Published in final edited form as:
Cancer Res. 2008 April 15; 68(8): 3043–3048. doi:10.1158/0008-5472.CAN-07-5852.

Genetic variation in the inhibin pathway and risk of testicular germ cell tumors

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Abstract

Gene-knockout studies in mice suggest that *INHA*, encoding a subunit of gonadotropin-regulating proteins known as inhibins, is a tumor suppressor for testicular stromal cell tumors. It is not known whether genetic variation in the inhibin pathway also influences susceptibility to testicular germ cell tumors (TGCT), the most common testicular cancer in young men. To address this question, we conducted a case-control analysis (577 cases, 707 controls) of single-nucleotide polymorphisms (SNPs) in genes in the inhibin pathway among participants in the U.S. Servicemen's Testicular Tumor Environmental and Endocrine Determinants (STEED) Study. Thirty-eight tagging SNPs in six genes (*INHA, INHBA, INHBB, INHBC, INHBE, SMAD4*) were genotyped. Odds ratios (OR) and 95% confidence intervals (CI) relating variant genotypes to TGCT risk were calculated using unconditional logistic regression. Among White subjects, an elevated risk of TGCT was observed for carriers of the T allele of the *INHA* variant rs2059693 (CT genotype: OR 1.33, 95% CI 1.04–1.71; TT: OR 1.60, 95% CI 1.01–2.52; P trend=0.008). The association with rs2059693 was stronger for nonseminomas, and for teratomas and teratocarcinomas in particular (N=58; CT: OR 1.63, 95% CI 0.89–2.99; TT: OR 4.54, 95% CI 2.00–10.3, P trend=0.0008). We found no evidence of association with variants in the other investigated genes. These findings suggest that genetic variation in the *INHA* locus influences TGCT development.

Keywords

inhibin; *INHA*; polymorphisms; case-control study; testicular cancer; USA

INTRODUCTION

The pathogenesis of testicular germ cell tumors (TGCT), the most common type of testicular cancer diagnosed among adolescent and young adult males, remains largely unknown; the only well described risk factors are cryptorchidism, Caucasian ethnicity, and family history and personal history of TGCT (1). Gonadotropin dysregulation has been hypothesized as one possible cause of TGCT (2;3). The gonadotropin follicle-stimulating hormone (FSH), necessary for germ cell maturation, is regulated by growth factors known as inhibins and

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activins (4). These dimeric glycoproteins share common subunits; inhibins, so-named for their inhibitory effect on FSH secretion, are α : β heterodimers, while activins – which stimulate FSH secretion – are β : β dimers. Two inhibin isoforms (Inhibin A and B) are produced, depending upon the type of β subunit (βA, βB) present. The testis is the primary site of inhibin production among males, with Sertoli cells the principal source of expression.

Deletion of *INHA*, the gene encoding the α subunit, has been shown to induce testicular and ovarian stromal tumors in mice with nearly 100% penetrance, suggesting that inhibin may be a tumor suppressor within the testis (5). The importance of inhibin genes in the development of TGCT, however, is unknown. To explore whether genetic variation in the inhibin pathway influences TGCT pathogenesis, we investigated the association between TGCT and single-nucleotide polymorphisms (SNPs) in *INHA* and other inhibin pathway genes (*INHBA*, *INHBB*, *INHBC*, *INHBE*, *SMAD4*) within a population-based case-control study, the U.S. Servicemen's Testicular Tumor Environmental and Endocrine Determinants (STEED) Study.

MATERIALS AND METHODS

Study Population

A detailed description of the study methodology is provided elsewhere (6;7). Briefly, the STEED study is a case-control study of TGCT conducted within the population of men who served in the U.S. military between 1987 and 2002, and have at least one serum sample stored in the Department of Defense Serum Repository (DoDSR, Silver Spring, MD). Using a person-specific ID, specimens in the DoDSR computerized database were linked to the Defense Medical Surveillance System (DMSS) (8) and to other military medical databases in order to determine which military personnel had developed TGCT.

All men with a sample in the DoDSR who subsequently developed TGCT while on active duty and who were aged ≤ 45 years at diagnosis were eligible to participate as cases. Diagnoses of TGCT, based on the original pathology reports, were limited to classic seminoma or nonseminoma (embryonal carcinoma, yolk sac carcinoma, choriocarcinoma, teratoma/teratocarcinoma, mixed germ cell tumor). Nine hundred sixty-one eligible case participants were initially identified. Of these men, 76 could not be traced, 27 had died, 3 were deployed to a combat zone, 2 were found to be ineligible, 22 did not complete study enrollment prior to the cut-off date and 77 refused to participate. Participating cases (N=754) completed a telephone interview and were asked to donate a buccal cell sample collected in mouthwash. Of these men, 590 provided buccal cell samples. Five hundred and seventy seven of these cases were successfully genotyped for at least one SNP.

