Gene variations in sex hormone pathways and the risk of testicular germ cell tumour: A case-parent triad study in a Norwegian-Swedish population

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Keywords:	testicular germ cell tumour, sex hormone pathway genes, polymorphisms, case-parent triad

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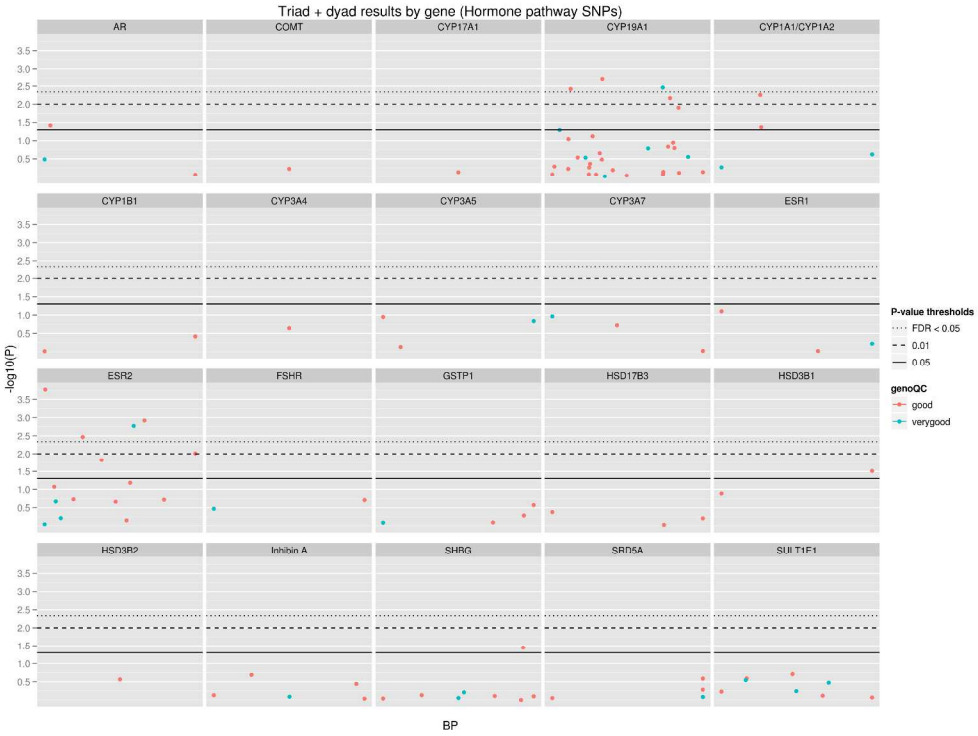

Triad + dyad results by gene (Hormone pathway SNPs)
AR
COMT
CYP17A1
CYP19A1
CYP1A1/CYP1A2
CYP1B1
CYP3A4
CYP3A5
CYP3A7
ESR1
ESR2
FSHR
GSTP1
HSD17B3
HSD3B1
HSD3B2
Inhibin A
SHBG
SRD5A
SULT1E1
-log10(P)
BP
P-value thresholds
FDR < 0.05
0.01
0.05
genoQC
good
verygood

Figure 1

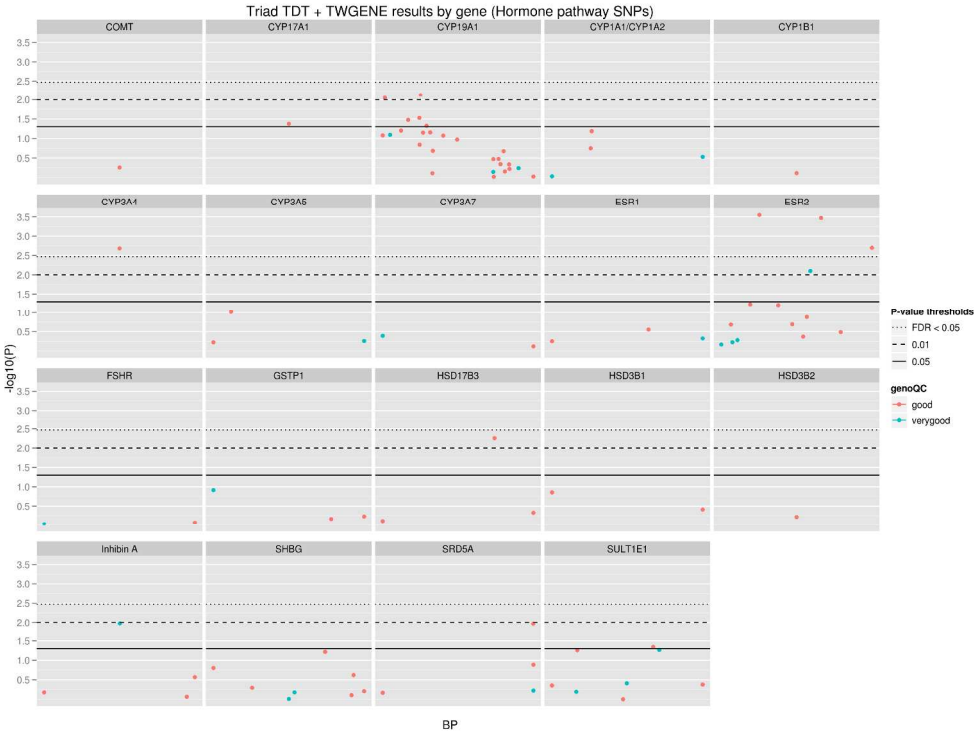
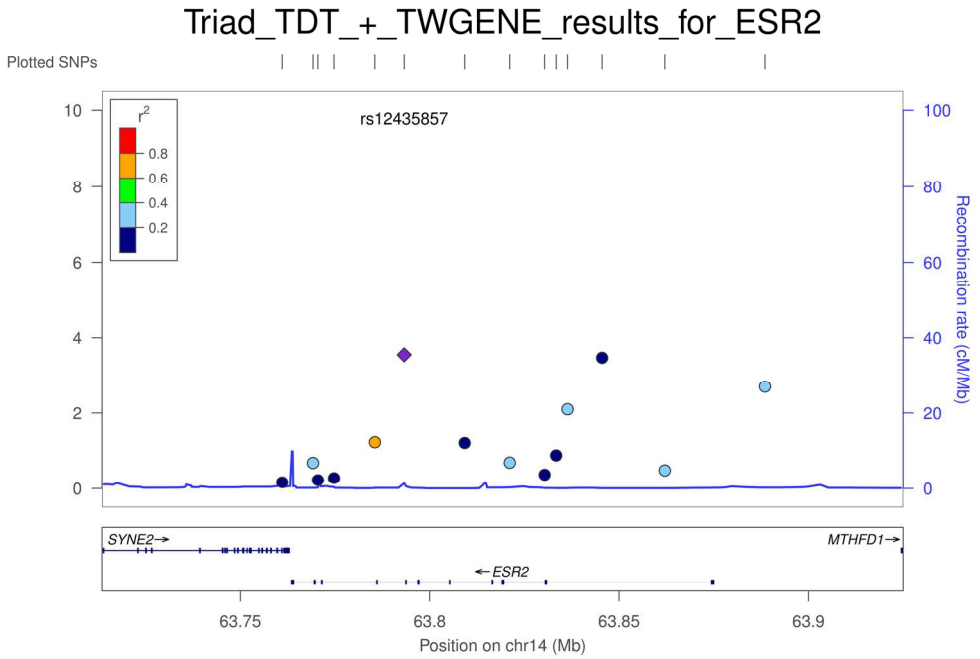

Triad TDT + TWGENE results by gene (Hormone pathway SNPs)
COMT
CYP17A1
CYP19A1
CYP1A1/CYP1A2
CYP1B1
CYP3A4
CYP3A5
CYP3A7
ESR1
ESR2
FSHR
GSTP1
HSD17B3
HSD3B1
HSD3B2
Inhibin A
SHBG
SRD5A
SULT1E1
-log10(P)
BP
P-value thresholds
FDR < 0.05
0.01
0.05
genoQC
good
verygood

Figure 2



177x124mm (300 x 300 DPI)

Gene variations in sex hormone pathways and the risk of testicular germ cell tumour: A case-parent triad study in a Norwegian-Swedish population

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Running title: Gene variation in sex hormone pathways genes and the risk of testicular cancer.

