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

Carol A.Davis-Dao[1], Kimberly D.Siegmund[1], David J.Vandenberg[1], Eila C.Skinner[2], Gerhard A.Coetzee[1,2], Duncan C.Thomas[1], Malcolm C.Pike[1,3] and Victoria K.Cortessis[1,*]

[1]Department of Preventive Medicine, Keck School of Medicine, University of Southern California, 1441 Eastlake Avenue, MC-9175, Los Angeles, CA 90033, USA, [2]Department of Urology, Keck School of Medicine, University of Southern California, Los Angeles, CA 90033, USA and [3]Department of Epidemiology and Biostatistics, Memorial Sloan-Kettering Cancer Center, New York, NY 10004, USA

*To whom correspondence should be addressed. Tel: +1 323 865 0544; Fax: +1 323 865 0134;
Email: cortessi@usc.edu

Increasing rates of testicular germ cells tumors (TGCTs) overtime suggest that environmental factors 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 CAG_n 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 nonseminomatous histology, a clinically and biologically important distinction.

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 factors acting early in life predispose to tumor development. A two-phase model has been postulated to explain TGCT etiology (5), hypothesizing that *in utero* estrogen exposure interferes with normal development of fetal germ cells (i.e. gonocytes), leading to formation of the presumed TGCT precursor, namely carcinoma *in situ*/intratubular germ cell neoplasia unclassified (CIS/ITGCNU) (6). Risk of adult TGCT begins with puberty, in the second stage of the proposed model, when activation of the sex hormone/gonadotropin axis is postulated to lead CIS/ITGCNU cells that were dormant during childhood to progress to TGCT (7).

Abbreviations: AR, androgen receptor; CIS/ITGCNU, carcinoma in situ/intratubular germ cell neoplasia unclassified; TGCT, testicular germ cell tumor.

Consistent with this model, a role for hormones has been suggested in TGCT etiology through an estrogen/androgen imbalance caused by *in utero* exposure to endogenous or exogenous hormones and/or genetic susceptibility (8). Exposure to compounds with estrogenic or antiandrogenic properties has also been proposed as an explanation for rising TGCT incidence rates (1).

The classical genomic actions of androgens are mediated by the androgen receptor (AR). Expression of the AR has been demonstrated in human and rodent gonocytes (9,10), and gonocyte proliferation is reportedly enhanced among testicular feminized mice, suggesting that AR deficiency leads to increased gonocyte numbers during the fetal period (10). CIS/ITGCNU cells reportedly express the AR (11), as well, and may thereby respond directly to androgens following puberty. As proposed for normal adult male germ cells, which do not express the AR (12), CIS/ITGCNU cells may also experience paracrine effects of androgens mediated through adult Sertoli cells, which express the AR and create the immediate microenvironment for regulating adult germ cell functions. Thus, it seems plausible that AR action could influence malignant potential of testicular germ cells in both the fetal and postpubertal periods.

Functional variants in the *AR* gene are implicated based on greatly increased TGCT risk among men with *AR* mutations, which disrupt AR function sufficiently to cause androgen insensitivity and high levels of circulating testosterone and estrogen (13). We and others suspected that *AR* variants with more subtle functional consequences may also influence TGCT risk since most TGCT cases have no clinical signs of androgen insensitivity.

One of the most extensively characterized functional polymorphisms in the human AR, a poly-glutamine (poly-Q) tract located in the transactivation domain, is encoded by a polymorphic CAG repeat. Poly-Q length is inversely correlated with AR transactivation *in vitro* (14,15), such that lesser transactivation is predicted for men with longer repeat lengths. Males have one copy of the *AR*, located at Xq11–12, and observational studies consistently report higher levels of serum testosterone and estradiol among men with longer repeat lengths (16). Therefore, men with longer CAG repeat lengths may experience milder forms of two features of androgen insensitivity: lower AR transactivation and higher circulating levels of androgens. We therefore postulated that longer CAG repeat length may be associated with increased TGCT risk.

To investigate this hypothesis, we analyzed data from mother–son pairs participating in a population-based family study of TGCT, comparing each TGCT case's *AR* CAG repeat length with that of his mother's second allele at this locus, which was not transmitted to her affected son. Employing a transmission/disequilibrium test for an X-linked marker in males, thus precluding bias from cryptic population structure (17), we estimated associations between CAG repeat length and TGCT risk, overall and within subgroups defined by tumor histology.