Men with a sample in the DoDSR who did not subsequently develop TGCT were eligible to participate as controls. The study was designed as a pair-matched case-control study. Using the computerized DMSS database, all available controls were identified for each potential case participant. From the list of possible controls, four individuals who matched each case on birth year (within 1 year), race (White, Black, other) and date of serum donation (within 30 days) and were on active duty at the time of serum donation were chosen at random as the control set. The first man on the list was designated as the primary control. Every attempt was made to enroll this man for thirty days (average number of attempted contacts=90). The effort included tracing attempts, multiple letters and telephone calls. If the man could not be traced, was deployed to a combat zone, deceased, refused to participate, or could not be contacted within a 30-day period, attempts began to enroll the next possible control in the set.

Among the controls, 2579 were evaluated for inclusion. Three hundred eighty-five men could not be traced, 18 had died, 64 were deployed to a combat zone, 2 were found to be ineligible, 32 did not complete study enrollment prior to the cut-off date, 928 were lost because they did not respond to attempts to contact them within 30 days and 222 refused to participate. Of the remaining 928 controls who were successfully enrolled and completed a telephone interview, 712 provided buccal cell samples. Seven hundred and seven of these controls were successfully genotyped for at least one SNP. Among the 577 cases and 707 controls with genotyping data, there were 547 matched case-control pairs.

The study was approved by Institutional Review Boards of the National Cancer Institute, Rockville, MD and the Walter Reed Army Institute for Research, Forest Glen, MD.

Genotyping and Quality Control

Tagging SNPs (N=38) were selected within the six inhibin pathway genes from among common variants (minor allele frequency ≥0.05) in the CEU (Caucasian) population sample of the HapMap Project (Data Release 20/Phase II, NCBI B35 assembly, dbSNP b125). Tagging SNP selection was done using the software application TagZilla[1], which employs the pairwise binning algorithm of Carlson et al (9). For each gene, SNPs within the region 20kb 5′ of the ATG-translation initiation codon and 10kb 3′ of the translation termination codon were grouped ("binned") using a binning threshold of $r^2 > 0.80$. The selected tagging SNPs, and corresponding percent coverage of common variation across each gene region, are summarized in Table 1.

Genotyping was performed at the National Cancer Institute Core Genotyping Facility (Gaithersburg, MD). The SNPlex multiplex genotyping system (Applied Biosystems Inc., Foster City, CA) was used to genotype the entire panel of tagging SNPs. One hundred and two samples (42 cases, 60 controls) failed genotyping due to insufficient DNA. Genotyping for one SNP, rs2059693, was repeated (i.e., including the 102 subjects) by TaqMan assay (Applied Biosystems Inc., Foster City, CA); genotypes were 99% concordant across platforms.

The genotyping success rates and quality control concordance rates ranged between 93%–100% and 99%–100% respectively. The variant rs1548039 had a poor genotyping success rate (83%) and deviated from Hardy-Weinberg Equilibrium (HWE) at a high level of statistical significance (*P*=0.0006), and was excluded from the analysis. Four other SNPs deviated from HWE to a lesser extent (rs907141, *P*=0.03; rs507562, *P*=0.02; rs11902591, *P*=0.03, rs17719440, *P*=0.001). These four SNPs were included in the analysis, given their high rates of assay completion and QC concordance (>95% assay completion, >98% concordance for each SNP).

Statistical Analysis

The analysis was conducted both among all subjects and restricted to White men (508 cases, 608 controls, 481 matched case-control pairs genotyped; 87% of study subjects). Odds ratios (OR) and 95% confidence intervals (CI) estimating the relative risk of TGCT in relation to SNP genotype were calculated using unconditional logistic regression adjusting for race, age at reference date (case's date of diagnosis) and date of serum sample collection (a study design variable). The latter two variables were modeled as continuous covariates. Similar results were obtained from conditional logistic regression analysis of individually matched pairs (results not shown). Tests for trend were conducted by assigning the ordinal values 0,

[1]obtainable at http://tagzilla.nci.nih.gov/

1, and 2 to homozgyous wild type, heterozygous and homozygous variant genotypes respectively, and by modeling these scores as a continuous variable.