Key words: testicular germ cell tumour, sex hormone pathway genes, polymorphisms, case-parent triad

Abstract

BACKGROUND: Testicular germ cell tumour (TGCT) is the most common cancer in young men, and an imbalance between the oestrogen and androgen levels *in utero* is hypothesized to influence TGCT risk. Thus, polymorphisms in genes involved in the action of sex hormones may contribute to variability in an individual's susceptibility to TGCT.

METHODS: We conducted a Norwegian-Swedish case-parent study. 105 SNPs in 20 sex hormone pathway genes were genotyped using Sequenom MassArray iPLEX Gold, in 831 complete triads and 474 dyads. To increase the statistical power, the analysis was expanded to include 712 case singletons and 3922 Swedish controls, thus including triads, dyads, and the case-control samples in a single test for association. Analysis for allelic associations was performed with the UNPHASED program, using a likelihood-based association test for nuclear families with missing data, and odds ratios (ORs) and 95 % confidence intervals (CIs) were calculated. False discovery rate (FDR) was used to adjust for multiple testing.

RESULTS: Five genetic variants across the *ESR2* gene (encoding ERβ) were statistically significantly associated with the risk of TGCT. In the case-parent analysis, the markers rs12434245 and rs10137185 were associated with reduced risk of TGCT (OR=0.66 and OR=0.72, respectively; both FDRs < 5%), while rs2978381 and rs12435857 were associated with increased risk of TGCT (OR=1.21 and OR=1.19, respectively; both FDRs < 5%). In the combined case-parent/case-control analysis, rs12435857 and rs10146204 were associated with increased risk of TGCT (OR=1.15 and OR=1.13, respectively; both FDRs < 5%), while rs10137185 was associated with reduced risk of TGCT (OR=0.79, FDR < 5%). In addition, we found that three genetic variants in *CYP19A1* (encoding aromatase) were statistically significantly associated with the risk of TGCT in the case-parent analysis. The T alleles of the rs2414099, rs8025374 and rs3751592 SNPs were associated with an increased risk of TGCT (OR=1.30, 1.30 and 1.21, respectively; all FDRs < 5%). We found no statistically significant differences in allelic effect estimates between parental inherited genetic variation in the sex hormone pathway and TGCT risk in the offspring, and no evidence of heterogeneity between seminomas and non-seminomas, or between the Norwegian and the Swedish population in any of the SNPs examined.

CONCLUSION: Our findings provide support for ERβ and aromatase being implicated in the aetiology of TGCT. Exploring the functional role of the TGCT-risk associated SNPs will further elucidate the biological mechanisms involved.

Introduction

Testicular germ cell tumour (TGCT) accounts for only 1-2% of all neoplasms in males, but is the most common malignancy in young men (Huyghe *et al.*, 2003). The incidence rate of TGCT worldwide has increased 2-3 times during the last 50 years in several western countries (Weir *et al.*, 1999, Richiardi *et al.*, 2004, Walschaerts *et al.*, 2008, Chia *et al.*, 2010). The current age-adjusted incidence rate of TGCT in Norway is 11 per 100,000 male person-years (Engholm *et al.*, 2010). Norway and Denmark are among the countries with the highest incidence rates of TGCT, nearly twice as high as in Sweden.

The aetiology of TGCT is largely unknown, but a commonly held view is that carcinoma *in situ* (CIS) cells originate from primordial germ cells delayed in maturation during early embryonic development (reviewed by (Hoei-Hansen *et al.*, 2005)). The subsequent malignant transformation from CIS cells to invasive seminomatous or non-seminomatous TGCT is believed to be regulated by endocrine mechanisms during puberty (Oosterhuis and Looijenga, 2005, Rajpert-De Meyts, 2006). There is evidence for a genetic contribution to the development of TGCT. Brothers of TGCT patients have an 8-10 fold increased risk of disease, while sons of men with TGCT have a 4-6 fold increased risk (Dong *et al.*, 2001, Hemminki and Li, 2004). Recently, three genome-wide association studies (GWAS) of TGCT have revealed genetic predisposition to TGCT linked to six genes (*KITLG, SPRY4, BAK1, TERT, ATF7IP, DMRT1*), central in normal primordial germ cell development (Kanetsky *et al.*, 2009, Rapley *et al.*, 2009, Turnbull *et al.*, 2010).

An association of TGCT with maternal levels of oestrogens and androgens in early pregnancy has recently been reported (Holl *et al.*, 2009). Offspring of mothers with high dehydroepiandrosterone sulphate (DHEAS) levels had a significantly decreased risk of TGCT, whereas high maternal androstenedione and total oestradiol level tended to be associated with an increased risk of TGCT. Exposure to environmental factors, such as endocrine disruptors, has also been postulated to play a role in the development of a testicular dysgenesis syndrome (TDS) including TGCT, by causing an imbalance of the androgen/oestrogen levels *in utero* (Sharpe, 2001, Skakkebaek *et al.*, 2001, Sharpe, 2003, Rajpert-De Meyts, 2006, Wohlfahrt-Veje *et al.*, 2009). The concept of TDS has, however, lately been disputed due to lack of epidemiologic assessment (Akre and Richiardi, 2009).

Only a few studies regarding TGCT and polymorphisms in genes involved in the sex hormone pathway have been reported (Starr *et al.*, 2005, Ferlin *et al.*, 2008, Figueroa *et al.*,

2008, Ferlin *et al.*, 2010). Of these only one (Starr *et al.*, 2005) examined TGCT risk in relation to maternal genetic polymorphisms in oestrogen-metabolizing genes, by incorporating maternal genetic markers.

We conducted a large Norwegian-Swedish population-based, case-parent triad study to examine if there is any association between polymorphisms in sex hormone genes and TGCT risk. Furthermore, we wanted to investigate whether such association is present also for the histologic subtype's seminoma and non-seminoma, and to study whether maternal genetic variants have any impact on the son's risk of TGCT. The final aim was to examine if there is any difference in gene variants between the Norwegian and the Swedish population that could contribute to an explanation to the difference in TGCT risk between these two countries.

Materials and Methods

Sample description

Study participants were recruited between September 2008 and September 2010. Men previously diagnosed with TGCT were contacted by mail and invited to participate in the study. They were asked to sign an informed consent document, donate a saliva sample which could be delivered by pre-paid mail, and grant us permission to contact their parents for possible inclusion in the study. Invited parents were also asked to sign an informed consent document and donate a saliva sample which could be delivered by pre-paid mail.

The study was approved by the Regional Committee for Medical Research Ethics, Southern Norway, the Norwegian Social Science Data Services and the Regional Research Ethics Committee in Stockholm, Sweden. The dedicated Research Biobank in Oslo was approved by the Ministry of Health and Care Services.

Norwegian population. Recruitment of Norwegian TGCT patients diagnosed between 1990 and 2008 was based on data from the Cancer Registry of Norway. In this period, 4354 males were diagnosed with this disease, out of which 132 had died (3%). Verification of diagnosis was assessed by the treating physician at the regional oncology centres. 1855 TGCT patients were invited to participate in the study, out of which 974 consented (53%). A total of 2132 Norwegian participants divided into 483 complete triads, 192 dyads (150 mother and son, 42 father and son) and 299 singletons, were included in the study.

500 of the tumours were pure seminoma and 471 were non-seminoma with or without a seminomatous component, while 3 were unclassifiable. Age at diagnosis was 15 to 65 years (mean: 33 years).

Swedish population. Recruitment of Swedish TGCT patients diagnosed between 1995 and 2006 was based on data from the Swedish National Cancer Registry. Verification of diagnosis was assessed by record linkage with the Swedish National Inpatient Register. In total, 2443 men were identified with the disease, out of whom 70 had died (3%). 2327 were invited, out of whom 1188 (51%) consented to participate in the study. In total 521 complete triads, 248 dyads (178 mother and son, 70 father and son), and 419 singletons were included in the study.

672 of the tumours were seminoma and 503 were pure non-seminoma or non-seminoma with a seminomatous component, while 13 were of unknown histology. Age at diagnosis was 18 to 45 years (mean: 32 years).

