Genetic variations associated with the effect of testicular cancer treatment on gonadal hormones

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STUDY QUESTION: Do genetic variations in the testosterone pathway genes modify the effect of treatment on the levels of testosterone and LH in long-term testicular cancer (TC) survivors (TCSs)?

SUMMARY ANSWER: Variations in LH receptor (LHR) and in 5α-reductase II (SRD5A2) genes may modify the effect of TC treatment on testosterone levels, whereas genetic variations in the androgen receptor (AR) may modify the effect on LH levels.

WHAT IS KNOWN ALREADY: TCSs experience variable degrees of long-term reduction in gonadal function after treatment. This variability can in part be explained by treatment intensity, but may also be due to individual variations in genes involved in the function and metabolism of reproductive hormones.

STUDY DESIGN, SIZE, DURATION: Cross-sectional study on testosterone and LH levels in 637 Norwegian TCSs in relation to genetic variants and TC treatment.

PARTICIPANTS/MATERIALS, SETTING, METHODS: The single nucleotide polymorphisms LHR Asn291Ser (rs12470652) and Ser312Asn (rs2293275), as well as SRD5A2 Ala49Thr (rs9282858) and Val89Leu (rs523349) were analyzed by allele-specific PCR. The insertion polymorphism LHR InsLQ (rs4539842) was analyzed by sequencing. The numbers of AR CAG and GGN repeats were determined by capillary electrophoresis. Blood samples were collected 5–21 years after diagnosis (median 11 years) and serum total testosterone and LH were analyzed by commercial immunoassays. The TCSs were divided into four groups according to their treatment; surgery only, radiotherapy and chemotherapy with ≤ 850 or > 850 mg of cisplatin. Polymorphisms presenting P, 0.1 for the interaction term with treatment in an initial two-way analysis of covariance (ANCOVA) were investigated further in two consecutive one-way ANCOVA analyses to elucidate the interaction between treatment and genotype.

MAIN RESULTS AND THE ROLE OF CHANCE: For the whole group of TCSs, there were no significant differences between the hormone levels in homozygotes for the wild type and carriers of at least one polymorphic allele for the investigated polymorphisms. Three of the polymorphisms showed signs of interaction with treatment, i.e. LHR InsLQ, SRD5A2 A49T and the AR CAG repeat. Follow-up analyses revealed three situations where only one of the genotypes of the polymorphism where associated with significantly different hormone levels after surgery compared with after additional cytotoxic treatment: For LHR InsLQ, only the wild-type allele was associated with lower testosterone levels after cisplatin > 850 mg compared with after surgery (24% lower, P < 0.001). For SRD5A2 A49T, testosterone levels were lower after radiotherapy compared with after surgery, but only for the heterozygotes for the polymorphism (39% lower, P = 0.001). In comparison, the testosterone levels were just slightly lower after radiotherapy (6% lower, P = 0.039) or cisplatin ≤ 850 mg (7% lower, P = 0.041), compared with surgery, independent of genotypes. For AR CAG, only the reference length of CAG = 21–22 had significantly higher LH levels after cisplatin ≤ 850 mg compared with after surgery (70% higher, P < 0.001). Independent of genotypes, however, LH levels after cisplatin ≤ 850 mg were only 26% higher than after surgery (P = 0.005).

LIMITATIONS, REASONS FOR CAUTION: Unadjusted P-values are presented. For analysis involving genotypes, the level of statistical significance was adjusted for the total number of polymorphisms tested, n = 7, i.e. to P < 0.007 (0.5/7). The rather weak associations indicate that additional polymorphisms are involved in the modulation.

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Introduction

Testicular germ cell tumor (TGCT, hereafter called TC) is the most common malignancy in young men, aged from 15 to 40 years, with a 5-year relative survival rate of 95%. After orchectomy, metastatic TC is treated with cisplatin-based chemotherapy, usually followed by resection of residual masses in non-seminoma patients. Patients with non-metastatic seminoma have previously received adjuvant radiotherapy, but are nowadays included into a surveillance program or receive carboplatin (Oldenburg et al., 2013).