We assessed the robustness of the individual SNP findings in relation to the overall number of SNPs investigated using the False Discovery Rate (FDR) method of Benjamini and Hochberg (9). The FDR is the expected ratio of erroneous rejections of the null hypothesis to the total number of rejected hypotheses among all the variants assessed. Statistically significant findings (as determined by the p-value of the test of trend) with a FDR of 20% or less were considered to be noteworthy.

Haplotype analyses were also conducted among White subjects. We estimated the haplotype block structure, defined using the method of Gabriel et al. (10), across each gene region within the CEU population sample in HapMap using the software package Haploview 3.2[2]. Haplotypes were reconstructed using the estimation-maximization algorithm in SAS/ Genetics (SAS Institute, Cary, North Carolina). The effects of individual haplotypes were estimated by fitting an additive model (11) adjusted for age and serum collection date. The overall difference in haplotype frequencies between cases and controls was assessed using the likelihood ratio test.

Separate analyses for seminoma and nonseminoma and among nonseminoma histologic types (embryonal carcinoma, teratoma/teratocarcinoma, other tumors) were conducted to explore the possible existence of subtype-specific effects. Heterogeneity between seminomas and nonseminomas was examined by conducting case-case comparisons for each variant (analyzed assuming the additive model). Analyses of nonseminomas excluding mixed germ cell tumors (N=21) yielded virtually identical findings.

All statistical analyses were conducted using SAS Version 9.1 (SAS Institute, Cary, North Carolina). All statistical tests were two-sided with an alpha level of 0.05.

RESULTS

The distributions of demographic variables in the study population are displayed in Table 2. As cases and controls were matched on age and race, there were no differences in the overall distributions of these variables. As anticipated, the mean age of the nonseminoma cases (26.2 years; standard deviation (SD) 5.6) was lower than that of the seminoma cases (30.6; SD 5.9). Approximately 87% of the study population was White, 4 percent were Black, and 11 percent were members of other racial/ethnic groups. Cases were more likely than controls to have a history of cryptorchidism (P<0.01) and to have a family history of testicular cancer (P=0.03).

Findings for *INHA* SNPs are shown in Table 3. The *INHA* variant rs2059693 was significantly associated with TGCT, with cases more likely than controls to carry the T allele (CT genotype: OR 1.26, 95% CI 1.00–1.60; TT: OR 1.36, 95% CI 0.90–2.05; P trend=0.04). This association was stronger among White participants (CT: OR 1.33, 95% CI 1.04–1.71; TT: OR 1.60, 95% CI 1.01–2.52; P_{trend}=0.008), and not present among subjects of other race/ethnic groups (CT: OR 0.93, 95% CI 0.48–1.84; TT: OR 0.57, 95% CI 0.21–1.60; P_{trend}=0.35). Among White men, the variant rs6729914 was associated with TGCT at a borderline level of statistical significance (P_{trend}=0.06), as was rs1039900 (P_{trend}=0.07). Variants in *INHBA*, *INHBB*, *INHBC*, *INHBE* and *SMAD4* were not associated with TGCT (online supplementary table 1). When the FDR method was applied to assess the robustness of the results among White men while taking into account the number of evaluated SNPs,

[2]obtainable at http://www.broad.mit.edu/personal/jcbarret/haploview/

the rs2059693 finding remained noteworthy controlling for a FDR of 30%, which was above our *a priori* threshold of 20%.

A plot illustrating pairwise disequilibrium among common HapMap SNPs in the *INHA* region within the CEU population sample of HapMap is provided in Figure 1. Two haplotype blocks were identified in this region; the first block includes the tagging SNPs rs907141 and rs3731920, while the second block includes rs2059693 and rs6729914. A global test of rs2059693 – rs6729914 haplotype frequencies between TGCT cases and controls was statistically significant (P=0.007); in particular, the T-C haplotype was significantly associated with increased risk (Table 3; *P*=0.005), whereas no association with C-C was observed. The distribution of rs907141 - rs3731920 haplotypes did not differ between cases and controls (global test: P=0.41).

Associations with rs2059693 for different histologic subtypes are summarized in Table 4. Excess risk with carriage of the T allele was clearly observed for nonseminomas (P_{trend}=0.003). The variant was not clearly associated with seminoma (P_{trend}=0.19), although the odds ratio for TT carriers, though non-significant (P=0.10), was similar to that for nonseminoma (1.62 and 1.73 respectively). A test of odds ratio heterogeneity between seminomas and nonseminomas was not statistically significant. Associations with rs2059693 were observed for different nonseminoma subtypes, and for teratomas and teratocarcinomas in particular (N=58; CT: OR 1.63, 95% CI 0.89–2.99; TT: OR 4.54, 95% CI 2.00–10.3, P_{trend}=0.0008).