Control group. The TwinGene project, conducted between 2004 and 2008, is a population-based Swedish study of twins born between 1911 and 1958. In total, 12591 individuals participated by donating blood to the study, and by answering questionnaires about lifestyle and health (Rahman *et al.*, 2009). The study was approved by the local ethics committee at Karolinska Institutet, and all participants gave informed consent. DNA has been extracted for all individuals, and for the majority (n=9836), genome wide genotyping with Illumina OmniExpress bead chip has been performed. For the present study, 3922 unrelated males were randomly selected from the TwinGene population as controls.

Treatment of saliva samples and DNA isolation

Genomic DNA was extracted from whole saliva samples collected with the Oragene® DNA sample collection kit (DNA Genotek Inc., Kanata, Ontario, Canada). These are easy-to-use kits, in which the donors simply just spit into a vial. When the vial is capped a solution containing antibacterial and DNA preserving chemicals mixes with the saliva, resulting in immediate conservation of the sample (Rylander-Rudqvist *et al.*, 2006). Storage of saliva and DNA samples, as well as isolation of DNA, was performed according to the manufacturer's protocol in "Laboratory Protocol for Manual Purification of DNA from 4.0 mL of Oragene® DNA/saliva" (http://www.dnagenotek.com/DNA_Genotek_Industry_CGT_SCA_P.html). In

brief, DNA was purified from the saliva samples using ethanol precipitation. Measurement of DNA yield and purity of the DNA samples were analysed using a NanoDrop® ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). In cases where the DNA yield was < 25µg, the participants were asked to provide a new sample.

A total of 35 Norwegian and 79 Swedish cases were excluded from the study due to low DNA yield (< 25 µg).

Selection of SNPs

Because TGCT has been hypothesized to be a hormone related cancer, candidate genes in sex hormone pathways were considered for study inclusion. First, we selected some genes that could affect either androgen or oestrogen levels or function: *AR, CYP191A, CYP1A1/CYP1A2, CYP3A5, CYP3A7, ESR2, GSTP1, SHBG and SULT1E1*. In these genes, SNPs were selected for genotyping if the minor allele frequency (MAF) was above 5% and had at least 90% genotyping success rate in HapMap2 CEU individuals. Haplotype block structure, based on confidence bounds of D prime values (Gabriel *et al.*, 2002), was inferred using data from the catalogue of common genetic variants generated from the International HapMap Project (The International HapMap Consortium, 2003). Within each haplotype block, htSNPs were selected using the Tagger software (de Bakker *et al.*, 2005), applying aggressive tagging and a minimal coefficient of determination equal to 0.95 in order to capture the common genetic variation across the genes.

In addition, we included additional sporadic SNPs in some genes, based on previously published biological function or associations in relevant populations. The non-tagged genes were *COMT*, *CYP17A1*, *CYP1B1*, *CYP3A4*, *ESR1*, *FSHR*, *HSD17B3*, *HSD3B1*, *HSD3B2*, *INHA* and *SRD5A2*.

A total of 127 SNPs in 20 genes were selected for genotyping. 105 of these SNPs were successfully genotyped and passed our genotype quality control procedure (Supplementary table 1 and 2).

Genotyping

The DNA samples were genotyped using the Sequenom MassARRAY® iPLEX Gold chemistry at the Centre for Integrative Genetics (CIGENE), Norwegian University of Life

Sciences, Ås, Norway. CIGENE is a core facility under the Norwegian Functional Genomics Programme (FUGE) and part of the Norwegian Genotyping and Sequencing Consortium (NGSC).

The Sequenom MassARRAY® iPLEX Gold assay uses PCR amplification followed by a single base pair primer extension reaction, resulting in an allele-specific difference in mass between extension products. This mass difference allows the data analysis software to differentiate between SNP alleles using MALDI-TOF MS (Matrix assisted laser desorption ionisation time-of-flight mass spectrometry). The assay uses three sequence specific primers and Taq-polymerase with no reverse transcriptase activity, circumventing most problems caused by co-precipitated bacterial DNA or RNA, respectively, in the samples.

Statistical analysis

Sample and SNP quality control. Samples with more than 20% missing genotypes or a heterozygosity rate more than three standard deviations from the sample mean (indicating possible sample contamination), were excluded from further analyses (n=277).

Within families we examined pairwise genotype identity-by-state (IBS) to confirm parent-offspring relations. For parent-offspring pairs where the standard deviation of the number of alleles per SNP shared IBS exceeded 0.55 (indicating that the parent may not be biological), we excluded the parent from further analyses. The threshold 0.55 was selected based on visual inspection of a plot of mean alleles shared IBS versus standard deviation of alleles shared IBS between pairs of related and unrelated individuals. 41 samples were excluded in this step. After excluding these samples, most of the 548 Mendelian errors observed in the pedigrees could be resolved. 47 remaining Mendelian errors were attributed to genotyping error, and the corresponding genotypes were set to missing in the subsequent analyses. The triad design has great ability to detect bacteria-caused errors because the inheritance will not be consistent between parents and sons.

Furthermore we examined pairwise IBS across the entire sample in order to uncover any unplanned duplicates. 17 pairs (34 samples) with identical or nearly identical genotypes were found. These samples were excluded since genotype and phenotype could not be unambiguously matched.

Samples which were part of a case-parent triad or dyad where the proband was lost to quality control, were also removed from the final analysis (n=111). SNPs with more than 10%

missing genotypes or MAF less than 0.01, were excluded from further analysis (n=9). 13 more SNPs were removed due to being marked 'deleted' or 'problematic' from CIGENE.

After sample and SNP quality control, 831 triads, 474 dyads and 712 case singletons remained for final analysis.

Imputation and quality control of TwinGene control samples. To increase the number of SNPs available for case-control analysis, we used full-genome imputed data in the TwinGene controls. Imputation was performed using IMPUTE2 (Howie *et al.*, 2009), and CEU reference haplotypes from the HapMap project, release 22. After imputation, genotypes with maximum posterior probabilities > 0.9 were called as the most likely genotype, while the more uncertain genotypes were called as missing. We extracted TwinGene genotypes for all SNPs that were genotyped and passed quality control in the case-parent sample, and were either directly genotyped or imputed in the TwinGene dataset (n=98). The same SNP quality control measures as for the cases were then applied to the control genotypes: SNPS with MAF less than 0.01 or missingness greater than 10% were removed (n=7).

Association analysis for the case-parent triads. For the main analysis we used a likelihood-based association test for nuclear families and unrelated subjects with missing data, implemented in the software package UNPHASED (Dudbridge, 2008). As a first step, we performed a purely family-based test, including only complete case-parent triads and dyads in the analysis. This test is robust to population stratification. An allelic main effect model was assumed, leading to a 1-df likelihood-ratio test. We furthermore investigated whether the allelic effect on TGCT risk was modified by histological subgroups (seminomas and non-seminomas) by including an interaction term in the statistical model. Formal tests of interaction were made using likelihood ratio tests. Similarly, whether the allelic effect was modified by country (Norway and Sweden) or the gender of the parent from whom the allele was transmitted (parent of origin effect), was investigated by an interaction term in the statistical model.

Association analysis for the combined case-parent/case-control population. To increase the power to detect associations, we next expanded the analysis to include case singletons and controls from the TwinGene project. Triads, dyads, and the case-control sample were

included in a single test for association. An unmatched analysis was performed, including both family-based controls (untransmitted alleles) and unrelated controls in the same test.

Controlling for multiple testing. To assess multiple testing issues a false discovery rate (FDR) approach was applied. This approach controls the expected proportion of falsely rejected null hypotheses and is less conservative than the commonly used Bonferroni adjustment. FDR was estimated by applying a semi parametric approach based on a modified Grenander density estimator and truncated maximum-likelihood estimation (Strimmer, 2008), as implemented in the R package fdrtool (Strimmer, 2008). SNPs with q values < 0.05 were considered significant, which resulted in an FDR < 5% among the significant SNPs.

Results

All single nucleotide polymorphism (SNP) positions in this section are reported in genomic build hg18 coordinates, and all alleles are reported relative to the positive (+) strand.

