Human Reproduction, Vol.28, No.7 pp. 1995–2002, 2013
Advanced Access publication on May 2, 2013 doi:10.1093/humrep/det127

human reproduction

ORIGINAL ARTICLE ***Reproductive genetics***

Genetic variation in *AKT1*, *PTEN* and the 8q24 locus, and the risk of testicular germ cell tumor

K.E. Andreassen[1,†*], W. Kristiansen[2,†], R. Karlsson[3], E.L. Aschim[2], O. Dahl[4,5], S.D. Fosså[6,7], H.-O. Adami[3,8], F. Wiklund[3], T.B. Haugen[2,‡], and T. Grotmol[9,‡]

[1]Department of Oncology, Oslo University Hospital, P.O. Box 4950, Nydalen, Oslo NO-0434, Norway [2]Faculty of Health Sciences, Oslo and Akershus University College of Applied Sciences, Oslo NO-0130, Norway [3]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm SE-17177, Sweden [4]Section of Oncology, Institute of Medicine, University of Bergen, Bergen NO-5020, Norway [5]Department of Oncology, Haukeland University Hospital, Bergen NO-5021, Norway [6]Department of Clinical Cancer Research, Radium Hospital, Oslo University Hospital, Oslo NO-0424, Norway [7]Faculty of Medicine, University of Oslo, Oslo NO-0316, Norway [8]Department of Epidemiology, Harvard School of Public Health, Boston, MA 02115, USA [9]Department of Research, Cancer Registry of Norway, Oslo NO-0304, Norway

*Correspondence address. Tel: +47-91-62-25-30; Fax: +47-23-02-68-31; E-mail: kristine.engen.andreassen@ous-hf.no

Submitted on October 24, 2012; resubmitted on February 19, 2013; accepted on March 22, 2013

STUDY QUESTION: Is there an association between testicular germ cell tumor (TGCT) and genetic polymorphisms in *AKT1*, *PTEN* and the 8q24 locus?

SUMMARY ANSWER: Our findings suggest that genetic variation in *PTEN* may influence the risk of TGCT.

WHAT IS KNOWN ALREADY: There is strong evidence that genetic variation influences the risk of TGCT. The oncogene, *AKT1*, the tumor suppressor gene, *PTEN* and the chromosome 8q24 locus play important roles in cancer development in general.

STUDY DESIGN, SIZE, DURATION: We have conducted a population-based Norwegian-Swedish case–parent study, based on cases diagnosed in 1990–2008, including 831 triads (TGCT case and both parents), 474 dyads (TGCT case and one parent) and 712 singletons (only the TGCT case). In addition we expanded the study to include 3922 unrelated male controls from the TwinGene project.

PARTICIPANTS/MATERIALS, SETTING, METHODS: We genotyped 26 single nucleotide polymorphisms (SNPs) in *AKT1*, *PTEN* and the 8q24 locus. First, triads and dyads were included in a likelihood-based association test. To increase the statistical power, case singletons and controls from the TwinGene project were included in a single test for association. We examined if the allelic effect on TGCT risk differed by histological subgroup, country of origin or parent of origin. Odds ratios (ORs) and 95% confidence intervals (CI) were calculated with Bonferroni correction (P_{bonf}) for multiple testing.

MAIN RESULTS AND THE ROLE OF CHANCE: In the case–parent analyses, none of the 26 SNPs were significantly associated with TGCT. Of the 23 SNPs investigated in the combined study, one SNP in *PTEN* (rs11202586) remained associated with TGCT risk after adjusting for multiple testing (OR = 1.16, 95% CI = 1.06–1.28, P_{bonf} = 0.040). We found no difference in risk according to histological subgroup, parent of origin or between countries.

LIMITATIONS, REASONS FOR CAUTION: Our study is strengthened by the population-based design and large sample size, which gives high power to detect risk alleles. The reported association was not highly significant, and although it was based on an *a priori* hypothesis of this tumor suppressor gene being implicated in the etiology of TGCT, replication studies, as well as functional studies of this polymorphism, are warranted.

WIDER IMPLICATIONS OF THE FINDINGS: We report, to our knowledge, a novel association between TGCT and a marker in the tumor suppressor gene *PTEN*. Previous studies have linked PTEN to TGCT etiology, and there is also a link between *PTEN* and *KITLG*, which contains TGCT susceptibility loci revealed through recent genome-wide studies.

† Indicates shared first authorship.

‡ Indicates shared last authorship.

© The Author 2013. Published by Oxford University Press on behalf of the European Society of Human Reproduction and Embryology. All rights reserved.
For Permissions, please email: journals.permissions@oup.com

Downloaded from https://academic.oup.com/humrep/article/28/7/1995/613969 by U.S. Department of Justice user on 16 August 2022

STUDY FUNDING/COMPETING INTEREST(S): This work was financially supported by the Norwegian Cancer Society (418975) and the Nordic Cancer Union (S-12/07). No competing interests are declared.

Key words: testicular cancer / single nucleotide polymorphism / Pten / Akt1 / 8q24

Introduction

Testicular germ cell tumors (TGCTs) are believed to originate from transformed primordial germ cells (PGCs) during early embryonic development (Hoei-Hansen *et al.*, 2005; Rajpert-De Meyts and Hoei-Hansen, 2007). These tumors are relatively rare, but the incidence has increased several folds during the last decades in most western countries (Huyghe *et al.*, 2003; Huyghe *et al.*, 2007; Chia *et al.*, 2010). This rapid increase in incidence suggests an important role of environmental factors in the development of TGCT. However, the increased risk of TGCT among brothers and sons of affected men (Heimdal *et al.*, 1996; Hemminki and Li, 2004) and especially among men with an affected monozygotic twin-brother (Swerdlow *et al.*, 1997), points towards a strong genetic component. Several recent studies have explored the impact of single nucleotide polymorphisms (SNPs) on TGCT susceptibility. Three genome-wide association studies (GWASs) have revealed six genes associated with TGCT risk (Kanetsky *et al.*, 2009; Rapley *et al.*, 2009; Turnbull *et al.*, 2010): KIT ligand (*KITLG*), sprouty-4 (*SPRY4*), BCL-2 antagonist/killer 1 (*BAK1*), telomerase reverse transcriptase (*TERT*), activating transcription factor 7 interacting protein (*ATF7IP*) and double sex and mab-3-related transcription factor 1 (*DMRT1*). Because these genes are involved in the regulation of PGC development, telomerase activity and sex-determination, a causal role in the development of TGCT is biologically plausible.

