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Polymorphic variation in the androgen receptor gene: Association with risk of testicular germ cell cancer and metastatic disease

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ABSTRACT

Increasing incidence of testicular germ cell cancer (TGCC) is most probably related to environment and lifestyle. However, an underlying genetic predisposition may play a role and since sex steroids are assumed to be important for the rise and progression of TGCC, a study of androgen receptor (AR) gene polymorphisms in relation to the risk, histological type and progression of TGCC was undertaken.

In 367 TGCC cases and 214 controls, AR CAG and GGN repeat lengths were determined and 11 haplotype-tagging single nucleotide polymorphisms (SNPs) were genotyped. By binary logistic regression, odds ratios (ORs) and 95% confidence intervals (95% CI) were calculated for the risk of TGCC, non-seminoma versus seminoma and metastatic versus localised (stage I) disease.

For the non-coding SNP, *rs12014709*, the minor genotype (G) was found in 10% of the cases and in 5.1% of the controls, conferring an OR of 2.07 (95% CI: 1.03–4.15) for having TGCC. Furthermore, short GGN (<23) was associated with an increased risk of metastatic disease (OR: 2.15; 95% CI: 1.04–4.45).

The AR polymorphisms found by us might be involved in gene–environment interaction by increasing the susceptibility to the effect of endocrine disruptors. From a biological point of view, our findings strengthen the hypothesis of the importance of androgen action in the aetiology and pathogenesis of testicular malignancy. Future studies should focus on the impact of sex hormones on foetal germ cell development and the interaction between environmental factors and androgen receptor variants in relation to the risk of testicular malignancy.

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1. Introduction

The current view regarding the aetiology of testicular germ cell cancer (TGCC), which predominantly affects males in the age group 15–44, includes a transformation of primordial germ cells/gonocytes to the so-called carcinoma *in situ* (CIS) during early embryonic development.[1] The next step in the pathogenesis of TGCC is the progression of CIS cells to seminoma (S) or non-seminoma (NS). Since the timing of increase in the age-related risk of TGCC is parallel with the pubertal rise in the levels of sex hormones and gonadotropins, endocrine mechanisms are believed to be involved in the regulation of the progression from CIS to tumour stage.[2]

The epidemiological trends of TGCC are quite remarkable with a 3–4-fold increase in the incidence during the past 30–40 years among Caucasians.[3] The cause of this increase is unknown, but *in utero* exposure to environmental pollutants with endocrine disrupting properties has been suggested as a contributing factor.[4] The pathways concerning endocrine disruption in developing males were first focused on oestrogen receptor-mediated events and evidence for this hypothesis was demonstrated by the fact that sons of mothers treated in early pregnancy with the potent oestrogenic compound diethylstilbestrol (DES) seemed to have an increased risk of TGCC.[5] Furthermore, higher levels of persistent organohalogen pollutants in serum of mothers of TGCC patients as compared to those of controls were also reported.[6]

Other plausible mechanisms include suppression of testosterone production by the foetal testis and inhibition of androgen receptor (AR) expression or action. Subjects with AR gene mutations leading to androgen insensitivity are at significantly higher risk of developing TGCC,[7] this risk being most pronounced in those subjects having mutations leading to partial but not complete insensitivity to the male sex hormones.[8] Furthermore, since the AR was found to be expressed in CIS cells,[9] looking for genetic variants modifying the function of the AR is an obvious strategy when searching for candidate genes involved in the aetiology and pathogenesis of TGCC.

The AR has two polymorphic regions: a glutamine encoding (CAG)n CAA stretch and a glycine-encoding repeat consisting of (GGT)3 GGG $(GGT)_2$ GGC_n, generally designated the CAG and GGN repeats, respectively. In previous studies, based on relatively small patient groups, it has been suggested that CAG > 25 confers a higher risk of non-seminoma and metastasising cancer,[10] and that the combination CAG20/GGN23 would be more common in TGCCs than in controls.[11] However, in another study no association between CAG number and TGCC risk, histology or tumour stage was found.[12] Single nucleotide polymorphisms (SNP) in the AR gene have also recently been investigated,[13] but none of the SNP investigated was associated with an increased risk of TGCC. However, only three variants were tested, implying that the whole gene was not covered.

Since previous studies on the association between AR variants and the risk of TGCC were based on either CAG/GGN number or SNPs in relatively small patient groups, we have in the current study analysed both types of genetic polymorphisms in the AR in relation to the risk of TGCC, histological tumour type and the presence or absence of metastases. In order to increase the study population, this work was carried out as a joint Danish–Swedish study comprising nearly 400 cases.