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 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 for 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 genotype 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[a]: all cases, seminoma cases, mixed germ cell tumor cases and nonseminoma cases

Characteristic	All cases[a] (*N* = 273), *N* (%)	Seminoma (*N* = 140), *N* (%)	Mixed germ cell tumors (*N* = 53), *N* (%)	Pure nonseminoma (*N* = 68), *N* (%)
Age at diagnosis[b]				
≤25	48 (17.6)	9 (6.4)	9 (17.0)	28 (41.2)
26–30	52 (19.1)	18 (12.9)	14 (26.4)	16 (23.5)
31–35	67 (24.5)	39 (27.9)	13 (24.5)	13 (19.1)
36–40	57 (20.9)	35 (25.0)	13 (24.5)	7 (10.3)
>40	48 (17.6)	39 (27.5)	4 (7.6)	4 (5.9)
Unknown	1 (0.4)			
Diagnosis date				
1974–1984	24 (8.8)	11 (7.9)	—	8 (11.8)
1985–1989	35 (12.8)	17 (12.1)	3 (5.6)	14 (20.6)
1990–1994	60 (22.0)	27 (19.3)	14 (26.4)	19 (27.9)
1995–2000	85 (31.1)	51 (36.4)	18 (34.0)	12 (17.6)
2000–2006	68 (24.9)	34 (24.3)	18 (34.0)	15 (22.1)
Unknown	1 (0.4)			
Race/ethnicity				
Non-Hispanic white	244 (89.1)	125 (89.3)	48 (90.6)	62 (88.2)
Hispanic white	16 (6.2)	8 (5.7)	3 (5.7)	5 (7.4)
African-American	4 (1.5)	1 (0.7)	1 (1.9)	2 (2.9)
Asian	5 (1.8)	3 (2.1)	1 (1.9)	1 (1.5)
Unknown	4 (1.5)	3 (2.1)		
TGCT laterality				
Unilateral	244 (89.4)	126 (90.0)	41 (77.4)	65 (95.6)
Bilateral	29 (10.6)	14 (10.0)	12 (22.6)	3 (4.4)
Personal history of cryptorchidism				
None	219 (80.2)	112 (80.0)	44 (83.0)	53 (77.9)
Unilateral	40 (14.7)	19 (13.6)	6 (11.3)	13 (19.1)
Bilateral	6 (2.2)	4 (2.9)	2 (3.8)	0[b]
Laterality unknown	8 (2.9)	5 (3.6)	1 (1.9)	2 (3.0)
Family history				
None	98 (35.9)	40 (28.6)	26 (49.0)	31 (45.6)
TGCT only	76 (27.8)	45 (32.1)	11 (20.8)	12 (17.7)
CO only[c]	80 (29.3)	47 (33.6)	14 (26.4)	19 (27.9)
TGCT and CO[c]	19 (7.0)	8 (5.7)	2 (3.8)	6 (8.8)
Histology[d]				
Seminoma	140 (51.3)	140 (100)	—	—
Pure seminoma	134 (95.7)	134 (95.7)	—	—
Anaplastic seminoma	4 (2.9)	4 (2.9)	—	—
Spermatocytic seminoma	2 (1.4)	2 (1.4)	—	—
Mixed germ cell tumor	53 (19.4)	—	53 (100)	—
Pure Nonseminoma	68 (24.9)	—	—	68 (100)
Embryonal carcinoma	36 (13.2)	—	—	36 (52.9)
Teratocarcinoma	18 (26.5)	—	—	18 (26.5)
Teratoma	6 (8.8)	—	—	6 (8.8)
Yolk sac	3 (1.1)	—	—	3 (4.4)
Choriocarcinoma	5 (1.8)	—	—	5 (7.4)
Unknown	12 (4.4)			

[a]Histology is unknown for 12 TGCT cases.
[b]Age at first diagnosis is used for cases with two primary TGCTs (bilateral cases).
[c]CO, cryptorchidism.
[d]Histology 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.

Analyses were conducted among all races/ethnicities, with and without adjustment for race/ethnicity, and among non-Hispanic whites only. Reported *P* values are two sided.