The strongest reported GWAS associations for TGCT to date were those in *KITLG*, encoding the ligand to the receptor tyrosine kinase KIT, with reported allelic odds ratios (ORs) >2 (Kanetsky *et al.*, 2009; Rapley *et al.*, 2009). KIT signaling activates the downstream phosphoinositide 3-kinase (PI3K)-AKT pathway, which is important in cell proliferation and apoptosis (Osaki *et al.*, 2004). *PTEN* is a tumor-suppressor gene that through its protein product, phosphatase, negatively regulates the PI3K-AKT pathway (Rottapel *et al.*, 1991; Osaki *et al.*, 2004). *PTEN* is among the most commonly mutated genes in human cancer, and somatic mutations in this gene have been identified in several tumor forms, such as glioblastoma, endometrial carcinoma and prostate cancer (Cairns *et al.*, 1997; Li *et al.*, 1997; Steck *et al.*, 1997; Mutter *et al.*, 2000). There have also been a few case reports of TGCTs in patients with Cowden disease. In 2004, Mazereeuw-Hautier *et al.* (2004) reported a case of testicular seminoma in a 42-year-old man with Cowden disease. In 2007, Devi *et al.* reported a mixed germ cell tumor in a 17-year-old man with Cowden disease. Previous studies have investigated changes in the expression of *PTEN* and the oncogene *AKT1* in TGCT (Kimura *et al.*, 2003; Di Vizio *et al.*, 2005; Nakai *et al.*, 2005; Hennenlotter *et al.*, 2011), but to our knowledge, no candidate-gene studies have addressed the association between inherited genetic polymorphisms in these genes and TGCT risk.

The chromosome 8q24 locus has been linked particularly to cancer of the prostate, colon and breast (Ghoussaini *et al.*, 2008; Wokolorczyk *et al.*, 2008). In 2008, Cook *et al.* examined 15 specific SNPs in the 8q24 locus; they found no association with TGCT, but noted that an association might have been missed.

Our aim was to examine the association between TGCT and genetic polymorphisms in *AKT1*, *PTEN* and in the 8q24 locus. We also wanted to compare the results according to histology (seminoma/non-seminoma) and country of origin (Norway and Sweden). In addition, our study design allowed us to examine whether any allelic effect was modified by the gender of the parent from whom the allele was transmitted (parent of origin effect). In order to address these aims, we conducted a combined nation-wide case–parent/case–control study in Norway and Sweden.

Materials and Methods

Norwegian TGCT patients

All patients diagnosed with a histologically verified TGCT (patients with spermatocytic seminoma were excluded) in the period 1990–2008 and reported to the Cancer Registry of Norway were identified. Of these 4354 men, 4222 (97%) were alive and eligible for the study. From the group of eligible patients, 1855 men were randomly selected and invited to participate in the study; of these, 974 (53%) consented (Table I). There was no difference in tumor stage when comparing those identified in the Cancer Registry with those invited to participate in the study (23 versus 22.4% had metastatic disease). The diagnosis was verified and the invitation was approved by the treating physician at the regional oncology division.

Swedish TGCT patients

Patients diagnosed with TGCT (patients with spermatocytic seminoma were excluded) between 1995 and 2006 and registered in the Swedish National Cancer Registry were identified. The diagnosis was verified by record linkage with the Swedish National Inpatient Register. In total, 2443 men were identified and 2373 (97%) of them were alive and eligible for the study. From the group of eligible patients, 2327 were randomly selected and invited to participate in the study, out of whom 1188 (51%) consented (Table I).

Men consenting to the study were slightly older at time of diagnosis compared with those who did not consent (32.4 versus 31.1 years). There was no difference in tumor stage (22.4 versus 22.3% with metastatic disease) or lag time between diagnosis and study recruitment (8.5 years for both groups) between those consenting and those not consenting.

Enrollment

During the period September 2008 through September 2010, all eligible cases received an invitation to participate in the study. The mailing included an information letter, an informed consent form and an Oragene DNA-self collection kit. If they consented, the participants were asked to provide saliva into the container (2 ml) according to the manufacturer's instruction and return it to the Cancer Registry of Norway (for Norwegian participants) and to the Karolinska Institutet (for Swedish participants) along with the signed consent form. In the information letter, the participants were asked for approval to contact their parents and, if they gave their consent, to write down contact information. The parents were then invited to participate in the study and asked to provide a saliva sample as described above.

Downloaded from https://academic.oup.com/humrep/article/28/7/1995/613969 by U.S. Department of Justice user on 16 August 2022

Table I Characteristics of cases and controls.

	Norwegian	Swedish	Total	Included in the final analysis[a]
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	499	672	1171	1103
Non-seminoma	467	503	970	900
Age at diagnosis (mean)	15–65 (33)	18–45 (32)	15–65 (32)	15–65 (32)
Unrelated controls (TwinGene)		3922	3922	3922

[a]After excluding samples with low DNA yield or not approved in the quality control.

Ethical approval

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, Norway.