The testicular cancer (TC) survivors (TCSs) constitute a diverse group, with several factors affecting their long-term post-treatment hormone levels, including the inherent gonadal function of the contralateral testis, pretreatment gonadal function and severity of the cancer in addition to the cytotoxicity of the treatment. Several studies have indicated increased risk of gonadal dysfunction in TC patients on long-term follow-up, dependent on the type and intensity of the treatment. High cumulative doses of chemotherapy (Nord et al., 2003) or the combination of chemotherapy and radiotherapy (Huddart et al., 2005) are shown to have the most adverse effect. However, the wide range in hormone levels within the treatment groups indicates individual differences in the endocrine response to TC treatment.

Luteinizing hormone/choriogonadotrophin receptor (LHCGR, in this article called LHR), 5α-reductase II (SRD5A2) and androgen receptor (AR) are key elements in androgen action. LHR is located to the Leydig cells, and upon LH binding it stimulates synthesis and secretion of testosterone. Testosterone is the major circulating androgen and is converted to the more potent androgen dihydrotestosterone (DHT) by SRD5A2 in several target tissues. Testosterone and DHT mediate their effects through binding and activating AR. Testosterone, however, also regulates the release of LH from the pituitary through a negative feedback loop. Thus, polymorphisms in the LHR, SRD5A2 and AR genes may alter the level of serum testosterone or LH in vivo, and possibly modify the effects of TC treatment on hormone levels.

In exon 1 of the LHR gene, a polymorphic CTGCAG insertion (Insl3Q; rs4539842), encoding leucine and glutamine, has been shown to increase the receptor sensitivity 2-fold and expression 40% in vitro (Piersma et al., 2006). A single nucleotide polymorphism (SNP) in exon 10, resulting in the change of asparagine 291 into serine (Asn291Ser; rs12470652), increased the receptor sensitivity 2-fold, whereas another SNP in the same exon, Ser312Asn (rs2293275), did not show any functional consequences in vitro (Piersma et al., 2007). However, Ser312Asn has been reported 15% less frequent in men with contradictionism compared with controls (Simoni et al., 2008).

In SRD5A2, the Ala49Thr polymorphism (rs9282858) in exon 1, has been shown to increase enzyme activity 5-fold in vitro, and this polymorphism was associated with 7- and 3-fold increased risk of prostate cancer in African-American and Hispanic men, respectively (Makridakis et al., 1999). In contrast, another SNP in the same exon, Val89Leu (rs523349), was shown to decrease enzyme activity by almost 30% in vivo. The 89Leu allele was almost twice as common among Asians, a population with low risk for prostate cancer, compared with Caucasians (Makridakis et al., 1997).

Exon 1 of the AR gene, which is located on the X chromosome, encodes the transactivation domain, which contains a glutamine repeat, encoded by (CAG)nCAA and a glycine repeat, encoded by (GGT)3(GGG)(GGG)2(GGC)n (Lubahn et al., 1989), commonly referred to as the CAG and GGN repeat, respectively. In the early studies, the length of the CAG repeat was found to be inversely related to the transactivation activity of AR both in vivo (La Spada et al., 1991) and in vitro, where AR with 30 CAG had only 64% of the transactivation activity of the 14 CAG AR (Tut et al., 1997). Recently, however, a non-linear AR activity pattern was demonstrated, i.e. AR containing the median CAG length of 22 exhibited 5 and 8 times higher transcriptional activity as compared with CAG repeat lengths in the outer normal ranges (Nenonen et al., 2010). Regarding the GGN repeat, in one study, a net reduction in transactivation activity per cell due to a 2.7 times reduction in AR protein level by going from a GGN repeat length of 19–23 was observed, but no effect on AR activity per se (Ding et al., 2005). Another study found that a median length of 23 GGN resulted in a 50–140% higher AR activity compared with repeat lengths of 10, 24 or 27 (Lundin et al., 2007). We have previously found that subjects with contradictionism were more likely to have a GGN repeat length of 24 than controls, where the median was 23 (Aschim et al., 2004).

The aim of the present study was to examine if polymorphisms in the testosterone pathway genes LHR, SRD5A2 and AR modify the effect of different treatment regimens on the levels of testosterone and LH in long-term TC survivors.

Material and Methods

Study population

The study includes 637 long-term TC survivors (TCSs) who were treated for unilateral germ cell TC at the Norwegian Radium Hospital, Oslo, Norway between 1980 and 1994, and did not receive androgen replacement therapy. Blood samples for DNA isolation and hormone analysis were collected as part of a Norwegian national follow-up survey of TCSs from 1998 to 2000 (Nord et al., 2003). Three hundred and twelve of the cancers were seminomas, and 325 were non-seminomas including mixed tumors. All TCSs were of Norwegian Caucasian origin.