2. Patients and methods

2.1. Swedish TGCC patients

All TGCC patients under the age of 50 referred to the Department of Oncology, Lund University Hospital, since March 1996 and to the Department of Oncology, Radiumhemmet and Södersjukhuset, Karolinska University Hospital, Stockholm since November 1998, were asked to participate in a study focusing on their reproductive function. Until October 2006, in total 460 patients were eligible for the study. Seventy-five declined to participate and 45 were excluded due to linguistic difficulties, bilateral testicular cancer, physical handicap or moving to another region. Seven patients were excluded due to compromising mental conditions, 10 were excluded due to contralateral testicular cancer diagnosed after inclusion and 3 died of progressive disease before blood samples were obtained. Among the 320 participants in the study on reproductive function, no DNA was available for 39, leaving 281 patients. Additionally three patients were excluded due to extragonadal germ cell cancer, leaving the total at 278 (Table 1).

The TGCC patients were divided according to the tumour histology into S and NS groups. Clinical staging was performed according to the Royal Marsden staging system, but only categories like local disease (stage I) or disseminated disease were used in the present study.

All men participated after giving written informed consent according to protocols approved by the ethical review boards at Lund University and the Karolinska Institute, respectively.

2.2. Danish TGCC patients

Samples of DNA from patients with TGCC were collected in the period 1999–2008 at the Copenhagen University Hospital (Rigshospitalet), either at the Department of Oncology (during the treatment) or at the Department of Growth and Reproduction (on the occasion of semen banking prior to surgery or fertility assessment after treatment).

From approximately 300 DNA samples collected in total, 100 were randomly selected for this study; the criterion for selection being sufficient amount and technical quality of the sample and an *a priori* determined CAG and GGN length. Subsequently, 11 patients were excluded because of wrong diagnosis ($n = 6$), purely extragonadal tumour ($n = 4$) and in one case because the genetic SNP analysis failed. Among the remaining 89 patients, 6 presented with CIS only (not in the analysis S versus NS) and in 5 patients no information on stage was available.

The patients had previously given informed consent to participate in genetic analysis of the Y chromosome (Regional Medical Research Ethics Committee in Copenhagen & Frederiksberg, nr KF 01-019/99) and subsequently, the use of these samples was permitted for studies of other genetic polymorphisms (KF 01-0832 and KF-11-013/04).

Table 1 – Background characteristics of cases.

	Age	Total	Histology		Stage			
	Mean (S.D.)		Seminoma	Non-seminoma	I	II	III	IV
TGCC-Sweden	39 (7.3)	278	127	151	200	54	5	19
TGCC-Denmark	42 (7.9)	89[a]	46	37	66	16	2	0

[a] Six Danish patients with pre-invasive CIS only (no tumour) are included in the TGCC risk part of the study; they are also counted as stage I. Information on stage lacking for 5 Danish patients.

2.3. Controls

In 2000–2001, a study of reproductive function among Swedish military conscripts aged 18–20 years was undertaken.[14] This group can be considered as a representative for the adolescent general population of men.[14,15] As a part of the investigation scrotal palpation and ultrasound were performed in order to exclude testicular tumour or microcalcification, which is indicative of an increased risk of CIS. Among the 305 men without any abnormal signs, the 214 having a Swedish mother were selected as controls for the present study.

2.4. Genotyping

Genomic DNA was prepared from peripheral leucocytes using QIAamp DNA Maxi Kit (Qiagen, Germany). The DNA concentrations were determined using Pico Green and all the samples were normalised to the same DNA concentration. The genotypes were determined using the Sequenom MassARRAY MALDI-TOF system which analyses allele-specific primer extension products using mass-spectrometry. Assay design was made using the MassARRAY Assay Design ver. 2.0 software (Sequenom Inc., USA).