Control group

A population-based Swedish study conducted in 2004–2008 (TwinGene project), included blood samples and questionnaires from 12 591 twins born in the period 1911–1958. All participants had given informed consent, and the study was approved by the local ethics committee at Karolinska Institute (Rahman *et al.*, 2009). From this population, 3922 unrelated men were randomly selected as a control group for the present study. For these individuals, DNA was already extracted and genome-wide genotyping with Illumina OmniExpress bead chip had been performed.

Selection of SNPs

Regarding *AKT1*, SNPs were selected for genotyping if the minor allele frequency (MAF) was >5% and had at least 90% genotyping success rate in HapMap2 CEU individuals. Haplotype block structure, based on confidence bounds of D prime values, was inferred using data from the catalog of common genetic variants generated from the International HapMap Project (The International HapMap Consortium, 2003). Within each haplotype block, haplotype tagging SNPs (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 gene.

For *PTEN* and the 8q24 locus, SNPs were selected based on previously published evidence of an association with TGCT or other relevant cancers.

In total, 31 SNPs were selected, and 26 of those were successfully genotyped and passed quality control.

Genotyping

Saliva was collected using the Oragene® DNA sample collection kit (DNA Genotek, Inc., Kanata, ON, Canada). 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). Cases with a DNA yield <25 μg were excluded from the study (35 Norwegian and 79 Swedish cases). Genotyping was performed using the Sequenom MassARRAY® iPLEX Gold platform (Sequenom, Inc., San Diego, CA, USA) at the Center for Integrative Genetics (CIGENE), Norwegian University of Life Sciences, Ås, Norway. The Sequenom assay uses a locus-specific PCR amplification, followed by a primer extension reaction where the mass-modified dideoxynucleotide terminators of the primer anneals immediately upstream of the polymorphic site. Using matrix-assisted laser desorption ionization time-of-flight mass spectrometry, the distinct mass of the extended primer differentiates between the SNP alleles (Gabriel *et al.*, 2009).

Statistical analysis

The statistical methods used in this study have been described in detail previously (Kristiansen *et al.*, 2012), and an abridged version is presented here.

Sample and SNP quality control

Samples with >20% missing genotypes or a heterozygosity rate more than three standard deviations from the sample mean (indicating possible sample contamination) were excluded ($n = 277$). To confirm parent–offspring relations, pairwise genotype identity-by-state (IBS) was examined. If the standard deviation of the number of alleles per SNP shared IBS exceeded 0.55, indicating that the parent is not biological, the parent was excluded ($n = 41$). After these steps in the quality control, 21 remaining Mendelian errors were resolved by setting the offending genotypes to missing. Furthermore, 34 samples identified as duplicates were excluded. If the proband was lost to quality control, samples from the parents were excluded from the final analysis ($n = 111$). SNPs with >10% missing genotypes or an MAF of <0.01 were also excluded from further analysis ($n = 3$). Two more SNPs were removed because they were considered 'problematic' by CIGENE. After completing quality control, 831 triads, 474 dyads and 712 case singletons were included in the final analysis (Table I).

Imputation and quality control of TwinGene control samples

In the TwinGene controls, we used full-genome imputed data to increase the number of SNPs available. Imputation was performed using IMPUTE2 (Howie *et al.*, 2009), and CEU reference haplotypes from the HapMap project, release 22. We extracted TwinGene genotypes for all SNPs that passed quality control in the case–parent sample, and were either directly genotyped or imputed in the TwinGene dataset. The same SNP quality control measures as for the cases were then applied to the control genotypes.

Downloaded from https://academic.oup.com/humrep/article/28/7/1995/613969 by U.S. Department of Justice user on 16 August 2022

Association analysis for the case–parent population

We used a likelihood-based association test for nuclear families and unrelated subjects with missing data in the main analysis, using the software package UNPHASED (Dudbridge, 2008), This test is robust to population stratification. Initially, only complete case–parent triads and dyads were included in the analysis. The assumption of an allelic main effect led to a 1-df likelihood-ratio test. Further, we included interaction terms in the model to examine if the allelic effect on TGCT risk differed by histological subgroups (seminoma and non-seminoma), country of origin (Norway or Sweden) or parent of origin.

Association analysis for the combined case–parent/case–control population

To increase the power to detect associations, we included case singletons and controls from the TwinGene project in the analysis. A single test for association was performed, including triads, dyads and case–control samples. Both family-based controls and unrelated controls were included in an unmatched analysis.

To account for multiple comparisons, *P*-values were adjusted using the Bonferroni correction (P_{bonf}). *P*-values < 0.05 were considered statistically significant.

Results

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

Case–parent study

In the case–parent part of the study, including 831 triads and 474 dyads, we studied a total of 26 SNPs in the three different genes. None of these SNPs were significantly associated with TGCT (Table II).

Combined case–parent/case–control study

Three of the *AKT1* SNPs were not genotyped or successfully imputed in the TwinGene control group, and could not be included in the combined case–parent/case–control study. Therefore, in the expanded study that included 718 singletons and 3922 controls in addition to the persons from the case–parent study, we only investigated 23 SNPs. Of these, three SNPs appeared associated with TGCT risk: one SNP in the 8q24 locus (rs7008482) and two SNPs in *PTEN* (rs11202586 and rs1234220).

In the 8q24 locus, the T allele of rs7008482 was associated with reduced risk of TGCT [OR = 0.91, 95% confidence interval (CI) = 0.84–0.99, $P = 0.029$]. In *PTEN*, both the T allele of rs11202586 and the G allele of rs1234220 were associated with a 16% increased risk of TGCT (OR = 1.16, 95% CI = 1.06–1.28, $P = 0.002$ and OR = 1.16, 95% CI = 1.03–1.31, $P = 0.018$, respectively). Only one of these SNPs, rs11202586, remained significantly associated with TGCT risk after adjusting for multiple testing ($P_{bonf} = 0.040$) (Table II and Fig. 1). The risk estimates did not differ significantly after including interaction terms for histology, parent-of-origin or country of origin; the *P*-values for the interaction terms were in the range of 0.25–1 after Bonferroni correction.