The treatment principles have been described elsewhere (Nord et al., 2003). At the time the patients in this study were treated, four cycles of
cisplatin-based chemotherapy represented the standard initial therapy for metastatic TC. With a cisplatin dose of 100 mg/m² per cycle the standard cumulative dose was 800–850 mg. Patients with recurrent cancer or those with a large tumor burden received additional treatment cycles. Our TCSs cumulative dose was 800–850 mg. Patients with recurrent cancer or those and/or radiotherapy.

of cisplatin radiotherapy only (radiotherapy) or chemotherapy with cumulative doses without retroperitoneal lymph node dissection (RPLND). The remaining reference treatment group and included orchiectomized cases with or study, we aim to evaluate if genetic variation can modify this effect, therefore the cases were allocated into the same four treatment groups as the previous study (Nord et al., 2003). In the present study, we aim to evaluate if genetic variation can modify this effect, therefore the cases were allocated into the same four treatment groups as the previous study (Nord et al., 2003). The surgery only group (surgery) was chosen as the reference treatment group and included orchiectomized cases with or without retroperitoneal lymph node dissection (RPLND). The remaining treatment groups received additional treatment after orchietomy; either radiotherapy only (radiotherapy) or chemotherapy with cumulative doses of cisplatin <850 mg or >850 mg, alone or in combination with RPLND and/or radiotherapy.

Genotyping
The SNPs LHR Asn291Ser (rs12470652) and Ser312Asn (rs2293275), as well as SRD5A2 Ala49Thr (rs9282858) and Val89Leu (rs523349) were analyzed by allele-specific PCR, while the insertion polymorphism LHR InsLQ (rs4539842) was analyzed by sequencing, as previously described (Kristiansen et al., 2012). The minor allele frequencies among our 637 TCSs were 4.6, 40.2, 1.7, 30.6 and 27.8%, respectively. There were no homozygotes for the Ala49Thr polymorphism. The numbers of AR CAG and GGN repeats were determined by capillary electrophoresis, as previously described (Kristiansen et al., 2012). The median numbers of AR repeats were 22 CAG and 23 GGN.

Control samples with known genotype (sequenced several times in both directions) for each of the polymorphisms were included in each run. Typically, each plate run consisted of 92 case samples, one blank sample and three positive controls (homozygote wild-type, heterozygote and homozygote polymorphism). Finally, 10% of the samples were randomly chosen for additional direct sequencing and compared with the primary results, confirming the validity of the method’s accuracy.

The percentage genotyping success rate among these were 99.5, 100, 99.2, 99.8, 100, 97.5 and 97.5% for LHR Asn291Ser, LHR Ser312Asn, LHR InsLQ, SRD5A2 Ala49Thr, SRD5A2 Val89Leu, AR CAG and GGN, respectively.

Hormone analyses
Blood samples were drawn before 11 a.m. at the outpatient clinic of the respective university hospital where the patients had received their TC treatment. Serum levels of total testosterone and LH were analyzed by commercial immunoassays (Nord et al., 2003), and all samples were analyzed in the same laboratory. The serum samples included in our analyses were collected 5–21 years after diagnosis (median 11 years).

Statistical analyses
Statistical analyses were performed using IBM SPSS Statistics 20.0 (IBM Corp., Armonk, NY, USA). All statistical tests were two-sided, and unadjusted P-values are presented. For evaluating the impact of SNPs and the InsLQ insertion a dominant model was tested, assuming that both one and two copies of the polymorphic allele would have an effect, compared with being homozygous for the wild-type allele, which was used as reference. For all these polymorphisms, the wild-type allele was the most common in our material (Kristiansen et al., 2012). The two AR repeats were trichotomized, in order to produce three similar size-groups with the middle group including the median length: CAG repeat lengths were categorized into CAG <21, CAG = 21–22, CAG >22, with the middle length group as reference (Nenonen et al., 2010). GGN repeat lengths were categorized into GGN <23, GGN = 23, GGN >23, in which GGN = 23 was used as reference (Lundin et al., 2007).