By studying 105 SNPs in 20 genes in a case-parent study (Supplementary table 1), we found that six SNPs in or near the *ESR2* gene, two SNPs located in the intergenic region between the *CYP1A1* and *CYP1A2* genes, five SNPs in the *CYP19A1* gene, one SNP in the *HSD3B1* gene, one SNP in the *SHBG* gene and one SNP in the *AR* gene were nominally associated with the risk of TGCT in 831 triads and 474 dyads (P<0.05). Controlling for an FDR < 5% revealed that four SNPs in the *ESR2* gene and three SNPs in the *CYP19A1* gene remained significantly associated with TGCT risk. In the *ESR2* gene, the T alleles of rs12434245 and rs10137185 were associated with reduced risk of TGCT (OR=0.66, 95% CI=0.53-0.82, P=0.0002 and OR=0.72, 95% CI=0.59-0.88, P=0.001, respectively), while the T and G alleles of rs2978381 and rs12435857 were associated with increased risk of TGCT (OR=1.21, 95% CI=1.08-1.37, P=0.002 and OR=1.19, 95% CI=1.06-1.35, P=0.003, respectively). In the *CYP19A1* gene, the T alleles of rs2414099, rs8025374 and rs3751592 was associated with increased risk of TGCT (OR=1.30, 95% CI=1.10-1.53, P=0.002, OR=1.30, 95% CI=1.09-1.54, P=0.004 and OR=1.21, 95% CI=1.06-1.37, P=0.003, respectively) (Table 2).

In an expanded analysis also including an additional 718 case singletons and 3922 controls, 91 of the 105 above SNPs were explored (Supplementary table 2). Nominal association with TGCT risk was observed among four SNPs in or near the *ESR2* gene, one

SNP in the *CYP3A4* gene, five SNPs in the *CYP19A1* gene, one SNP in the *CYP17A1* gene, one SNP in the *HSD17B3* gene, one SNP in the *INHA* gene, one SNP in the *SRD5A2* gene and one SNP in the *SULT1E1* gene were associated with the risk of TGCT (Table 2). At an FDR < 5% three SNPs in the *ESR2* gene and one SNP in the *CYP3A4* gene remained significantly associated with TGCT risk. In the *ESR2* gene, the G alleles of rs12435857 and rs10146204 were associated with increased risk of TGCT (OR=1.15, 95% CI=1.07-1.24, *P*=0.000 and OR=1.13, 95% CI=1.05-1.22, *P*=0.002, respectively), while the T allele of rs10137185 was associated with reduced risk of TGCT (OR=0.79, 95% CI=0.70-0.90, *P*=0.000). In the *CYP3A4* gene, the T allele of rs2740574 was associated with reduced risk of TGCT (OR=0.74, 95% CI=0.62-0.89, *P*=0.002) (Table 3). Of note, none of the *CYP19A1* SNPs that were significantly associated with TGCT risk in the triad analysis were available for analysis in the combined case-parent/case-control analysis.

When analysing the histological subgroups, we found a reduced risk of developing seminoma rather than non-seminoma in two SNPs (rs1004984 and rs6493497) in the *CYP19A1* gene and one SNP (rs1691053) in the *SRD5A2* gene; however, after controlling for an FDR < 5% no statistically significant associations with histological subgroups remained (data not shown). When analysing offspring TGCT risk in relation to maternal genetic polymorphisms, we found that one SNP (rs2472304) in the *CYP1A2* gene, one SNP (rs2740574) in the *CYP3A4* gene, two SNPs (rs2059693 and rs3731920) in the *INHA* gene, one SNP (rs523349) in the *SRD5A2* gene, and one SNP in the *ESR1* gene had nominally significant different effects on disease risk depending upon whether their alleles were maternally or paternally inherited (data not shown). These associations were, however, no longer statistically significant after controlling for an FDR < 5%.

In addition, we found no statistically significant differences in allelic effect estimates between the Norwegian and the Swedish populations in any of the SNPs examined after controlling for an FDR < 5% (data not shown).

Discussion

The aetiology of TGCT is most probably multifactorial, and there are limited data on the risk factors in both sporadic and familial TGCTs. Exposure to environmental factors, with an emphasis on endocrine disruptors with oestrogenic or anti-androgenic properties resulting in

hormonal disturbances during early foetal life, has been postulated to play a role in the development of TGCT, but there is also evidence for a genetic contribution.

In this study we analysed the possible association between genetic variations in sex hormone pathway genes and the risk of TGCT. The most striking and novel result of the present study is the finding that oestrogen receptor beta (ERβ) may be implicated in the aetiology of TGCT. The main finding was that five genetic variants across the *ESR2* gene (encoding ERβ), were statistically significantly associated with the risk of TGCT in a population of Norwegian and Swedish men. In the case-parent analysis, the T alleles of the rs12434245 and rs10137185 SNPs were associated with a 34% and 28% reduced risk of TGCT, respectively. In addition, the T and G alleles of the rs2978381 and rs12435857 SNPs were associated with a 21% and 19% increased risk of TGCT, respectively. In the combined case-parent/case-control analysis, the G alleles of the rs12435857 and rs10146204 SNPs were associated with a 15% and 13% increased risk of TGCT, respectively, while the T allele of the rs10137185 was associated with a 21% decreased risk of TGCT. These five genetic variants are all located in intronic regions in or near the *ESR2* gene with unknown functional effect and none of them have previously been associated with any cancer disease. However, they are expected to be in linkage disequilibrium (LD) with functional sequence variations in regulatory regions of the *ESR2* gene. Variation in *ESR2* may cause conformational change in the ERβ with consequent alteration in transcriptional activity and downstream cellular events (Thomas and Gustafsson, 2011). ERβ is expressed in germ cells and Sertoli cells in human testis (Saunders *et al.*, 2002, Aschim *et al.*, 2004), and is shown to be down-regulated in seminomas and embryonal cell carcinomas compared to normal testicular cells (Hirvonen-Santti *et al.*, 2003, Pais *et al.*, 2003). ERβ seems to have tumour suppressor properties, based on results in one Esr2-knockout mouse model, and *in vitro* studies in cancer cells, showing that ERβ has anti-proliferative effects (reviewed by (Thomas and Gustafsson, 2011)). ERβ may control and limit cell proliferation during the progression of cancer of the breast, prostate, ovary, and colon (Pasquali *et al.*, 2001, Roger *et al.*, 2001, Weyant *et al.*, 2001, Staibano *et al.*, 2003). If the low-risk associated alleles of the SNPs of the *ESR2* gene in the present study are shown to increase the ERβ activity and *vice versa*, our findings would support the notion of an anti-proliferative activity of ERβ implicated in the aetiology of TGCT.

Our findings of an association between TGCT and polymorphisms in or near the *ESR2* gene are in some accordance with the only previous study which has investigated

355 polymorphisms in ERβ in relation to TGCT risk (Ferlin *et al*., 2010). In Italian men, Ferlin et
356 al. found a weak association with the *ESR2* SNP rs1256049, which is not in LD with any of
357 our risk-associated SNPs, but a limitation to Ferlin et al.'s study was a relatively small sample
358 size. In addition, we did not replicate the findings by Ferlin et al. (Ferlin *et al*., 2008) of a
359 decreased risk of TGCT with two SNPs (rs6165 and rs6166) in the *FSHR* gene in either the
360 case-parent or the combined case-parent/case-control material (Supplementary table 1 and 2).
361 In the combined case-parent/case-control analysis we found a statistically significantly
362 26% decreased risk of TGCT with the T allele of the rs2740574 SNP in *CYP3A4* (table 3).
363 This is in agreement with the findings of Starr et al. (Starr *et al.*, 2005), who found the C
364 allele of this SNP to be associated with increased risk of TGCT in the offspring. In the case-
365 parent material, we found a statistically non-significantly increased risk of TGCT with an
366 intergenic SNP (rs12441817; *P*=0.006, *FDR*=0.054) in the *CYP1A1/CYP1A2* genes, and a
367 similar tendency to decreased risk of TGCT with another intergenic SNP (rs4886605;
368 *P*=0.043, *FDR*=0.214) in the same gene, in regard to offspring carriage. Starr et al. (Starr *et*
369 *al.*, 2005), found that both offspring and maternal carriage of the polymorphic allele of
370 rs762551 in the *CYP1A2* gene was associated with a reduced risk of TGCT. The rs762551
371 SNP is in high LD with the *CYP1A2* rs2472304 SNP which has been associated with
372 increased enzyme activity (Sachse *et al.*, 1999, Nordmark *et al.*, 2002). Thus, both these
373 polymorphisms may lead to higher carcinogenic catechol oestrogen formation. However, we
374 did not find any association between the risk of TGCT and maternal carriage of the G allele of
375 the *CYP1A2* SNP rs2472304. Since we did not replicate the association of TGCT with either
376 offspring or maternal carriage of polymorphisms in the *CYP1A1/CYP1A2, CYP1B1* and
377 *CYP3A4* genes in our large study (Supplementary table 1 and 2), it is a possibility that the
378 results of Starr et al. were chance findings. Our overall lack of association between maternal
379 genetic variation in sex hormones pathway and TGCT risk in the offspring indicates that the
380 most important hormonal alterations implicated in the aetiology of TGCT have their origin in
381 the foetus and not the mother.
382 The production of oestrogens from androgens is mediated by the aromatase, encoded
383 by *CYP19A1*, the aberrant expression of which plays a critical role in the development of
384 malignancy in a number of tissues. The levels of oestrogen within the male reproductive tract
385 are higher than in the general circulation (Hess, 2000). Aromatase is expressed in the adult
386 testis and has been detected in Leydig cells and elongated spermatids in mice and humans
387 (Sierens *et al.*, 2005). Several polymorphisms in the *CYP19A1* gene have been studied and