Discussion

In this large case–parent study, the association between risk of TGCT and 26 SNPs in *AKT1*, *PTEN* and the 8q24 locus was investigated. In addition, we conducted an expanded case–parent/case–control analysis that included 23 of these SNPs. The principal finding was that for one SNP in *PTEN*, rs11202586, the T allele was associated with a 16% increased risk of developing TGCT. This association was not modified by the gender of the parent from whom the allele was transmitted, which might indicate that neither genomic imprinting nor any other epigenetic component, resulting from environment influences, is involved in the inheritance of this risk allele (Guilmatre and Sharp, 2012). We found no significant association between *AKT1* or 8q24 locus and TGCT risk.

Despite several studies indicating a role of *AKT1* and *PTEN* in TGCT pathogenesis, no previous candidate-gene studies have, to our knowledge, investigated the association between common genetic variants in these genes and TGCT. As such, our finding is novel and suggests a possible role for *PTEN* in the pathogenesis of TGCT. Although the association was not highly significant, it was based on an *a priori* hypothesis of this tumor-suppressor gene being implicated in the etiology of this cancer form, but replication studies and functional analyses of this polymorphism are warranted.

The serine–threonine protein kinase encoded by *AKT1* inhibits apoptosis and induces cell growth. PTEN is a tumor suppressor that negatively regulates the AKT1 signaling pathway (Datta *et al.*, 1999; Testa and Bellacosa, 2001; Song *et al.*, 2012). Both the oncogene, *AKT1* and the tumor-suppressor gene, *PTEN* are frequently mutated in human cancers (Staal, 1987; Dong *et al.*, 2001; Sun *et al.*, 2001).

In mice, estrogens increase the production of KITLG and thereby stimulate growth of PGCs via the AKT1/PTEN pathway (Moe-Behrens *et al.*, 2003). Further, it has been shown that knockout of *Pten* in male mice PGCs causes bilateral testicular teratomas (Kimura *et al.*, 2003). In 2005, a study by Nakai *et al.* indicated an activation of the KIT-PI3K-AKT1 pathway in seminomas. Another group found that *PTEN* was expressed in normal germ cells, whereas it was lost in more than half of all seminomas, 86% of embryonal carcinomas and almost all teratomas. *PTEN* was, however, abundantly expressed in intratubular germ cell neoplasia (ITGCN) suggesting a crucial role in the transition from ITGCN to invasive TGCT (Di Vizio *et al.*, 2005).

Recently, Hennenlotter *et al.* (2011) investigated *PTEN* expression in non-seminoma TGCTs. They found that *PTEN* expression was reduced in all non-seminoma subgroups with no altered expression or negative regulation of *AKT1*. This result was in contrast to the previously mentioned findings (Nakai *et al.*, 2005), and might reflect a difference in tumor biology between seminoma and non-seminoma TGCTs. Some evidence suggest that genetic variation in *CYP1A1/CYP1A2* or androgen receptor CAG repeat length is associated with histological subtype of TGCT, but the results are inconsistent (Figueroa *et al.*, 2008; Davis-Dao *et al.*, 2011; Kristiansen *et al.*, 2011). In a previous study among the same group of individuals as in the present one, the association of TGCT with polymorphisms in the sex hormone pathway genes did not differ significantly between histological subtypes of TGCT (Kristiansen *et al.*, 2012). Our observed lack of association between histological subtypes for genetic variation in *AKT1*, *PTEN* and the 8q24 locus is thus in accordance with the null results most often reported in genetic association studies on histological subtype of TGCT.

The gene-poor region 8q24 has been linked particularly to breast cancer, prostate cancer and colorectal cancer (Yeager *et al.*, 2009; He *et al.*, 2011; Li *et al.*, 2011). Several recent studies suggest that enhancers involved in tissue-specific MYC regulation are located in this region (Jia *et al.*, 2009; Ahmadiyeh *et al.*, 2010; Wasserman *et al.*, 2010). Since *MYC* is a well-known proto-oncogene located immediately downstream

Downloaded from https://academic.oup.com/humrep/article/28/7/1995/613969 by U.S. Department of Justice user on 16 August 2022

Downloaded from https://academic.oup.com/humrep/article/28/7/1995/613969 by U.S. Department of Justice user on 16 August 2022

Table II ORs for associations between polymorphisms in *AKT1*, *PTEN* and 8q24 locus and TGCT risk in the case–parent and the combined case–parent/case–control study (includes all triads and dyads from the case–parent study).