The statistical analyses can be summarized according to the study questions (Table I), where the first two were investigating the effect of either treatment independent of genotype or of genotype on LH and testosterone for the whole group of patients independent of treatment. According to the main aim of the study, interaction between genotype and treatment regarding the effect on LH and testosterone was investigated in three steps; first, looking at the interaction term between genotype and treatment, secondly, at differences in hormone levels between the polymorphic and the wild-type genotypes within one treatment group and thirdly, at differences in hormone levels between surgery only and an additional cytotoxic treatment within one genotype.

The effects of different TC treatment regimens on LH and testosterone levels, independent of genotype, were analyzed by one-way analysis of covariance (one-way ANCOVA) where hormone levels were entered as the dependent variable and treatment groups as categorical independent variable, adjusting for age and BMI as continuous covariates (Table II). One case had much higher LH levels than the rest of the study group, producing a strong outlier. The second highest LH level in the dataset was 34 IU/l, and to reduce the influence of the outlier without transforming the whole set of LH values, the maximum LH level was redefined from the original 54.5–36 IU/l. Still, this reduction resulted in only minimal changes in the P-values and adjusted means. These analyses only include two tests (Table I) and were not adjusted for multiple testing.

The effects of the different genotypes on LH and testosterone levels, independent of treatment, were analyzed by one-way ANCOVA, using genotype categories as categorical independent variable, adjusting for age and BMI as continuous covariates. All the seven polymorphisms were investigated for both testosterone and LH. For these analyses, the level of statistical significance was adjusted for the total number of polymorphisms investigated, n = 7 i.e. to P < 0.007 (0.05/7).
The first step of the interaction analysis was performed using two-way analysis of covariance (two-way ANCOVA) where hormone levels were entered as the dependent variable and genotype categories and treatment groups as categorical independent variables, adjusting for age and BMI as continuous covariates. In this step, all the seven polymorphisms were investigated for both testosterone and LH. The statistical models included genotype and treatment entered both as main effects and as the interaction term between the two. Only polymorphisms displaying P-values below 0.1 for the interaction term were considered as potentially interacting with treatment, for the hormone in question, and were selected for a follow-up by one-way ANCOVA for the specific hormone. In this analysis, the effect of genotype was analyzed after dividing the cases into the separate treatment groups, to see if the treatment-specific hormone levels in the polymorphic genotype group were significantly different from that of the reference genotype, the effect of treatment on the hormone levels was analyzed by one-way ANCOVA after dividing the cases into the separate genotype groups. This second round of follow-up was conducted to see if the treatment- and genotype-specific hormone level was significantly different from that of the reference treatment group, surgery, for the same genotype group. For these analyses, the level of statistical significance was adjusted for the total number of polymorphisms investigated, n = 7, i.e. to P < 0.007 (0.05/7).

The analysis of genotype effect on LH within the cisplatin ≤ 850 mg group included subgroups with unequal variances, violating an important assumption for ANCOVA. Thus, the analysis was repeated using linear regression on both untransformed and ln-transformed LH levels, producing similar P-values (P-value for ln-transformed LH is given under footnote b in Table IV). Two subjects had LH levels below detection level of 0.6 IU/l. Before transformation their LH levels were set to 0.5 to allow ln-transformation.

All hormone levels referred to in the results section are mean hormone levels adjusted for age and BMI, produced by the one-way ANCOVA procedure, unless otherwise stated. Unadjusted median hormone levels were also included in the tables for the sake of comparison, to indicate the underlying distribution.

**Ethical approval**

The study was conducted with all the participants’ informed written consent and approved by the Regional Committee for Medical Research Ethics, Southern Norway.

**Results**

**Effects of genotype on gonadal hormones**

There were no statistically significant overall associations between the levels of testosterone or LH and the investigated genotype categories when analyzing all TCSs together, independent of treatment (data not shown).
Effects of TC treatment on gonadal hormones

The levels of testosterone and LH for the different treatment groups are listed in Table II. For most groups, the mean values, adjusted for BMI and age, were similar to the unadjusted median hormone levels. The testosterone levels for all post-orchiectomy cytotoxic treatments were significantly lower than that of the SU group. While both RT and cisplatin ≤850 mg had 6% lower testosterone levels, the reduction was 14% in the cisplatin >850 mg group (Table II). For LH, cisplatin ≤850 mg and cisplatin >850 mg had significantly higher LH levels than surgery, 26 and 46%, respectively, while the LH levels in radiotherapy were not significantly increased (Table II).