The following SNPs, with minor allele frequency >0.05 or if identified as haplotype-tagging SNPs, were selected by use of dbSNP (www.ncbi.nlm.nih.gov/SNP): *rs962458*, *rs2070757*, *rs6152*, *rs2207040*, *rs2223823*, *rs1204039*, *rs1204038*, *rs7061037*, *rs2361634*, *rs12014709* and *rs5031002*. Primers were obtained from Metabion GmbH, Germany, and all reactions were run under the same conditions except for the primer annealing temperature of the primary PCR. PCR reactions were performed in a total volume of 6 μl containing 2.5 ng of template DNA, 1.25× Taq PCR buffer (HotStar), 0.15 U of Taq polymerase (HotStar), 3.5 mM $MgCl_2$, 0.5 mM dNTPs and 100 nM of each primer. Amplifications were performed using GeneAmp 9700 machines with dual-384 heads as follows: 95 °C for 15 min, 45 cycles at 95 °C for 20 s, 56 °C, 60 °C or 64 °C for 30 s, 72 °C for 60 s and finally 72 °C for 3 min. Dephosphorylation of unincorporated dNTPs was achieved using shrimp alkaline phosphatase. Concentrations of individual hME primer pairs were adjusted to even out peak heights in the mass spectrum. The extension reactions were then made by mixing the adjusted MassEXTEND primer mix (containing approximately 1 μM of each primer) with hME EXTEND mix containing buffer and 50 μM of each d/ddNTP mix and 1.25 U of Thermo Sequenase. PCR amplification of hME reactions was performed as follows: 94 °C for 2 min and 99 cycles at 94 °C for 5 s, 52 °C for 5 s and 72 °C for 5 s. The samples were then manually desalted by using 6 mg of Clean Resin and a dimple plate and subsequently transferred to a 384-well SpectroCHIP using a nanodispenser. A more extensive description can be found at: http://www.sequenom.com/applications/high_performance_genotyping.php.

The CAG and GGN repeats were amplified by PCR and subsequently analysed externally on a Beckman Coulter CEQ 2000XL sequencer (Beckman Coulter, Bromma, Sweden).

2.5. Statistical analysis

The raw data were parsed using a Perl script in a Linux environment. Polymorphisms *rs2070757* and *rs2223823* were not analysed as they were found to be invariant across the dataset. Using SPSS (SPSS for Windows, Rel. 11.0.1. 2001. Chicago, SPSS Inc.) binary logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (95% CI).

The CAG repeat lengths were categorised in: CAG < 21; CAG = 21; CAG = 22; CAG = 23–25; CAG > 25.This categorisation was selected in order to have categories of approximately equal size. The GGN lengths were trichotomised: <23, =23 and >23.

The objective was primarily to define the OR for each SNP and repeat length categories as predictors of TGCC versus non-TGCC (controls). Following this, we analysed the OR for S versus NS among the TGCC patients and for metastatic disease (stages II–IV) versus testis localised (stage I). In case a statistically significant association between a genotype and risk of metastasis was found, histological type was included in the analysis as a covariate, in order to investigate whether this association was independent of the tumour type. For the same reason, association between AR polymorphisms and metastatic disease was tested by splitting the dataset by histology.

Calculations were made both in the combined Swedish and Danish material and separately for the two countries to test the robustness of the trends. For a single SNP, the most common genotype was always used as reference, and the reference repeat length categories were set to CAG < 21 and GGN23. Short CAG was used as reference, to detect any relative differences between short, medium and long CAG lengths, since recent *in vitro* work showed that both short and long repeats are associated with a lower activity as compared to repeat length close to the mean.[16]

Association between CAG/GGN length in relation to the presence or absence of metastasis was tested by simultaneously using both histology and repeat length as covariates.

Since the SNPs are X-linked, Hardy–Weinberg equilibrium has not been evaluated. However, to assess whether the SNPs were in linkage disequilibrium, the absolute difference between the observed relative haplotype frequencies and the

expected relative haplotype frequencies (*D*-values) was calculated.

3. Results

The rare genotype G of SNP *rs12014709*, found in 10% of TGCC patients and in 5.1% of controls (Table 2) was associated with a 2.07 (95% CI: 1.03–4.15) OR for having TGCC. The same trend was found in both populations, however, not reaching the level of statistical significance among the Danish subjects. None of the other SNPs or CAG/GGN repeat lengths were associated with significantly increased or reduced risk of TGCC (Table 3).

GGN < 23 was associated with an increased risk of NS in the combined dataset, with an OR of 1.88 (95% CI 0.905–3.91). The trend was also seen in the country-specific datasets, but neither reached the level of statistical significance. None of the other genetic variations seemed to be linked to the histology of the tumour (Table 4).

Similarly, GGN < 23 was associated with increased risk of metastatic disease (Table 5) in the combined material with an OR of 2.15 (95% CI 1.04–4.45). This trend was replicated in the country-specific data sets, but only being statistically significant for the Swedish TGCC patients where the OR was 2.38 (95% CI 1.06–5.32). After including histology as covariate, the association between GGN length and metastasis risk was changed (OR: 1.91; 95% CI: 0.907–4.01). None of the SNP was associated with the risk of metastases, when S and NS were analysed separately.