Gene (CHR)	SNP	Genomic position	A1	A2	MAF	Genotype[a]	Case–parent		Combined		
							OR (95% CI)	*P*	OR (95% CI)	*P*	P_{bonf}[b]
AKT1 (14)	rs2494731	104308725	C	G	0.34		0.91 (0.80–1.03)	0.128	0.93 (0.86–1.01)	0.071	1.000
	rs2498794	104316296	A	G	0.48		1.03 (0.91–1.15)	0.670	NA		
	rs2494739	104318034	C	T	0.06		0.92 (0.73–1.16)	0.461	0.96 (0.83–1.11)	0.594	1.000
	rs2498789	104321090	A	G	0.09		1.02 (0.83–1.24)	0.889	NA		
	rs10138227	104330751	C	T	0.14		0.95 (0.80–1.12)	0.547	NA		
	rs2494752	104334653	A	G	0.06		1.14 (0.89–1.45)	0.317	0.95 (0.81–1.11)	0.502	1.000
	rs4983387	104339273	A	G	0.09		0.95 (0.77–1.16)	0.585	1.07 (0.95–1.22)	0.283	1.000
PTEN (10)	rs1234212	89598872	C	T	0.34		0.97 (0.86–1.10)	0.633	0.99 (0.92–1.07)	0.824	1.000
	rs11202586	89602004	C	T	0.18		1.16 (1.00–1.35)	0.054	1.16 (1.06–1.28)	**0.002**	**0.040**
						CC	1		1		
						CT	1.52 (0.98–2.38)		1.46 (1.06–2.03)		
						TT	1.68 (1.06–2.67)		1.64 (1.18–2.26)		
	rs1234221	89606459	A	C	0.24		0.99 (0.86–1.13)	0.828	0.97 (0.89–1.06)	0.537	1.000
	rs1234220	89635453	A	G	0.11		1.06 (0.88–1.28)	0.544	1.16 (1.03–1.31)	**0.018**	0.414
						AA	1		1		
						AG	1.08 (0.89–1.32)		1.18 (1.03–1.34)		
						GG	1.01 (0.51–1.99)		1.19 (0.67–2.12)		
	rs1234219	89639557	A	G	0.08		1.12 (0.90–1.38)	0.308	1.09 (0.95–1.25)	0.221	1.000
	rs2299939	89647130	A	C	0.18		0.93 (0.80–1.09)	0.363	1.04 (0.95–1.15)	0.386	1.000
	rs12357281	89690651	C	G	0.08		1.08 (0.87–1.33)	0.504	1.05 (0.91–1.20)	0.534	1.000
	rs2248293	89697245	C	T	0.34		1.05 (0.93–1.19)	0.448	0.95 (0.88–1.03)	0.233	1.000
	rs926091	89711392	C	T	0.13		0.89 (0.75–1.06)	0.181	0.91 (0.82–1.02)	0.105	1.000
8q24 locus (8)	rs7008482	126336812	G	T	0.30		0.95 (0.83–1.08)	0.401	0.91 (0.84–0.99)	**0.029**	0.674
						GG	1		1		
						GT	0.76 (0.59–0.98)		0.80 (0.66–0.96)		
						TT	0.79 (0.59–1.01)		0.78 (0.64–0.93)		
	rs13254738	128173525	A	C	0.33		1.03 (0.91–1.17)	0.617	1.02 (0.94–1.10)	0.707	1.000
	rs6983561	128176062	A	C	0.37		0.94 (0.69–1.28)	0.676	0.98 (0.80–1.19)	0.815	1.000
	rs16901979	128194098	A	C	0.04		1.02 (0.75–1.39)	0.906	0.94 (0.78–1.15)	0.566	1.000
	rs6983267	128482487	G	T	0.47		0.97 (0.86–1.09)	0.556	0.98 (0.91–1.05)	0.548	1.000
	rs7000448	128510352	C	T	0.39		1.08 (0.96–1.22)	0.190	0.97 (0.90–1.05)	0.424	1.000
	rs1447295	128554220	A	C	0.12		1.08 (0.90–1.30)	0.411	0.99 (0.88–1.11)	0.864	1.000
	rs4242382	128586755	A	G	0.11		1.11 (0.92–1.34)	0.277	1.00 (0.89–1.13)	0.986	1.000
	rs7017300	128594450	A	C	0.15		0.92 (0.77–1.08)	0.301	0.94 (0.85–1.05)	0.266	1.000
	rs7837688	128608542	G	T	0.11		0.94 (0.78–1.14)	0.545	1.06 (0.94–1.19)	0.333	1.000

Genomic positions refer to the UCSC hg18 version of the reference human genome. CHR, chromosome; SNP, single nucleotide polymorphism; A1, reference allele (allelic odds ratio = 1); MAF, minor allele frequency in founders; OR, odds ratio; CI, confidence interval. Bold denotes $P < 0.05$.

[a]Significant associations only.

[b]Bonferroni correction (*P* multiplied by the number of total SNP analyses, $n = 23$).

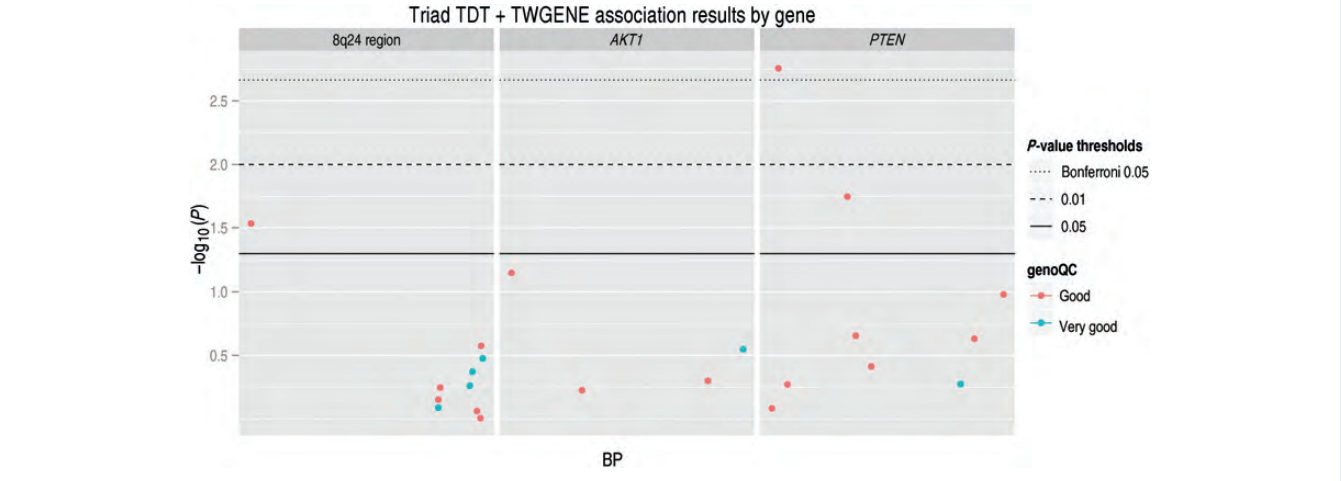

Figure 1 Plot of $-\log_{10}(P)$ values for each SNP in the combined case–parent/case–control study with significance thresholds indicated for $P = 0.05$, $P = 0.01$ and Bonferroni = 0.05 (23 SNPs).

of the 8q24 locus, this notion is appealing; although the results are not conclusive. In 2008, Cook *et al.* investigated the association between TGCT and 15 SNPs in this region with no significant findings. However, since neither this study nor the present study investigated all SNPs in this region, we cannot exclude the possibility of an association between the 8q24 locus and TGCT.