Interactions between genotypes and treatment regimens affecting testosterone levels

Both the SRD5A2 Ala49Thr and the LHR InsLQ polymorphisms displayed $P < 0.1$ for the interaction in treatment group with interaction analyses regarding the effect on testosterone levels ($P = 0.045$ and $P = 0.075$, respectively). Thus, these two polymorphisms were chosen for subsequent one-way ANCOVA analyses to elucidate the interactions. None of the other polymorphisms showed any signs of interaction with treatment, regarding testosterone levels (data not shown).

For the LHR InsLQ polymorphism, homo- or heterozygotes for the polymorphic allele had 23% higher testosterone levels compared with cases being homozygous for the wild-type allele among TCs receiving cisplatin >850 mg, although the difference was only borderline significant after adjusting for multiple testing ($P = 0.010$, Table III). When investigating this association further, we found that for cases with at least one polymorphic LHR InsLQ allele, those in the cisplatin >850 mg group did not have significantly different testosterone levels from those belonging to the surgery group (15.9 versus 16.5 nmol/l, respectively, $P = 0.643$). In comparison, among cases being homozygous for the wild-type allele, those in the cisplatin >850 mg group had significantly lower testosterone levels compared with the surgery group (12.9 versus 16.9 nmol/l, respectively, $P < 0.001$). There were no significant differences in testosterone levels between the two LHR InsLQ genotypic groups for the other treatment groups.

Regarding the rare SRD5A2 Ala49Thr polymorphism, the testosterone level was 27% higher among those that were heterozygous for the polymorphic 49Thr allele compared with homozygotes for the wild-type 49Ala allele in the surgery group, although the difference was not statistically significant after adjusting for multiple testing ($P = 0.0496$, Table III). In contrast, for cases in the radiotherapy group, the heterozygotes had 19% lower testosterone levels than wild-type homozygotes, but the difference was not statistically significant ($P = 0.092$). The difference in testosterone levels between SRD5A2 Ala49Thr heterozygotes in the surgery and radiotherapy groups was significant, however, despite low number of cases in both groups (21.1 nmol/l, n = 5 versus 12.9 nmol/l, n = 11, respectively, $P = 0.001$). There were no significant differences in testosterone levels between the two SRD5A2 Ala49Thr genotypic groups for the other treatment groups.

Interactions between genotypes and treatment regimens affecting LH levels

The AR CAG polymorphism displayed $P = 0.039$ for the interaction term between genotype and treatment group in regard to affecting the LH levels. Thus, only this polymorphism was chosen for subsequent one-way ANCOVA analyses to elucidate the interaction. None of the other polymorphisms showed any signs of interaction with treatment, regarding LH levels (data not shown).

The only difference in LH levels between AR CAG genotype groups was found within the treatment group cisplatin ≤850 mg, where cases with CAG > 22 had 28% lower LH-levels compared with those with the reference length of CAG = 21–22 ($P = 0.012$, Table IV), but this association did not remain statistically significant after adjusting for multiple testing. Owing to unequal variances in these groups, linear regression was also performed, producing $P = 0.032$ in a corresponding analysis using ln-transformed LH levels. When investigating this association further, we found that for cases with CAG > 22, those in the cisplatin ≤850 mg group did not have significantly different LH levels from those in belonging to the surgery group (5.87 versus 6.19 IU/l, respectively, $P = 0.698$). In comparison, among cases with the reference CAG length of 21–22, those in the cisplatin ≤850 mg group had significantly higher LH levels compared with the surgery group (8.12 versus 4.77 IU/l, respectively, $P < 0.001$ with both one-way ANCOVA and linear regression). There were no significant differences in LH levels between the three AR CAG genotypic groups for the other treatment groups.

### Table IV LH levels (IU/l) in treatment and genotype groups.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Surgery</th>
<th>Radiotherapy</th>
<th>Cisplatin ≤850</th>
<th>Cisplatin &gt;850</th>
</tr>
</thead>
<tbody>
<tr>
<td>AR CAG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;21</td>
<td>38.569 (4.7, 1.3–20.3) 0.130</td>
<td>73.553 (5.0, 2.1–24.8) 0.903</td>
<td>58.693 (5.2, 1.8–54.5) 0.264</td>
<td>14.634 (6.6, 0.0–10.9) 0.177</td>
</tr>
<tr>
<td>21–22</td>
<td>46.477 (3.8, 0.6–10.3) ref</td>
<td>77.554 (5.0, 1.9–20.8) ref</td>
<td>55.812 (6.0, 1.5–31.9) ref</td>
<td>18.823 (5.6, 3.0–19.3) ref</td>
</tr>
<tr>
<td>&gt;22</td>
<td>41.619 (4.6, 0.0–17.1) 0.065</td>
<td>107.611 (5.2, 1.6–34.3) 0.354</td>
<td>76.587 (5.0, 1.3–17.3) 0.012</td>
<td>18.845 (6.7, 3.10–16.2) 0.916</td>
</tr>
</tbody>
</table>