found associated with hormone dependent cancers such as breast and prostate cancer (Haiman *et al.*, 2007, Cai *et al.*, 2008, Raskin *et al.*, 2009, Darabi *et al.*, 2011), albeit with conflicting results. In the case-parent material, we found that three genetic variants in *CYP19A1* were statistically significantly associated with the risk of TGCT. The T alleles of the rs2414099, rs8025374 and rs3751592 SNPs were associated with a 30%, 30% and 21% increased risk of TGCT, respectively, giving support of aromatase being implicated in the aetiology of TGCT.

Although most epidemiologic studies have shown little variation in risk factors between the two subtypes of TGCT (Bray *et al.*, 2006), hormonal exposures could potentially be modified by genetic variation in hormone metabolizing genes, and thus affect whether a seminoma or nonseminoma develop. There is some evidence suggesting that genetic variation in *CYP1A1* may be associated with histological subtype, the results are however inconsistent (Figueroa *et al.,* 2008, Kristiansen *et al.*, 2011). A recent study indicated that polymorphisms in the *AR* gene are associated with the histological subtypes of TGCT, by reporting 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 CIS to seminoma (Davis-Dao *et al.*, 2011). In the present study we were not able to show an influence of either *CYP1A1/CYP1A2* or *AR*, or any of the other studied sex hormone pathway genes, on the histological subtype, thus not lending support to a role of genetic variation in determining which subtype prevails.

The incidence rate of TGCT has for many years been twice as high in Norway as in Sweden, the reasons for which have remained elusive. The present study was not able to demonstrate any country-related interaction in the associations between the studied SNPs and the risk of TGCT, implying that there was no heterogeneity between the countries related to genetic susceptibility to TGCT. Accordingly, our results do not shed any light on the difference in the incidence rate between these two neighbouring countries.

Strengths of the present study include the population-based design and large sample size providing great power to study genetic risk alleles. A potential limitation is the rather low response rate of about 50% that could have introduced selection bias; however, since the mortality of TGCT is very low (3%) in both countries, the low response rate is not related to survival bias. We applied a combined design by comprehensively assessing a subset of the genes through a haplotype tagging approach while only earlier reported genetic variants were assessed for the remainder of the selected genes. Therefore we may have failed to observe associations between genetic variants and TGCT risk in untagged genes. In the combined

case-parent/case-control analysis population stratification may be an issue since all unrelated control individuals were of Swedish residence. However, since estimated allele effects from case-parent and combined case-parent/case-control analysis were similar, we argue that possible population stratification effects are of minor importance.

In conclusion, our findings provide supportive evidence for several genes in the sex hormone pathway being implicated in the aetiology of TGCT. This applies specifically to the *ESR2* (encoding ERβ) and *CYP19A1* (encoding aromatase), but also to a certain extent to *CYP3A4* and *CYP1A1/CYP1A2*. Although only some of these associations remained significant after controlling for multiple testing, our findings suggest that disturbance of the balance between the levels of oestrogens and androgens play a functional role in the aetiology of TGCT. The lack of association between maternal genetic variation and TGCT risk indicates that the most important hormonal alterations implicated in the aetiology of TGCT have foetal and not maternal origin. Exploring the functional role of the TGCT-risk associated SNPs will further elucidate the biological mechanisms involved.

Authors' roles

All authors have been involved in study design, data interpretation, and preparation and approval of the final manuscript.

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Conflict of interest

None declared.

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Figure legends

Figure 1. Plot of *P*-values for each SNP in the case-parent study with significance thresholds indicated for *P*=0.05, *P*=0.01, and FDR<0.05 (105 SNPs).

Figure 2. Plot of *P*-values for each SNP in the combined case-parent/case-control study with significance thresholds indicated for *P*=0.05, *P*=0.01, and FDR<0.05 (91 SNPs).

Figure 3. LocusZoom plot showing the TGCT associated region of the *ESR2* gene. Association results for SNPs (-log10 *P* value) as a function of genomic distance (NCBI Build 36.1, hg 18). Purple diamonds indicate SNP at each locus with the strongest association evidence. Each circle represents a SNP, with the color of the circle indicating the correlation between that SNP and the most strongly associated SNP (purple diamond). Light blue line indicate estimated recombination rate in HapMap phase II CEU samples.

Tables

Table 1. Characteristics of cases and controls.

	Norwegian	Swedish	Total	Included in the final analysis*
TGCT cases	974	1188	2162	2017
Triads	483	521	1004	831
Dyads (mothers/fathers)	192 (150/42)	248 (178/70)	440 (328/112)	474 (340/134)
Singletons	299	419	718	712
Seminoma	500	672	1135	1103
Non-seminoma or mixed tumour	471	503	935	900
Age at diagnosis (mean)	15-65 (33)	18-45 (32)	15-65	15-65 (32)
Controls (TwinGene)		3922	3922	3922

* After excluding low DNA yield samples, and sample and SNP quality control.

Table 2. Associations of TGCT with SNP markers in sex hormone pathways in the case-parent triad study.

Gene	Gene name	SNP	Allele1*	Allele2	Genomic position	CHR	OR	95 % CI	*P*	FDR q-value[#]
ESR2	Oestrogen receptor beta	rs12434245	C	T	63761606	14	0.66	0.53-0.82	0.0002	**0.018**
		rs10137185	C	T	63845529		0.72	0.59-0.88	0.001	**0.034**
		rs2978381	C	T	63836405		1.21	1.08-1.37	0.002	**0.037**
		rs12435857	A	G	63793278		1.19	1.06-1.35	0.003	**0.043**
		rs10146204	A	G	63888522		1.18	1.04-1.33	0.010	0.078
		rs1273196	A	G	63809258		0.71	0.53-0.94	0.015	0.101
CYP19A1	Cytochrome P450, family 19, subfamily A, polypeptide 1 "Aromatase"	rs2414099	C	T	49336074	15	1.30	1.10-1.53	0.002	**0.038**
		rs8025374	C	T	49305662		1.30	1.09-1.54	0.004	**0.043**
		rs3751592	C	T	49393870		1.21	1.06-1.37	0.003	**0.043**
		rs1004984	A	G	49400821		1.18	1.05-1.34	0.007	0.061
		rs2470144	C	T	49409017		1.16	1.03-1.31	0.013	0.091
CYP1A1/	Cytochrome P450, family 1,	rs12441817	C	T	72812867	15	1.36	1.10-1.70	0.006	0.054
CYP1A2	subfamily A, polypeptide 1/2	rs4886605	C	T	72813041		0.84	0.71-0.99	0.043	0.214
HSD3B1	Hydroxy-delta-5-steroid dehydrogenase	rs6428830	A	G	119856298	1	0.86	0.75-0.99	0.031	0.173
SHBG	Sex hormone-binding globulin	rs2543553	A	C	7479688	17	0.77	0.61-0.99	0.036	0.191
AR	Androgen receptor	rs5919402	C	T	66782221	23	0.80	0.64-0.99	0.038	0.200

* Reference allele.