The *D*-values (and haplotype counts) indicated that *rs12014709* and *rs962458* are in mutually exclusive coinheritance with four other SNPs (*rs6152*, *rs1204039*, *rs1204038* and *rs7061037*). This implies that *rs12014709* is commonly coinherited with four other SNPs.

4. Discussion

In this study, we found that the G variant of the tag SNP *rs12014709* in the AR gene was significantly more common in cases with TGCC (10%) as compared to controls (5.1%). The doubled risk for having TGCC is of same magnitude as found in other association studies focusing on genetic factors linked to the risk of testicular malignancy, for example, the SNPs reported in the recent whole genome scans.[17,18] Since this genetic variant was absent in most TGCC patients, the clinical relevance of this finding is limited.

Our findings could however be of importance from a biological point of view, since they support the hypothesis of a link between AR function and the aetiology of TGCC.

In a previous study on the association between SNP in the AR and hormone metabolising genes in relation to the risk of TGCC, three SNPs in the AR were investigated, but no association was observed.[13] The three polymorphisms selected covered approximately 75% of the common genetic variation of AR, but did not include *rs12014709*, which was found as a principal risk SNP in the current study. Two other previously analysed SNPs were also included in the current study (*rs6152* and *rs1204038*) and as previously reported, these were not associated with the risk of TGCC or disease progression. In our cohort, these two SNPs were coinherited with the *rs12014709*, and were also associated with slightly increased

Table 2 – Single nucleotide polymorphism minor allele frequencies and CAG/GGN repeat length category distribution in the controls and testicular cancer patients, stratified according to country of origin, histology, and presence or absence of metastases.

	Controls	Sweden	Denmark	Seminoma	Non-seminoma	Localised	Metastatic
N[a]	214	278	89	173	188	266	96
	%						
rs962458	9.3	9.7	7.9	11	7.4	11	5.2
rs6152	14	17	13	17	15	19	14
rs2207040	3.7	4.0	3.4	4.0	3.7	3.4	5.2
rs1204039	14	18	18	18	18	21	15
rs1204038	14	19	18	20	18	21	14
rs7061037	15	19	13	18	16	19	15
rs2361634	7.9	6.1	9.0	7.5	6.4	7.2	6.3
rs12014709	5.1	10	10	9.8	11	11	9.4
rs5031002	3.3	2.2	2.2	2.3	1.6	1.9	3.1
CAG21	17	16	25	18	18	20	14
CAG22	8.4	13	7.9	13	10	12	11
CAG23-25	27	29	26	25	31	26	34
CAG>25	14	9.0	6.7	9.2	8.0	8.8	8.3
CAG<21 (reference)	34	32	35	34	32	33	32
GGN<23	13	11	9.0	7.5	13	9.0	17
GGN>23	34	33	35	33	34	37	30
GGN23 (reference)	53	52	39	51	48	54	47

[a] Six Danish CIS patients are only included in the TGCC risk part of the study. Information on stage is lacking for 5 Danish patients. Numbers represent the allele frequencies as percentage of the total number of subjects in each group. As a result of missing observations, the numbers do not necessarily add up to *N*.

Table 3 – Odds ratios (OR) for associations between AR polymorphisms and risk of testicular germcell cancer.

Geneticvariant	OR[a] (95% confidence interval, CI) All	OR[a] (95% CI) Sweden	OR[a] (95% CI) Denmark
rs962458	0.986 (0.552–1.762)	1.04 (0.564–1.90)	0.830 (0.338–2.04)
rs6152	1.29 (0.801–2.08)	1.32 (0.804–2.18)	1.18 (0.569–2.45)
rs2207040	1.03 (0.423–2.49)	1.07 (0.422–2.70)	0.894 (0.232–3.45)
rs1204039	1.43 (0.895–2.29)	1.46 (0.890–2.39)	1.35 (0.695–2.63)
rs1204038	1.41 (0.887–2.26)	1.44 (0.884–2.35)	1.33 (0.684–2.59)
rs7061037	1.31 (0.818–2.09)	1.37 (0.839–2.22)	1.10 (0.532–2.27)
rs2361634	0.841 (0.443–1.60)	0.747 (0.372–1.50)	1.15 (0.476–2.77)
rs12014709	2.07 (1.03–4.15)	**2.06 (1.00–4.25)**	**2.08 (0.831–5.22)**
rs5031002	0.654 (0.234–1.83)	0.646 (0.214–1.95)	0.681 (0.139–3.35)
CAG21[b]	1.09 (0.661–1.79)	0.984 (0.577–1.68)	1.14 (0.703–2.71)
CAG22[b]	1.40 (0.750–2.62)	1.57 (0.823–3.01)	0.903 (0.343–2.38)
CAG23–25[b]	1.08 (0.697–1.66)	1.13 (0.714–1.79)	0.921 (0.485–1.75)
CAG > 25	0.641 (0.357–1.15)	0.697 (0.376–1.30)	0.481 (0.181–1.27)
GGN < 23[c]	0.844 (0.491–1.45)	0.828 (0.468–1.47)	0.914 (0.382–2.19)
GGN > 23[c]	1.04 (0.716–1.51)	0.963 (0.649–1.43)	1.36 (0.771–2.39)