The polymorphisms were selected by initial interaction analysis (two-way ANCOVA). Data are mean (median, range). Mean values are adjusted for age and BMI. Median values and range are not adjusted. Values significantly different from the wild-type within a treatment group are in bold type.

$^a$Unadjusted $P$-value for the difference between this polymorphic genotype and the reference genotype, 21–22 CAG length, analyzed by one-way ANCOVA.

$^b$Significantly different from the reference 21–22 CAG length in this treatment group, also with linear regression analysis ($P = 0.032$).
Discussion

Despite a lack of significant overall associations between genotype and hormone levels when looking at all TCSs together, we observed signs of interaction between the effects of treatment and genotype on hormone levels for the LHR InsLQ and SRD5A2 Ala49Thr polymorphisms on testosterone levels, and for the AR CAG polymorphic repeat on LH levels.

We found that all post-orchiectomy cytotoxic treatments, i.e. radio- or chemotherapy, of TC lead to reduced levels of testosterone compared with the surgery only group, with cisplatin \( > 850 \text{ mg} \) presenting the lowest levels. Interestingly, only cases being homozygous for the LHR InsLQ wild-type allele had significantly lower testosterone levels after cisplatin \( > 850 \text{ mg} \) compared with surgery, while there was no statistical difference between cisplatin \( > 850 \text{ mg} \) and surgery for cases being homo- or heterozygous for the polymorphic InsLQ allele. These findings may suggest that the common LHR InsLQ polymorphic allele counteracts the cytotoxic effect of high doses of chemotherapy on the Leydig cells. This is in accordance with functional studies showing increased receptor sensitivity and increased expression of the polymorphic InsLQ LHR receptor (Piersma et al., 2006). On the other hand, the lack of overall association between the LHR InsLQ genotype and testosterone and LH levels in our study, concurs with a previous study on men with testicular maldescent and healthy controls (Simoni et al., 2008).

The polymorphic SRD5A2 49Thr allele may seem to result in an increased testosterone level in the surgery group, but a possibly reduced testosterone level in the radiotherapy group. Cases being SRD5A2 Ala49Thr homozygotes had significantly higher testosterone levels after treatment with surgery only compared with radiotherapy. In contrast, wild-type homozygotes had similar testosterone levels in these two treatment groups. The SRD5A2 Ala49Thr polymorphism has been shown to increase enzyme activity substantially in vitro (Makridakis et al., 1999). Administration of high levels of DHT has been found to induce a drop in plasma testosterone levels in normal men, due to inhibition of gonadotrophin secretion (Santen, 1975; Veldhuis et al., 1992; Kuhn et al., 1984). Thus, it is difficult to explain a link between increased testosterone to DHT turnover and increased testosterone levels. Still, a non-significant tendency of 7–9% higher testosterone levels in heterozygotes for the 49Thr allele compared homozygotes for the wild-type 49Ala allele has been observed in control men (Allen et al., 2003; Hayes et al., 2007). The increased testosterone levels within the surgery only group presenting with the 49Thr allele may also be related to the fact that the TC cases are slightly hypogonadic based on serum hormone levels. Even before treatment, a few patients have relatively low testosterone and/or increased FSH, often combined with poor semen quality (Harland et al., 1998; Jacobsen et al., 2000). In addition, TCSs have after orchiectomy only half of the normal number of Leydig cells, possibly increasing the risk of hypogonadism (Bandak et al., 2011). A polymorphism that has been found to reduce SRD5A2 activity, Val89Leu (Makridakis et al., 1997), has been associated with reduced testosterone levels in elderly men (Schatz et al., 2002). We cannot exclude, however, that the associations observed in our study may be chance findings due to the rarity of SRD5A2 Ala49Thr and thereby a low number of polymorphic cases.