DISCUSSION

In the current study, the polymorphism rs2059693 was significantly associated with TGCT risk among White men. This association was stronger for nonseminomas than for seminomas, and strongest for teratomas and teratocarcinomas in particular. There was no evidence of association for variants in the other investigated inhibin/activin pathway genes (*INHBA*, *INHBB*, *INHBC*, *INHBE* and *SMAD4)*.

As rs2059693 is located 2kb 3′ of the *INHA* stop codon, beyond the gene's 3′ untranslated region (UTR), it is most likely that this SNP does not influence risk directly, but is rather a marker for another underlying causal variant. The rs2059693 SNP did not correlate with any other of the known common SNPs in HapMap at the time of tagging (i.e., it represents a "singleton" bin), although HapMap is an incomplete reference panel, particularly for uncommon SNPs (12;13); thus, it is likely that the causal variant is unmeasured in HapMap at this time. As shown in Figure 1, rs2059693 resides within a region of linkage disequilibrium that includes exon 2 and the 3′ UTR of *INHA*. A rare missense mutation (Ala257Thr; rs12720062) in exon 2 has been significantly associated with ovarian failure in three populations (14–16) and with impaired inhibin activity in a functional study (17). Given its rarity, this mutation is unlikely to account for our observed association, although it is possible that other exon 2 variants may play a role. Variants in the 3′ UTR can also potentially influence disease risk by affecting mRNA stability, translational efficiency and other factors affecting gene expression (18;19). If our rs2059693 finding is replicated in other studies, then fine mapping of the region surrounding *INHA* will be important for identifying the disease variant being tagged by this SNP.

The finding for rs2059693 is interesting given that *INHA* has been previously identified as a tumor suppressor gene for other testicular tumors, with *INHA*-knockout mice rapidly developing gonadal stromal cell tumors with nearly 100% penetrance (20). However, it not clear whether these experimental findings offer insight into the pathogenesis of TGCT, given that such stromal tumors do not arise from germ cells. *INHA* inactivation has also

been found to be a common event in prostate cancer cells (21;22), although there is also evidence suggesting that the alpha subunit is expressed in advanced tumors (23).

The mechanism through which *INHA* variation might influence TGCT risk is unclear. Activins, but not inhibins, have been shown to directly regulate germ cell differentiation (24;25). The possibility of autocrine or paracrine effects of the inhibin/activin system is suggested by evidence of positive immunohistochemical staining of inhibin and activin subunits in TGCT but not in normal germ cells (26;27). Alternatively, *INHA* may affect TGCT development indirectly through its role as a regulator of FSH. It has been suggested that elevated FSH levels at puberty could influence TGCT development by stimulating the progression of carcinoma in-situ cells formed during fetal development (2). In support of this hypothesis is the observation that some men undergoing surgery for TGCT have higher FSH levels than normal (28), particularly among patients who subsequently develop a second contralateral germ cell tumor (29). Indirect evidence suggesting a link between FSH exposure during gestation and TGCT risk includes reports of elevated levels of FSH among mothers of dizygous twins (30–32), a group at high risk of this cancer (33;34). Men with Down's syndrome, another group at increased risk of TGCT, also have elevated FSH levels (35). Additionally, FSH has been shown to influence expression of cyclin D2, a putative proto-oncogene frequently overexpressed in TGCT (36), and to enhance gonadal tumor progression in INHA-deficient mice (37). On the basis of this evidence, it is plausible that variation in *INHA* expression or polypeptide function could affect TGCT development through changes in FSH secretion. At present, it is not known whether prediagnostic FSH levels are higher among men who develop TGCT than among men who do not.

Strengths of this study include its population-based design and relatively large sample size; the STEED study is one of the largest case-control investigations of testicular cancer etiology conducted to date. This study also has limitations. First, only TGCT cases diagnosed during active duty where identified for enrollment in the case series of the study, thus potentially limiting the potential sample size of the study somewhat. This restricted follow-up could also have potentially introduced bias into our study if rs2059693 genotype was associated with the probability of TGCT diagnosis during active duty (vs. diagnosis after military service), although this scenario is unlikely. Second, given that we investigated several tagging SNPs across multiple genes, it is possible that the rs2059693 association is a chance finding. Indeed, the association for this SNP among white men remained noteworthy at a level of FDR control of 30%, which was greater than our *a priori* threshold of 20%. It is important that these results be replicated in other studies before meaningful inferences regarding causation can be drawn.

In conclusion, the results of the current study suggest that variation in *INHA* may influence susceptibility to TGCT. Additional investigations are needed to replicate these findings and, more generally, explore further the relevance of the inhibin/activin/FSH pathway to the pathogenesis of this poorly understood cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was supported by the Intramural Research Program of the NIH, National Cancer Institute.