For many years, the incidence rate of TGCT in Sweden has for unknown reasons been only about half of that in Norway. The present study was not able to demonstrate any interaction in the associations between the investigated SNPs and the risk of TGCT related to country, implying that the genetic susceptibility to TGCT concerning our investigated SNPs does not seem to be heterogeneous between the countries. Hence, our results thus do not shed any light on the difference in the incidence rate between these two neighboring countries. The same applies for the previously mentioned study on sex hormone pathway genes in the same study population (Kristiansen *et al.*, 2012).

Our study is strengthened by the population-based design and large sample size, which give high power to detect risk alleles. In this study we performed the analyses in two stages, where the first stage only considered case–parent triads and dyads. This analysis is robust to bias from population stratification, since the non-transmitted parental alleles act as 'controls' within each family unit, and the analysis is thus implicitly stratified by country. Adding the unrelated cases and population controls to the second stage analysis increases the power to detect SNP-disease associations, but is exposed to potential population stratification issues due to the control sample consisting of Swedes only. Since the effect estimate (OR, 1.16) for the most strongly associated marker rs11202586 barely changed from the population-robust Stage 1 to the less robust but higher powered Stage 2, we believe this association, though minute in effect, to be a true finding. Finally, in tests for effect modification of alleles by country, we detected no significant effect modifications. Although the absence of evidence should not be taken as evidence for absence, this is in agreement with the previous paragraph, and with our assumption that the Swedish and Norwegian populations are genetically similar.

Regarding the 8q24 locus and *PTEN*, the SNPs were selected based on results from previously published reports. Regarding these non-tagged genes, we have not captured all common variation, and thus, some unknown risk alleles cannot be excluded.

In conclusion, we report an association between a genetic polymorphism in the tumor-suppressor gene *PTEN* and risk of TGCT. Exploring the functional role of the SNPs associated with TGCT will further clarify the biological mechanisms involved. Further, our results show that common variants in *AKT1* probably do not substantially influence risk of TGCT, although we might have missed an association in the combined case–parent/case–control study because three of the htSNPs were not genotyped in the control group. Regarding the 8q24 locus more studies are needed to elucidate any possible association with TGCT risk.

Acknowledgements

We are grateful to the study participants for contributing to our research. We thank Gerd Agerberg at the Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden, and Gjøril B. Aas and Jan Ivar Martinsen at the Department of Research, Cancer Registry of Norway, Oslo, Norway, for organizing the collection of the triad study samples and database management. We wish to thank Christine Ø. Buen, Hilde Herning, Manpret Kaur and Susanne S. Windju at the Faculty of Health Sciences, Oslo and Akershus University College of Applied Sciences, Oslo, Norway for help with the DNA isolation. We also thank Roy M. Bremnes, Department of Oncology, University Hospital North Norway, Tromsø, Norway, Olbjørn Klepp, Department of Oncology, Ålesund Hospital, Ålesund, Norway, Carl W. Langberg, Department of Oncology, Oslo University Hospital, Ullevål, Oslo, Norway and Arne Solberg, Department of Oncology, St Olavs University Hospital, Trondheim, Norway for help in selecting cases. We thank Patrik K.E. Magnusson for giving us access to genotypes from the TwinGene Project to use as a control group. And finally, we express gratitude to Steinar Tretli, at the Department of Research, Cancer Registry of Norway, Oslo, Norway for valuable discussion.

Downloaded from https://academic.oup.com/humrep/article/28/7/1995/613969 by U.S. Department of Justice user on 16 August 2022

Authors' roles

E.L.A., H.-O.A., F.W., T.B.H. and T.G. designed the study. K.E.A., O.D., S.D.F., F.W. and T.G. recruited cases. K.E.A., W.K. and E.L.A. extracted genomic DNA. R.K. and F.W. performed statistical analyses. K.E.A., W.K., R.K., E.L.A., H.-O.A., T.B.H. and T.G. drafted the manuscript. All authors read and approved the final version of the manuscript.

Funding

This work was financially supported by the Norwegian Cancer Society (418975) and the Nordic Cancer Union (S-12/07).

Conflict of interest

None declared.

References

Ahmadiyeh N, Pomerantz MM, Grisanzio C, Herman P, Jia L, Almendro V, He HH, Brown M, Liu XS, Davis M *et al.* 8q24 prostate, breast, and colon cancer risk loci show tissue-specific long-range interaction with MYC. *Proc Natl Acad Sci USA* 2010;**107**:9742–9746.

Cairns P, Okami K, Halachmi S, Halachmi N, Esteller M, Herman JG, Jen J, Isaacs WB, Bova GS, Sidransky D. Frequent inactivation of PTEN/MMAC1 in primary prostate cancer. *Cancer Res* 1997; **57**:4997–5000.

Chia VM, Quraishi SM, Devesa SS, Purdue MP, Cook MB, McGlynn KA. International trends in the incidence of testicular cancer, 1973–2002. *Cancer Epidemiol Biomarkers Prev* 2010;**19**:1151–1159.

