 (0.16–1.28) | 22/14 | 0.28 (0.10–0.79) |
| ≥24 | 19/11 | 0.36 (0.13–1.03) | 19/15 | 0.27 (0.10–0.78) |
| P_trend | 0.036 | 0.0039 |

A| Includes unilateral and bilateral TGCT cases with histology, race, ethnicity and age at diagnosis available.  
The observed association between shorter CAG repeat length and seminoma risk also suggests that environmental agents, such as exogenous androgens or androgen agonists (6), may act jointly with polymorphic AR variants to influence malignant potential of germ cells.

Chance seems an unlikely explanation for our findings for several reasons. Firstly, we observed both a monotonic trend of increasing seminoma risk over decreasing CAG repeat length and statistically significant heterogeneity between trends for risk of seminoma and nonseminoma. Secondly, the association between repeat length and seminoma risk persisted in all sensitivity analyses (excluding additional pairs from the same family as well as pairs in which cases had anaplastic or spermatoctytic seminoma, personal history of cryptorchidism and/or family history of TGCT or cryptorchidism; supplementary Results, available at Carcinogenesis Online). Finally, mothers are an ideal reference group for testing association between a male-limited disease such as TGCT and an X-chromosome variant (17). However, the possibility remains that other X-chromosome variants in linkage disequilibrium with CAG repeat genotypes could influence TGCT risk to some extent.

Strengths of our analysis include that cases analyzed were similar to cases from the base population on features that we did not oversample, and among features upon which we oversampled by design (personal history of cryptorchidism and family history of TGCT or cryptorchidism; supplementary Results, available at Carcinogenesis Online), sensitivity analyses revealed consistency of results. Moreover, comparing cases to mothers allowed us to estimate genotype–disease associations in a manner free of confounding by population structure, often a concern in studying conditions, like TGCT, that vary greatly by race/ethnicity. One consideration regarding the case–parent design is that parental genotypes associated with disease risk may interfere with reproductive ability. However, there is no evidence that CAG repeat length influences female fertility, thus mothers’ untransmitted alleles seem an appropriate control in this investigation. A limitation is that we enrolled only surviving TGCT cases, which may have excluded cases with most severe disease. However, survival rates are exceedingly high, so this concern seems minor.

In summary, we found shorter AR CAG repeat length is associated with risk of seminoma, suggesting the AR may be involved in progression from CIS/ITGCNU to seminoma, or that the activity of androgens, possibly through non-genomic mechanisms, may influence testicular carcinoma in utero. Mechanistic insights may be provided by investigating both AR expression and AR methylation in TGCT tissue of distinct histologic types and the role of AR transactivation and varying testosterone levels on the development and malignant potential of gonocytes. Further association studies are warranted to confirm these findings, including associations of shorter AR CAG repeats with seminoma risk, longer AR CAG repeats with nonseminoma risk and an apparently null association with mixed GCT risk. Such confirmation would further implicate molecules of androgen action and response in TGCT etiology, establishing two new TGCT research priorities: epidemiologic investigation of potential joint effects of functional AR variants and exposure to hormonally active compounds of both endogenous and exogenous origin, and exploration of cellular mechanisms underlying AR variant–TGCT associations.

Supplementary material

Supplementary Materials and Methods, Results, Supplementary Table 1 and Figure 1 can be found at http://carcin.oxfordjournals.org/

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Conflict of Interest Statement: None declared.

References


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