For LH, only chemotherapy lead to significantly increased levels in our study, with the group treated with the highest doses of cisplatin presenting the highest levels, compared with the group only treated with surgery. Among the surgery group, cases presenting with the AR CAG reference length of 21–22 had the lowest LH level, compared with those with shorter or longer CAG, repeat, although not significantly different. Still, this is in accordance with functional studies suggesting that a CAG length of around 22 has results in higher AR activity than either shorter or longer repeats (Nenonen et al., 2010). Since AR mediates the negative feedback of testosterone on LH production, a greater AR activity would be expected to reduce the LH levels. Interestingly, we found that cases in the cisplatin \( \leq 850 \text{ mg} \) group with 21–22 CAGs had higher LH levels than those with longer CAG repeats, but the statistical significance of this finding was lost after adjusting for multiple testing. Still, this might suggest that despite the relatively low ‘background’ level of LH, these patients are especially sensitive to chemotherapy.

The increased testosterone levels within the surgery only group presenting with the highest doses of cisplatin \( > 850 \text{ mg} \) may be explained by a minor role of these genetic variants when very high doses of chemotherapy are applied.

Our 637 cases have previously been analyzed with regard to testosterone and LH as part of a larger study, including 1 182 TC cases and 200 controls (Nord et al., 2003). We used the same four treatment groups as in this study, except that they used controls as the reference group. They found a slight and gradual, but statistically non-significant decrease in testosterone levels with increasing treatment intensity (surgery—radiotherapy—cisplatin \( \leq 850 \text{ mg} \)—cisplatin \( > 850 \text{ mg} \)), with radiotherapy and cisplatin \( \leq 850 \text{ mg} \) being very similar (Nord et al., 2003). Thus, the testosterone levels in their analyses showed the same tendency as we found, but did not reach statistical significance, possibly because Nord et al. adjusted for dichotomized age and did not adjust for BMI. In our contrast, we chose to adjust for age as a continuous variable, and also adjust for BMI, which is strongly associated with the hormones in our study. In regard to LH, Nord et al. found that all the TC treatment groups had higher age-adjusted mean LH levels than controls, and that the levels gradually increased with treatment intensity (Nord et al., 2003), in a manner similar to our results.

We have chosen to present unadjusted \( P \)-values and adjust the level of significance for the total number of polymorphisms tested, for all statistical analyses that include genotypes. The main aim, to investigate if genetic variations are associated with the effect of TC treatment on the hormone levels, creates an \textit{a priori} hypothesis, and the selection of associations for follow-up analyses keeps the number of analyses to a minimum. Furthermore, this study includes several small groups, and a stricter level of significance would mask several biologically plausible associations—and the connection between them—thus, making it more difficult to get an idea on which associations seems to be true and which seems to be due to chance. It is important, however, to be careful in interpreting the results, since we cannot rule out the possibility of false positives among our analyses.

To our knowledge, this is the first study supporting the notion that polymorphisms may explain at least some of the individual differences in endocrine response to TC treatment. A previous study on sperm concentration, however, found an inverse correlation with AR CAG length \( 12–24 \) months after receiving high doses of chemotherapy, but not at other time periods or treatment groups (Eberhard et al., 2004). Further research will be necessary to confirm our findings, and to elucidate the mechanisms behind these associations. Moreover, the rather weak associations indicate that additional polymorphisms are involved in the modulation.
In conclusion, our findings indicate that the impact of certain TC treatment regimens on testosterone and LH levels in long-term TCSs may be associated with genetic variations, suggesting that individuals with certain genotypes might be more vulnerable to these treatments. Knowledge on genetic predisposition concerning genetically determined treatment-related endocrine sensitivity to different treatment regimens may help tailoring TC treatment when possible. Vulnerable TCSs might benefit from intensified endocrine monitoring and early treatment of hypogonadism.

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Authors’ roles

E.L.A.: Conception and design, analysis and interpretation of data, drafting and revising the article and final approval. J.O.: Conception, critical revision and final approval. W.K.: S.D.F.: Conception, acquisition of data, critical revision and final approval. A.G.: Interpretation of data, critical revision and final approval. O.W.: Acquisition of data, critical revision and final approval. T.B.H.: Conception and design, acquisition and interpretation of data, critical revision and final approval.

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Conflict of interest

There were no competing interests.

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