Cook MB, Graubard BI, Quraishi SM, Yeager M, Chanock SJ, Crenshaw A, Erickson RL, Rubertone MV, Thomas G, McGlynn KA. Genetic variants in the 8q24 locus and risk of testicular germ cell tumors. *Hum Genet* 2008;**123**:409–418.

Datta SR, Brunet A, Greenberg ME. Cellular survival: a play in three Akts. *Genes Dev* 1999;**13**:2905–2927.

Davis-Dao CA, Siegmund KD, Vandenberg DJ, Skinner EC, Coetzee GA, Thomas DC, Pike MC, Cortessis VK. Heterogenous effect of androgen receptor CAG tract length on testicular germ cell tumor risk: shorter repeats associated with seminoma but not other histologic types. *Carcinogenesis* 2011;**32**:1238–1243.

de Bakker PI, Yelensky R, Pe'er I, Gabriel SB, Daly MJ, Altshuler D. Efficiency and power in genetic association studies. *Nat Genet* 2005; **37**:1217–1223.

Devi M, Leonard N, Silverman S, Al-Qahtani M, Girgis R. Testicular mixed germ cell tumor in an adolescent with cowden disease. *Oncology* 2007; **72**:194–196.

Di Vizio D, Cito L, Boccia A, Chieffi P, Insabato L, Pettinato G, Motti ML, Schepis F, D'Amico W, Fabiani F *et al.* Loss of the tumor suppressor gene PTEN marks the transition from intratubular germ cell neoplasias (ITGCN) to invasive germ cell tumors. *Oncogene* 2005;**24**:1882–1894.

Dong JT, Li CL, Sipe TW, Frierson HF Jr. Mutations of PTEN/MMAC1 in primary prostate cancers from Chinese patients. *Clin Cancer Res* 2001; **7**:304–308.

Dudbridge F. Likelihood-based association analysis for nuclear families and unrelated subjects with missing genotype data. *Hum Hered* 2008; **66**:87–98.

Figueroa JD, Sakoda LC, Graubard BI, Chanock S, Rubertone MV, Erickson RL, McGlynn KA. Genetic variation in hormone metabolizing genes and risk of testicular germ cell tumors. *Cancer Causes Control* 2008; **19**:917–929.

Gabriel S, Ziaugra L, Tabbaa D. SNP genotyping using the Sequenom MassARRAY iPLEX platform. *Curr Protoc Hum Genet* 2009;Chapter 2: Unit 2.12 (2.12.1–2.12.18).

Ghoussaini M, Song H, Koessler T, Al Olama AA, Kote-Jarai Z, Driver KE, Pooley KA, Ramus SJ, Kjaer SK, Hogdall E *et al.* Multiple loci with different cancer specificities within the 8q24 gene desert. *J Natl Cancer Inst* 2008;**100**:962–966.

Guilmatre A, Sharp AJ. Parent of origin effects. *Clin Genet* 2012;**81**:201–209.

He J, Wilkens LR, Stram DO, Kolonel LN, Henderson BE, Wu AH, Le Marchand L, Haiman CA. Generalizability and Epidemiologic characterization of eleven colorectal cancer GWAS Hits in multiple populations. *Cancer Epidemiol Biomarkers Prev* 2011;**20**:70–81.

Heimdal K, Olsson H, Tretli S, Flodgren P, Borresen AL, Fossa SD. Familial testicular cancer in Norway and southern Sweden. *Br J Cancer* 1996; **73**:964–969.

Hemminki K, Li X. Familial risk in testicular cancer as a clue to a heritable and environmental aetiology. *Br J Cancer* 2004;**90**:1765–1770.

Hennenlotter J, Amend B, Vogel U, Renninger M, Springer C, Kuehs U, Stenzl A, Bedke J. Differential Akt signalling in non-seminomatous testicular germ cell tumors. *Anticancer Res* 2011;**31**:3783–3788.

Hoei-Hansen CE, Rajpert-De Meyts E, Daugaard G, Skakkebaek NE. Carcinoma *in situ* testis, the progenitor of testicular germ cell tumours: a clinical review. *Ann Oncol* 2005;**16**:863–868.

Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS Genet* 2009;**5**:e1000529.

Huyghe E, Matsuda T, Thonneau P. Increasing incidence of testicular cancer worldwide: a review. *J Urol* 2003;**170**:5–11.

Huyghe E, Plante P, Thonneau PF. Testicular cancer variations in time and space in Europe. *Eur Urol* 2007;**51**:621–628.

Jia L, Landan G, Pomerantz M, Jaschek R, Herman P, Reich D, Yan C, Khalid O, Kantoff P, Oh W *et al.* Functional enhancers at the gene-poor 8q24 cancer-linked locus. *PLoS Genet* 2009;**5**:e1000597.

Kanetsky PA, Mitra N, Vardhanabhuti S, Li M, Vaughn DJ, Letrero R, Ciosek SL, Doody DR, Smith LM, Weaver J *et al.* Common variation in KITLG and at 5q31.3 predisposes to testicular germ cell cancer. *Nat Genet* 2009;**41**:811–815.

Kimura T, Suzuki A, Fujita Y, Yomogida K, Lomeli H, Asada N, Ikeuchi M, Nagy A, Mak TW, Nakano T. Conditional loss of PTEN leads to testicular teratoma and enhances embryonic germ cell production. *Development* 2003;**130**:1691–1700.

Kristiansen W, Haugen TB, Witczak O, Andersen JM, Fossa SD, Aschim EL. CYP1A1, CYP3A5 and CYP3A7 polymorphisms and testicular cancer susceptibility. *Int J Androl* 2011;**34**:77–83.

Kristiansen W, Andreassen KE, Karlsson R, Aschim EL, Bremnes RM, Dahl O, Fossa SD, Klepp O, Langberg CW, Solberg A *et al.* Gene variations in sex hormone pathways and the risk of testicular germ cell tumour: a case–parent triad study in a Norwegian-Swedish population. *Hum Reprod* 2012;**27**:1525–1535.

Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, Puc J, Miliaresis C, Rodgers L, McCombie R *et al.* PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science* 1997;**275**:1943–1947.

Li J, Humphreys K, Heikkinen T, Aittomaki K, Blomqvist C, Pharoah PD, Dunning AM, Ahmed S, Hooning MJ, Martens JW *et al.* A combined analysis of genome-wide association studies in breast cancer. *Breast Cancer Res Treat* 2011;**126**:717–727.

Mazereeuw-Hautier J, Assouère MN, Moreau-Cabarrot A, Longy M, Bonafè JL. Cowden's syndrome: possible association with testicular seminoma. *Br J Dermatol* 2004;**150**:378–379.

Downloaded from https://academic.oup.com/humrep/article/28/7/1995/613969 by U.S. Department of Justice user on 16 August 2022

Moe-Behrens GH, Klinger FG, Eskild W, Grotmol T, Haugen TB, De Felici M. Akt/PTEN signaling mediates estrogen-dependent proliferation of primordial germ cells *in vitro*. *Mol Endocrinol* 2003;**17**:2630–2638.

Mutter GL, Lin MC, Fitzgerald JT, Kum JB, Baak JP, Lees JA, Weng LP, Eng C. Altered PTEN expression as a diagnostic marker for the earliest endometrial precancers. *J Natl Cancer Inst* 2000;**92**:924–930.

Nakai Y, Nonomura N, Oka D, Shiba M, Arai Y, Nakayama M, Inoue H, Nishimura K, Aozasa K, Mizutani Y *et al*. KIT (c-kit oncogene product) pathway is constitutively activated in human testicular germ cell tumors. *Biochem Biophys Res Commun* 2005;**337**:289–296.

Osaki M, Oshimura M, Ito H. PI3K-Akt pathway: its functions and alterations in human cancer. *Apoptosis* 2004;**9**:667–676.

Rahman I, Bennet AM, Pedersen NL, de Faire U, Svensson P, Magnusson PK. Genetic dominance influences blood biomarker levels in a sample of 12,000 Swedish elderly twins. *Twin Res Hum Genet* 2009;**12**:286–294.

Rajpert-De Meyts E, Hoei-Hansen CE. From gonocytes to testicular cancer: the role of impaired gonadal development. *Ann N Y Acad Sci* 2007; **1120**:168–180.

Rapley EA, Turnbull C, Al Olama AA, Dermitzakis ET, Linger R, Huddart RA, Renwick A, Hughes D, Hines S, Seal S *et al*. A genome-wide association study of testicular germ cell tumor. *Nat Genet* 2009;**41**:807–810.

Rottapel R, Reedijk M, Williams DE, Lyman SD, Anderson DM, Pawson T, Bernstein A. The Steel/W transduction pathway: kit autophosphorylation and its association with a unique subset of cytoplasmic signaling proteins is induced by the Steel factor. *Mol Cell Biol* 1991;**11**:3043–3051.

Song MS, Salmena L, Pandolfi PP. The functions and regulation of the PTEN tumour suppressor. *Nat Rev Mol Cell Biol* 2012;**13**:283–296.

Staal SP. Molecular cloning of the akt oncogene and its human homologues AKT1 and AKT2: amplification of AKT1 in a primary human gastric adenocarcinoma. *Proc Natl Acad Sci USA* 1987;**84**:5034–5037.

Steck PA, Pershouse MA, Jasser SA, Yung WK, Lin H, Ligon AH, Langford LA, Baumgard ML, Hattier T, Davis T *et al*. Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. *Nat Genet* 1997;**15**:356–362.

Sun M, Wang G, Paciga JE, Feldman RI, Yuan ZQ, Ma XL, Shelley SA, Jove R, Tsichlis PN, Nicosia SV *et al*. AKT1/PKBalpha kinase is frequently elevated in human cancers and its constitutive activation is required for oncogenic transformation in NIH3T3 cells. *Am J Pathol* 2001; **159**:431–437.

Swerdlow AJ, De Stavola BL, Swanwick MA, Maconochie NE. Risks of breast and testicular cancers in young adult twins in England and Wales: evidence on prenatal and genetic aetiology. *Lancet* 1997;**350**:1723–1728.

Testa JR, Bellacosa A. AKT plays a central role in tumorigenesis. *Proc Natl Acad Sci USA* 2001;**98**:10983–10985.

The International HapMap Consortium. The International HapMap Project. *Nature* 2003;**426**:789–796.

Turnbull C, Rapley EA, Seal S, Pernet D, Renwick A, Hughes D, Ricketts M, Linger R, Nsengimana J, Deloukas P *et al*. Variants near DMRT1, TERT and ATF7IP are associated with testicular germ cell cancer. *Nat Genet* 2010; **42**:604–607.

Wasserman NF, Aneas I, Nobrega MA. An 8q24 gene desert variant associated with prostate cancer risk confers differential *in vivo* activity to a MYC enhancer. *Genome Res* 2010;**20**:1191–1197.

Wokolorczyk D, Gliniewicz B, Sikorski A, Zlowocka E, Masojc B, Debniak T, Matyjasik J, Mierzejewski M, Medrek K, Oszutowska D *et al*. A range of cancers is associated with the rs6983267 marker on chromosome 8. *Cancer Res* 2008;**68**:9982–9986.

Yeager M, Chatterjee N, Ciampa J, Jacobs KB, Gonzalez-Bosquet J, Hayes RB, Kraft P, Wacholder S, Orr N, Berndt S *et al*. Identification of a new prostate cancer susceptibility locus on chromosome 8q24. *Nat Genet* 2009; **41**:1055–1057.

Downloaded from https://academic.oup.com/humrep/article/28/7/1995/613969 by U.S. Department of Justice user on 16 August 2022