False discovery rate (FDR) was applied to control for multiple testing.

Table 3. Associations of TGCT with SNP markers in sex hormone pathways in the combined case-parent/case-control study (includes all triads and dyads from the case-parent study in Table 2).

Gene	Gene name	SNP	Allele1 *	Allele2	Genomic position	CHR	OR	95 % CI	*P*	FDR q-value[#]
ESR2	Oestrogen receptor beta	rs12435857	A	G	63793278	14	1.15	1.07-1.24	0.000	**0.011**
		rs10137185	C	T	63845529		0.79	0.70-0.90	0.000	**0.011**
		rs10146204	A	G	63888522		1.13	1.05-1.22	0.002	**0.034**
		rs2978381	C	T	63836405		1.11	1.03-1.19	0.008	0.065
CYP3A4	Cytochrome P450, family 3, subfamily A, polypeptide 4	rs2740574	C	T	99220032	7	0.74	0.62-0.89	0.002	**0.034**
CYP19A1	Cytochrome P450, family 19, subfamily A, polypeptide 1	rs10851498	C	T	49324304	15	0.90	0.84-0.97	0.007	0.064
		rs4646	A	C	49290136		1.12	1.03-1.21	0.009	0.067
	"Aromatase"	rs28757162	A	G	49323177		1.18	1.01-1.36	0.030	0.136
		rs12592697	C	T	49312465		0.92	0.85-0.99	0.033	0.145
		rs12911554	C	T	49330049		1.08	1.00-1.16	0.047	0.169
CYP17A1	Cytochrome P450, family 17, subfamily A, polypeptide 1	rs743572	A	G	104587142	10	0.92	0.86-1.00	0.042	0.161
HSD17B3	Hydroxysteroid (17-beta) dehydrogenase 3	rs8190495	A	G	98101705	9	0.90	0.83-0.97	0.006	0.057
INHA	Inhibin A	rs3731920	C	T	220142289	2	1.19	1.04-1.36	0.011	0.070
SRD5A2	Steroid-5-alpha-reductase	rs2208532	A	G	31642493	2	0.91	0.84-0.98	0.011	0.071
SULT1E1	Sulfotransferase family 1E	rs3775770	C	T	70758859	4	1.09	1.00-1.18	0.046	0.166

* Reference allele.

False discovery rate (FDR) was applied to control for multiple testing.

Supplementary table 1. Case-parent study.

SNP	GENE	CHR	Genomic position	Allele1 reference	Allele2	OR	L95*	U95*	*P*	FDR q-value
rs5919402	*AR*	23	66782221	C	T	0.796	0.64	0.989	0.0383	0.200
rs2361634	*AR*	23	66779568	A	G	0.865	0.647	1.156	0.326	0.512
rs12011518	*AR*	23	66849761	G	T	1.022	0.761	1.374	0.883	0.740
rs4680	*COMT*	22	18331271	A	G	1.032	0.916	1.164	0.601	0.659
rs743572	*CYP17A1*	10	104587142	A	G	0.98	0.869	1.106	0.745	0.706
rs2414099	*CYP19A1*	15	49336074	C	T	1.297	1.1	1.528	0.0019	0.038
rs3751592	*CYP19A1*	15	49393870	C	T	1.205	1.063	1.366	0.00346	0.043
rs8025374	*CYP19A1*	15	49305662	C	T	1.295	1.087	1.542	0.00379	0.043
rs1004984	*CYP19A1*	15	49400821	A	G	1.184	1.048	1.339	0.0068	0.061
rs2470144	*CYP19A1*	15	49409017	C	T	1.161	1.032	1.305	0.0125	0.091
rs17601241	*CYP19A1*	15	49295166	A	G	1.21	1	1.464	0.0508	0.239
rs12591359	*CYP19A1*	15	49326660	A	G	0.895	0.792	1.012	0.0758	0.299
rs12439137	*CYP19A1*	15	49303596	A	G	0.865	0.73	1.023	0.0907	0.326
rs4774585	*CYP19A1*	15	49403772	A	G	1.137	0.97	1.333	0.113	0.359
rs1902584	*CYP19A1*	15	49398946	A	T	0.847	0.677	1.059	0.146	0.396
rs2445762	*CYP19A1*	15	49405000	C	T	1.099	0.964	1.254	0.159	0.408

rs17523880	*CYP19A1*	15	49379835	A	C	1.131	0.952	1.343	0.163	0.411
rs7172156	*CYP19A1*	15	49333590	A	G	1.081	0.954	1.224	0.222	0.450
rs6493497	*CYP19A1*	15	49418127	A	G	1.106	0.922	1.327	0.279	0.479
rs12592697	*CYP19A1*	15	49312465	C	T	1.069	0.944	1.211	0.29	0.484
rs10519295	*CYP19A1*	15	49319939	C	T	0.9	0.739	1.096	0.293	0.485
rs2008691	*CYP19A1*	15	49335602	A	G	1.082	0.923	1.267	0.331	0.516
rs10851498	*CYP19A1*	15	49324304	C	T	0.954	0.848	1.074	0.434	0.583
rs4646	*CYP19A1*	15	49290136	A	C	1.044	0.917	1.189	0.517	0.625
rs10459592	*CYP19A1*	15	49323433	G	T	1.038	0.919	1.171	0.55	0.639
rs2899472	*CYP19A1*	15	49303347	A	C	0.965	0.843	1.103	0.599	0.658
rs749292	*CYP19A1*	15	49346023	A	G	0.973	0.865	1.095	0.649	0.676
rs2470151	*CYP19A1*	15	49394361	C	T	0.976	0.848	1.122	0.731	0.702
rs1458928	*CYP19A1*	15	49432291	G	T	1.026	0.884	1.19	0.739	0.704
rs7174997	*CYP19A1*	15	49409420	G	T	1.022	0.874	1.196	0.783	0.716
rs17602308	*CYP19A1*	15	49394257	C	T	1.026	0.802	1.312	0.84	0.730
rs3751591	*CYP19A1*	15	49394002	A	G	1.015	0.861	1.197	0.859	0.734
rs28757162	*CYP19A1*	15	49323177	A	G	1.021	0.809	1.289	0.861	0.735
rs2255192	*CYP19A1*	15	49288127	C	T	0.987	0.849	1.147	0.863	0.735
rs12911554	*CYP19A1*	15	49330049	C	T	0.99	0.881	1.113	0.867	0.736

rs8029807	*CYP19A1*	15	49359329	A	G	0.992	0.823	1.197	0.936	0.751
rs17601842	*CYP19A1*	15	49338422	A	G	1.004	0.768	1.313	0.978	0.759
rs12441817	*CYP1A1/CYP1A2*	15	72812867	C	T	1.364	1.095	1.699	0.00555	0.054
rs4886605	*CYP1A1/CYP1A2*	15	72813041	C	T	0.838	0.706	0.994	0.0427	0.214
rs2472304	*CYP1A1/CYP1A2*	15	72831291	A	G	0.928	0.819	1.051	0.238	0.459
rs2470893	*CYP1A1/CYP1A2*	15	72806502	C	T	1.04	0.916	1.182	0.544	0.636
rs10012	*CYP1B1*	2	38155894	C	G	0.944	0.83	1.074	0.382	0.551
rs1056836	*CYP1B1*	2	38151707	C	G	1.003	0.887	1.134	0.964	0.756
rs2740574	*CYP3A4*	7	99220032	C	T	0.839	0.631	1.116	0.226	0.453
rs4646457	*CYP3A5*	7	99083016	A	C	1.183	0.96	1.458	0.113	0.359
rs776745	*CYP3A5*	7	99129273	G	T	0.865	0.71	1.053	0.146	0.396
rs28365094	*CYP3A5*	7	99088411	C	T	1.031	0.857	1.241	0.743	0.705
rs2687145	*CYP3A7*	7	99156580	A	G	0.853	0.702	1.037	0.109	0.354
rs2687134	*CYP3A7*	7	99168978	G	T	1.154	0.932	1.428	0.189	0.431
rs2014764	*CYP3A7*	7	99185442	C	T	1.005	0.854	1.183	0.951	0.754
rs2234693	*ESR1*	6	152205028	C	T	1.112	0.988	1.252	0.0792	0.305
rs722208	*ESR1*	6	152364578	A	G	1.035	0.909	1.179	0.601	0.659
rs1801132	*ESR1*	6	152307215	C	G	0.996	0.861	1.152	0.956	0.755
rs12434245	*ESR2/SYNE2*	14	63761606	C	T	0.66	0.531	0.821	0.000169	0.014