[a] Reference genotype = the most common allele.
[b] Reference CAG < 21.
[c] Reference GGN = 23.

Table 4 – Odds ratios for associations between AR polymorphisms and risk of non-seminoma.

Genetic variant	OR[a] (95% CI) of non-seminoma All	OR[a] (95% CI) of non-seminoma Sweden	OR[a] (95% CI) of non-neminoma Denmark
rs962458	0.660 (0.320–1.36)	0.776 (0.346–1.70)	0.234 (0.0260–2.10)
rs6152	0.873 (0.495–1.54)	0.801 (0.426–1.51)	1.18 (0.323–4.32)
rs2207040	0.911 (0.313–2.65)	1.00 (0.298–3.36)	0.611 (0.0530–7.02)
rs1204039	0.932 (0.545–1.60)	0.968 (0.523–1.79)	0.800 (0.256–2.50)
rs1204038	0.890 (0.525–1.51)	0.911 (0.500–1.66)	0.796 (0.255–2.48)
rs7061037	0.908 (0.526–1.57)	0.833 (0.455–1.53)	1.22 (0.333–4.44)
rs2361634	0.844 (0.374–1.90)	0.943 (0.353–2.52)	0.745 (0.166–3.35)
rs12014709	1.10 (0.555–2.17)	0.966 (0.441–2.12)	1.69 (0.420–6.83)
rs5031002	0.689 (0.152–2.12)	0.838 (0.166–4.23)	N/A
CAG21[b]	1.03 (0.562–1.88)	0.853 (0.416–1.75)	1.80 (0.577–5.62)
CAG22[b]	0.835 (0.410–1.70)	0.771 (0.352–1.69)	0.818 (0.128–5.23)
CAG23–25[b]	1.27 (0.748–2.17)	1.19 (0.646–2.18)	1.49 (0.477–4.64)
CAG > 25[b]	0.906 (0.411–2.00)	0.754 (0.310–1.83)	1.64 (0.279–9.58)
GGN < 23[c]	1.88 (0.905–3.91)	1.77 (0.773–4.04)	2.56 (0.522–12.6)
GGN > 23[c]	1.08 (0.680–1.72)	0.986 (0.583–1.67)	1.65 (0.601–4.52)

N/A, odds ratio analysis not possible because *N* was zero in one of the cells.
[a] Reference genotype = the most common allele.
[b] Reference CAG < 21.
[c] Reference GGN = 23.

OR of TGCC, however, without reaching the level of statistical significance.

Two genome wide association studies on TGCC patients were also recently conducted.[17,18] In both studies, SNP markers within the *KITLG* gene, which encodes the ligand (also called stem cell factor) for the membrane-bound receptor tyrosine kinase KIT showed strong association with TGCC risk. KIT/KITLG signalling is involved in regulation of proliferation and migration of germ cells and steroidogenesis[19] and a heterozygous deletion of a variant of this gene conferred a 2-fold increased risk of TGCC in a mouse model.[20]

Leydig cells of mice expressing mutant KIT were unable to respond effectively to KITLG stimulation. However, mutant animals had normal serum testosterone levels. The findings suggested a model in which the mutant Leydig cells initially produced lower levels of testosterone, reducing testosterone negative feedback on the hypothalamic–pituitary axis, which lead to elevated luteinising hormone secretion and subsequent restoration of normal serum testosterone levels. This effect is mediated by the AR and in this context timing might be of importance. Once the adult hypothalamus–testis–axis regulation is established, variations in either KITLG or AR

Table 5 – Odds ratios for associations between AR polymorphisms and risk of metastasing testicular germ cell cancer.