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 histologic 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 $\geq$ 20 versus CAG $\leq$ 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 $\leq$ 19: OR = 0.82 (95% CI: 0.43–1.58), CAG 22–23 versus CAG $\leq$ 19: OR = 0.39 (95% CI: 0.19–0.83) and CAG $\geq$ 24 versus CAG $\leq$ 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 those for seminoma and nonseminoma cases [CAG $\geq$ 20 versus CAG $\leq$ 19, OR = 1.10 (95% CI: 0.47–2.59)], possibly indicating a mixture of effects. Case–case analyses (Table IV) revealed monotonic patterns of shorter CAG repeat length in seminoma cases 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 cutpoints 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-moderating 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

	Range	Mean ± SD	Median	*P* value[a]
All TGCT				
Case alleles ($N = 273$)	12–30	21.12 ± 3.01	21.0	0.45
Control alleles ($N = 273$)	9–33	21.33 ± 3.30	21.0	
Seminoma only				
Case alleles ($N = 140$)	12–29	20.71 ± 2.92	20.0	0.014
Control alleles ($N = 140$)	12–30	21.61 ± 3.19	22.0	
Mixed germ cell tumor only				
Case alleles ($N = 53$)	17–30	21.92 ± 3.00	22.0	0.69
Control alleles ($N = 53$)	13–33	21.66 ± 3.66	22.0	
Nonseminoma only				
Case alleles ($N = 68$)	14–29	21.51 ± 3.00	21.0	0.060
Control alleles ($N = 68$)	9–29	20.51 ± 3.15	20.0	

[a]*P* value from paired *t*-test comparing mean length of case (transmitted) alleles and control (untransmitted) alleles.

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

	All TGCT case/control	Seminoma[a] case/control	Mixed GCT[a] case/control	Nonseminoma[a] case/control
A. All races/ethnicities				
No. CAG repeats	(N = 546)	(N = 280)	(N = 106)	(N = 136)
≤19	83/73	48/31	12/13	17/25
≥20	190/200	92/109	41/40	51/43
20–21	80/73	45/36	14/13	19/19
22–23	56/61	24/37	13/12	17/11
≥24	54/66	23/36	14/15	15/13
OR (95% CI)[b]				
≤19	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
≥20	0.84 (0.58–1.21)	0.54 (0.31–0.93)	1.10 (0.47–2.59)	1.80 (0.83–3.90)
20–21	0.96 (0.61–1.49)	0.82 (0.43–1.58)	1.13 (0.40–3.20)	1.53 (0.61–3.87)
22–23	0.81 (0.50–1.29)	0.39 (0.19–0.83)	1.13 (0.40–3.20)	2.11 (0.83–5.34)
≥24	0.72 (0.44–1.17)	0.42 (0.20–0.86)	1.01 (0.35–2.94)	1.79 (0.66–4.85)
	P_{trend} = 0.13	P_{trend} = 0.0030	P_{trend} = 0.98 $P^{c}_{heterogeneity}$ = 0.17	P_{trend} = 0.17 $P^{c}_{heterogeneity}$ = 0.012
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)[b]				
≤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 (0.37–3.35)	1.97 (0.70–5.53)
	P_{trend} = 0.10	P_{trend} < 0.001	P_{trend} = 0.98 $P^{c}_{heterogeneity}$ = 0.055	P_{trend} = 0.12 $P^{c}_{heterogeneity}$ = 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

	Seminoma cases/mixed GCT cases[a]	OR (95% CI)[b]	Seminoma cases/ nonseminoma cases[a]	OR (95% CI)[b]
A. All races/ethnicities				
No. CAG repeats	(N = 188)		(N = 203)	
≤19	46/12	1.0 (ref)	46/17	1.0 (ref)
≥20	90/40	0.55 (0.26–1.17)	90/50	0.56 (0.27–1.17)
20–21	45/14	0.81 (0.33–1.98)	45/18	1.00 (0.41–2.45)
22–23	24/13	0.47 (0.18–1.24)	24/16	0.36 (0.14–0.96)
≥24	21/13	0.36 (0.13–0.95)	21/16	0.34 (0.13–0.92)
		P_{trend} = 0.021		P_{trend} = 0.0096
B. Non-Hispanic whites				
No. CAG repeats	(N = 171)		(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		P_{trend} = 0.0039

[a]Includes unilateral and bilateral TGCT cases with histology, race, ethnicity and age at diagnosis available.
[b]Adjusted for age at diagnosis (≤25, 26–30, 31–35, 36–40, >40).

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 though 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 though additional effectors (27). Understanding of this pathway's signaling requirements for normal male development and spermatogenesis is just beginning to emerge (28).

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 spermatocytic 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 carcinogenesis *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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