rs10137185	*ESR2*	14	63845529	C	T	0.72	0.59	0.879	0.00119	0.034
rs2978381	*ESR2*	14	63836405	C	T	1.214	1.075	1.37	0.00169	0.037
rs12435857	*ESR2*	14	63793278	A	G	1.194	1.06	1.345	0.00343	0.043
rs10146204	*ESR2*	14	63888522	A	G	1.175	1.04	1.329	0.00976	0.078
rs1273196	*ESR2*	14	63809258	A	G	0.705	0.531	0.935	0.0152	0.101
rs2987983	*ESR2*	14	63833406	A	G	0.884	0.776	1.008	0.065	0.275
rs8006145	*ESR2*	14	63769203	A	C	1.123	0.985	1.281	0.0838	0.314
rs17766755	*ESR2*	14	63785526	A	G	1.086	0.961	1.227	0.185	0.428
rs3020443	*ESR2*	14	63862093	G	T	1.094	0.957	1.252	0.19	0.431
rs1256064	*ESR2*	14	63770492	A	G	0.874	0.708	1.08	0.212	0.445
rs3783736	*ESR2*	14	63821125	G	T	0.927	0.823	1.045	0.215	0.447
rs10144225	*ESR2*	14	63774747	A	G	1.068	0.828	1.379	0.612	0.663
rs1887994	*ESR2*	14	63830364	A	C	0.964	0.794	1.17	0.708	0.695
rs8020646	*ESR2*	14	63761073	A	G	1.017	0.763	1.355	0.908	0.745
rs6165	*FSHR*	2	49044545	C	T	1.083	0.96	1.223	0.194	0.434
rs6166	*FSHR*	2	49043425	C	T	1.059	0.942	1.191	0.338	0.521
rs1138272	*GSTP1*	11	67110155	C	T	0.883	0.71	1.099	0.267	0.474
rs1695	*GSTP1*	11	67109265	A	G	0.959	0.846	1.088	0.518	0.625
rs6591256	*GSTP1*	11	67106475	A	G	0.985	0.871	1.114	0.807	0.722

rs688878	*GSTP1*	11	67096525	A	G	1.025	0.835	1.258	0.817	0.724
rs2066479	*HSD17B3*	9	98037631	C	T	0.887	0.663	1.186	0.418	0.573
rs2026002	*HSD17B3*	9	98124002	A	G	1.048	0.869	1.265	0.622	0.667
rs8190495	*HSD17B3*	9	98101705	A	G	0.995	0.881	1.125	0.94	0.751
rs6428830	*HSD3B1*	1	119856298	A	G	0.86	0.75	0.987	0.0308	0.173
rs3765945	*HSD3B1*	1	119852969	A	G	1.105	0.971	1.257	0.129	0.379
rs1538989	*HSD3B2*	1	119791376	A	G	0.934	0.827	1.055	0.271	0.476
rs907141	*INHA*	2	220137490	C	G	0.923	0.816	1.045	0.204	0.440
rs2059693	*INHA*	2	220150734	C	T	1.063	0.932	1.212	0.362	0.538
rs1039900	*INHA*	2	220132731	A	G	0.98	0.872	1.103	0.741	0.704
rs3731920	*INHA*	2	220142289	C	T	1.024	0.839	1.25	0.814	0.724
rs6729914	*INHA*	2	220151760	C	T	0.994	0.883	1.119	0.921	0.748
rs2543553	*SHBG*	17	7479688	A	C	0.772	0.606	0.985	0.0356	0.191
rs9913778	*SHBG*	17	7474626	C	T	0.947	0.765	1.172	0.616	0.665
rs858520	*SHBG*	17	7470996	C	T	1.021	0.904	1.153	0.739	0.704
rs6259	*SHBG*	17	7477252	A	G	1.027	0.855	1.234	0.777	0.714
rs1641544	*SHBG*	17	7480591	C	T	1.036	0.793	1.355	0.794	0.719
rs1799941	*SHBG*	17	7474148	A	G	1.01	0.886	1.151	0.883	0.740
rs9898876	*SHBG*	17	7467687	G	T	1.009	0.865	1.176	0.911	0.746

rs2955617	*SHBG*	17	7479510	A	C	1	0.883	1.133	0.999	0.763
rs523349	*SRD5A2*	2	31659210	C	G	0.929	0.818	1.055	0.258	0.470
rs2208532	*SRD5A2*	2	31642493	A	G	0.962	0.853	1.084	0.52	0.626
rs9282858	*SRD5A2*	2	31659330	C	T	1.046	0.698	1.567	0.827	0.727
rs1691053	*SRD5A2*	5	6730165	C	T	1.014	0.843	1.219	0.885	0.740
rs3775775	*SULT1E1*	4	70752871	A	G	1.171	0.922	1.489	0.194	0.434
rs3775779	*SULT1E1*	4	70743796	A	T	1.074	0.95	1.215	0.255	0.468
rs1238574	*SULT1E1*	4	70743612	C	T	0.866	0.664	1.129	0.285	0.482
rs1881668	*SULT1E1*	4	70760045	C	G	1.069	0.933	1.224	0.336	0.519
rs11573763	*SULT1E1*	4	70753626	A	G	1.073	0.84	1.37	0.573	0.648
rs1220726	*SULT1E1*	4	70738795	C	T	0.954	0.803	1.132	0.588	0.654
rs3775770	*SULT1E1*	4	70758859	C	T	0.98	0.86	1.118	0.765	0.711
rs1590128	*SULT1E1*	4	70768648	A	G	0.989	0.871	1.122	0.858	0.734

* L95 and U95 refer to the lower and upper range, respectively, of the 95% CI.

Supplementary table 2. Combined case-parent/case-control study.

SNP	GENE	CHR	Genomic position	Allele1 reference	Allele2	OR	L95*	U95*	*P*	FDR q-value
rs4680	*COMT*	22	18331271	A	G	1.023	0.95	1.101	0.556	0.597
rs743572	*CYP17A1*	10	104587142	A	G	0.924	0.856	0.997	0.0422	0.161
rs10851498	*CYP19A1*	15	49324304	C	T	0.904	0.839	0.973	0.0074	0.064
rs4646	*CYP19A1*	15	49290136	A	C	1.115	1.027	1.21	0.00899	0.067
rs28757162	*CYP19A1*	15	49323177	A	G	1.175	1.014	1.36	0.0297	0.136
rs12592697	*CYP19A1*	15	49312465	C	T	0.918	0.849	0.994	0.0334	0.145
rs12911554	*CYP19A1*	15	49330049	C	T	1.078	1.001	1.161	0.0474	0.169
rs8025374	*CYP19A1*	15	49305662	C	T	1.111	0.994	1.242	0.0629	0.188
rs7172156	*CYP19A1*	15	49333590	A	G	1.074	0.994	1.16	0.0698	0.195
rs12591359	*CYP19A1*	15	49326660	A	G	1.072	0.994	1.157	0.071	0.196
rs17601241	*CYP19A1*	15	49295166	A	G	1.115	0.986	1.262	0.0807	0.205
rs2255192	*CYP19A1*	15	49288127	C	T	0.919	0.835	1.012	0.0835	0.207
rs749292	*CYP19A1*	15	49346023	A	G	0.937	0.87	1.009	0.0849	0.208
rs8029807	*CYP19A1*	15	49359329	A	G	1.102	0.979	1.241	0.107	0.239
rs10459592	*CYP19A1*	15	49323433	G	T	0.946	0.877	1.02	0.145	0.282
rs2414099	*CYP19A1*	15	49336074	C	T	1.069	0.963	1.186	0.207	0.355