	OR[a] (95% CI) of metastasing disease All	OR[a] (95% CI) of metastasing disease Sweden	OR[a] (95% CI) of metastasing disease Denmark
rs962458	0.463 (0.173–1.24)	0.551 (0.201–1.51)	N/A
rs6152	0.780 (0.398–1.53)	0.644 (0.302–1.37)	1.76 (0.396–7.84)
rs2207040	1.57 (0.514–4.82)	2.22 (0.658–7.51)	N/A
rs1204039	0.675 (0.354–1.29)	0.613 (0.296–1.27)	0.946 (0.234–3.82)
rs1204038	0.605 (0.314–1.17)	0.541 (0.257–1.14)	0.900 (0.225–3.61)
rs7061037	0.777 (0.406–1.49)	0.640 (0.310–1.32)	1.84 (0.414–8.16)
rs2361634	0.863 (0.334–2.23)	0.778 (0.246–2.46)	1.23 (0.226–6.68)
rs12014709	0.868 (0.394–1.91)	0.667 (0.260–1.71)	1.97 (0.440–8.79)
rs5031002	1.68 (0.393–7.16)	2.63 (0.519–13.3)	N/A
CAG21[b]	0.710 (0.341–1.48)	0.677 (0.284–1.61)	0.821 (0.206–3.28)
CAG22[b]	1.01 (0.452–2.24)	1.08 (0.454–2.59)	0.548 (0.0560–5.35)
CAG23–25[b]	1.36 (0.758–2.43)	1.43 (0.743–2.75)	1.03 (0.276–3.82)
CAG > 25[b]	0.987 (0.400–2.44)	1.05 (0.391–2.84)	0.657 (0.0650–6.61)
GGN < 23[c]	**2.15 (1.04–4.45)**	**2.38 (1.06–5.32)**	**1.50 (0.241–9.34)**
GGN > 23[c]	0.952 (0.556–1.63)	0.867 (0.474–1.59)	1.50 (0.438–5.14)

N/A, odds ratio analysis not possible because N was zero in one of the cells.
[a] Reference genotype = the most common allele.
[b] Reference CAG < 21.
[c] Reference GGN = 23.

may have only weak effect, if any, whereas the same polymorphism may exert stronger influence in the foetal period, when the initial step of the neoplastic transformation of germ cells takes place, which is hypothesised to be an arrest of gonocyte maturation. A recent global gene expression-profiling study confirmed this long-standing hypothesis that the CIS cells are a transformed genocyte that failed to differentiate, most likely due to the improper hormonal function of the foetal somatic cells.[21]

Opposite to our previous study on a small series of 83 Swedish TGCC patients,[10] but in concert with a previous report on Danish patients,[12] the length of the CAG repeats of the AR was neither predictive for the risk of having testicular cancer nor for the histology or metastatic spread of the disease. On the other hand, TGCC patients having short GGN repeats were more prone to aggressive TGCC, presenting more often with NS and metastases at the time of diagnosis. Previous studies have indicated that GGN < 23 is associated with lower AR activity, as compared to the most common GGN = 23 genotype,[22] meaning that low androgen response could play a role in disease progression.

The strength of our study was the access to a relatively large patient and control groups of two separate populations, which has enabled analyses of two independent patient cohorts. Furthermore, both study populations were of the same ethnic origin thus excluding genetic heterogeneity across populations. Since the same increase in the OR of TGCC was found among the Swedish as well the Danish subjects, the robustness of the trend indicates that our finding may have a biological relevance rather than being a chance finding.

The limitation of our study is the lack of knowledge which coinherited genetic factors or regulatory sequences are associated with the identified SNP. In addition we lack the knowledge of the possible biological mechanisms linking the identified risk polymorphisms to processes involved in neoplastic transformation of germ cells and further progression from CIS to the stage of invasive tumour. A possible mechanism of action might be that the G variant of the tag SNP *rs12014709* is associated with higher sensitivity of the AR to endocrine-disrupting effect of environmental toxicants. Thus, such gene–environment interaction might be responsible for the increase in the TGCC incidence observed in many countries.[3]

In conclusion, we found that short GGN repeats are associated with an increased risk of metastatic TGCC. Furthermore, we have identified one SNP variant in the AR gene that implies an increased risk of TGCC. Although, this SNP is located in non-coding parts of the gene it could be in linkage disequilibrium with other genes that are of importance for this malignancy.

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Conflict of interest statement

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