rs4774585	*CYP19A1*	15	49403772	A	G	1.063	0.965	1.171	0.212	0.361
rs1902584	*CYP19A1*	15	49398946	A	T	1.077	0.927	1.252	0.335	0.471
rs3751591	*CYP19A1*	15	49394002	A	G	1.051	0.949	1.164	0.339	0.474
rs1004984	*CYP19A1*	15	49400821	A	G	0.971	0.898	1.049	0.452	0.546
rs2470144	*CYP19A1*	15	49409017	C	T	1.029	0.955	1.108	0.459	0.550
rs6493497	*CYP19A1*	15	49418127	A	G	1.035	0.917	1.167	0.575	0.605
rs7174997	*CYP19A1*	15	49409420	G	T	0.974	0.882	1.076	0.603	0.616
rs2445762	*CYP19A1*	15	49405000	C	T	0.984	0.906	1.069	0.699	0.650
rs3751592	*CYP19A1*	15	49393870	C	T	1.015	0.938	1.098	0.716	0.656
rs2008691	*CYP19A1*	15	49335602	A	G	0.986	0.893	1.088	0.776	0.674
rs1458928	*CYP19A1*	15	49432291	G	T	0.997	0.905	1.097	0.947	0.716
rs2470151	*CYP19A1*	15	49394361	C	T	0.998	0.913	1.089	0.957	0.718
rs4886605	*CYP1A1/CYP1A2*	15	72813041	C	T	0.902	0.808	1.007	0.065	0.190
rs12441817	*CYP1A1/CYP1A2*	15	72812867	C	T	1.102	0.956	1.27	0.178	0.323
rs2472304	*CYP1A1/CYP1A2*	15	72831291	A	G	1.042	0.965	1.126	0.295	0.440
rs2470893	*CYP1A1/CYP1A2*	15	72806502	C	T	0.997	0.921	1.078	0.931	0.712
rs1056836	*CYP1B1*	2	38151707	C	G	1.011	0.938	1.09	0.77	0.672
rs2740574	*CYP3A4*	7	99220032	C	T	0.742	0.615	0.894	0.00207	0.034
rs28365094	*CYP3A5*	7	99088411	C	T	1.104	0.982	1.242	0.0963	0.225

rs776745	*CYP3A5*	7	99129273	G	T	0.962	0.849	1.091	0.551	0.594
rs4646457	*CYP3A5*	7	99083016	A	C	1.036	0.908	1.182	0.597	0.614
rs2687145	*CYP3A7*	7	99156580	A	G	0.948	0.837	1.074	0.405	0.519
rs2687134	*CYP3A7*	7	99168978	G	T	1.022	0.891	1.172	0.76	0.669
rs1801132	*ESR1*	6	152307215	C	G	1.051	0.961	1.15	0.28	0.427
rs722208	*ESR1*	6	152364578	A	G	1.03	0.95	1.117	0.472	0.557
rs2234693	*ESR1*	6	152205028	C	T	1.022	0.949	1.101	0.56	0.598
rs12435857	*ESR2*	14	63793278	A	G	1.148	1.065	1.236	0.000282	0.011
rs10137185	*ESR2*	14	63845529	C	T	0.794	0.699	0.903	0.000338	0.011
rs10146204	*ESR2*	14	63888522	A	G	1.129	1.045	1.219	0.002	0.034
rs2978381	*ESR2*	14	63836405	C	T	1.107	1.027	1.194	0.00797	0.065
rs17766755	*ESR2*	14	63785526	A	G	1.078	0.997	1.165	0.0586	0.184
rs1273196	*ESR2*	14	63809258	A	G	0.847	0.711	1.01	0.0614	0.187
rs2987983	*ESR2*	14	63833406	A	G	0.94	0.867	1.019	0.131	0.268
rs3783736	*ESR2*	14	63821125	G	T	0.952	0.883	1.027	0.203	0.351
rs8006145	*ESR2*	14	63769203	A	C	1.055	0.971	1.146	0.207	0.355
rs3020443	*ESR2*	14	63862093	G	T	1.043	0.959	1.136	0.326	0.464
rs1887994	*ESR2*	14	63830364	A	C	1.051	0.93	1.188	0.425	0.531
rs10144225	*ESR2*	14	63774747	A	G	1.051	0.903	1.223	0.524	0.582

rs1256064	*ESR2*	14	63770492	A	G	0.965	0.846	1.1	0.593	0.612
rs8020646	*ESR2*	14	63761073	A	G	1.037	0.871	1.234	0.682	0.645
rs6165	*FSHR*	2	49044545	C	T	1.007	0.934	1.085	0.859	0.696
rs6166	*FSHR*	2	49043425	C	T	0.997	0.926	1.074	0.938	0.714
rs688878	*GSTP1*	11	67096525	A	G	0.905	0.797	1.028	0.122	0.258
rs1695	*GSTP1*	11	67109265	A	G	1.022	0.945	1.105	0.592	0.612
rs6591256	*GSTP1*	11	67106475	A	G	1.015	0.942	1.094	0.688	0.647
rs8190495	*HSD17B3*	9	98101705	A	G	0.898	0.832	0.969	0.00555	0.057
rs2026002	*HSD17B3*	9	98124002	A	G	0.958	0.851	1.078	0.473	0.557
rs2066479	*HSD17B3*	9	98037631	C	T	0.975	0.812	1.17	0.782	0.675
rs3765945	*HSD3B1*	1	119852969	A	G	1.061	0.981	1.147	0.14	0.277
rs6428830	*HSD3B1*	1	119856298	A	G	0.964	0.887	1.048	0.39	0.509
rs1538989	*HSD3B2*	1	119791376	A	G	0.981	0.909	1.057	0.61	0.619
rs3731920	*INHA*	2	220142289	C	T	1.189	1.043	1.357	0.0105	0.070
rs6729914	*INHA*	2	220151760	C	T	1.043	0.968	1.123	0.271	0.419
rs1039900	*INHA*	2	220132731	A	G	0.984	0.913	1.06	0.664	0.639
rs2059693	*INHA*	2	220150734	C	T	1.007	0.928	1.094	0.86	0.696
rs6259	*SHBG*	17	7477252	A	G	1.116	0.994	1.252	0.0607	0.186
rs9898876	*SHBG*	17	7467687	G	T	0.935	0.851	1.027	0.157	0.297

rs2543553	*SHBG*	17	7479688	A	C	0.915	0.791	1.06	0.239	0.389
rs858520	*SHBG*	17	7470996	C	T	1.026	0.951	1.107	0.507	0.574
rs1641544	*SHBG*	17	7480591	C	T	1.044	0.881	1.238	0.619	0.622
rs9913778	*SHBG*	17	7474626	C	T	0.97	0.848	1.111	0.663	0.638
rs2955617	*SHBG*	17	7479510	A	C	0.989	0.915	1.069	0.782	0.675
rs1799941	*SHBG*	17	7474148	A	G	0.999	0.919	1.086	0.98	0.723
rs2208532	*SRD5A2*	2	31642493	A	G	0.908	0.843	0.978	0.0107	0.071
rs523349	*SRD5A2*	2	31659210	C	G	0.941	0.869	1.018	0.13	0.267
rs9282858	*SRD5A2*	2	31659330	C	T	1.072	0.828	1.389	0.598	0.614
rs1691053	*SRD5A2*	5	6730165	C	T	0.977	0.874	1.092	0.679	0.644
rs3775770	*SULT1E1*	4	70758859	C	T	1.088	1.002	1.182	0.0455	0.166
rs1881668	*SULT1E1*	4	70760045	C	G	0.921	0.847	1.002	0.0542	0.178
rs3775779	*SULT1E1*	4	70743796	A	T	1.079	0.998	1.166	0.0552	0.180
rs11573763	*SULT1E1*	4	70753626	A	G	1.071	0.915	1.253	0.389	0.509
rs1590128	*SULT1E1*	4	70768648	A	G	0.969	0.896	1.047	0.418	0.527
rs1220726	*SULT1E1*	4	70738795	C	T	0.958	0.86	1.068	0.442	0.540
rs1238574	*SULT1E1*	4	70743612	C	T	0.963	0.82	1.13	0.642	0.631
rs3775775	*SULT1E1*	4	70752871	A	G	1	0.866	1.156	0.995	0.726

* L95 and U95 refer to the lower and upper range, respectively, of the 95% CI.