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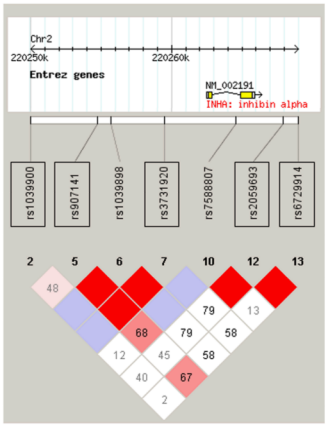

Figure 1.

Linkage disequilibrium plot of HapMap SNPs within the *INHA* gene region. The plot was generated using data from the CEU population sample of HapMap using Haploview software. The five tagging SNPs included in the study are enclosed in boxes. The level of pairwise LD is expressed by color (bright red, D′ = 1 and LOD ≥ 2, shades of pink/red, D′ < 1 and LOD ≥ 2; blue, D′ = 1 and LOD < 2; white, D′ < 1 and LOD < 2).

Table 1

Summary of evaluated genes and tagging single-nucleotide polymorphisms (SNPs)

Gene(s) (Region)	Total Number of SNPs*	Bins			Tagging SNPs			% Coverage of total SNPs
					Assayed		Not Assayed	
		Singleton	Non-singleton	Total	N	SNPs	N	
INHA (2q33-36)	7	5	1	6	5	rs1039900, rs907141, rs3731920, rs2059693, rs6729914	1†	86%
INHBA (7p15-p13)	19	7	4	11	11	rs17776182, rs17719440, rs7782324, rs1003291, rs998190, rs1122291, rs2877098, rs3801158, rs2237432, rs11770488, rs10486719	0	100%
INHBB (2cen-q13)	13	10	1	11	11	rs934716, rs7593535, rs17625845, rs7589138, rs7578624, rs11902591, rs7581178, rs745723, rs7576183, rs7579169, rs1548039	0	100%
INHBC/*INHBE* (12q13)	23	2	6	8	8	rs7964492, rs543410, rs560048, rs3741414, rs3809114, rs507562, rs2242578, rs4760148	0	100%
SMAD4 (18q21.1)	30	0	3	3	3	rs948589, rs9304407, rs12456284	0	100%

*Number of common SNPs (minor allele frequency ≥0.05) located within gene and flanking regions (20kb 5′ of initiation codon, 10kb 3′ of translation termination codon) in CEU population sample of the HapMap Project.

†rs7588807, which tagged a singleton bin, was excluded from SNPlex panel due to assay QC issues.

Table 2

Characteristics of study participants, Servicemen's Testicular Tumor Environmental and Endocrine Determinants Study, 2002–2005

	Controls (N=707)		All testicular germ cell tumors (N=577)		Seminomas (N=254)		Nonseminomas (N=323)	
	N	%	N	%	N	%	N	%
Reference age (years)								
<25	223	31.5	201	34.8	47	15.8	154	47.7
25–29	202	28.6	157	27.2	72	28.4	85	26.3
30–34	141	19.9	103	17.9	58	22.8	45	13.9
35–39	106	15.0	85	14.7	56	22.1	29	9.0
≥40	35	5.0	31	5.4	21	8.3	10	3.1
P-value*			0.72		<0.01		<0.01	
Race								
White	608	86.0	508	88.0	211	83.1	297	92.0
Black	24	3.4	13	2.3	8	3.2	5	1.6
Other	75	10.6	56	9.7	35	13.8	21	6.5
P-value*			0.4		0.02		0.02	
Height (cm)								
≤177.8	398	56.3	285	49.4	127	50.0	158	48.9
>177.8	309	43.7	292	50.6	127	50.0	165	51.1
P-value*			0.01		0.05		0.05	
Cryptorchidism								
No	692	98.6	544	95.8	245	97.6	299	94.3
Yes	10	1.4	24	4.2	6	2.4	18	5.7
P-value*			<0.01		<0.01		<0.01	
Family history of testicular cancer†								
Absent	694	98.2	555	96.2	243	95.7	312	96.6
Present	13	1.8	22	3.8	11	4.3	11	3.4
P-value*			0.03		0.08		0.08	

*Based on chi-square tests comparing case groups to controls

†Among first- and second-degree relatives

Table 3

Genetic variants in *INHA* and risk of testicular germ cell tumors; results from analyses of all subjects and of White subjects only

		All Subjects ***(577 cases, 707 controls)***				**White Subjects** ***(508 cases, 608 controls)***			
SNP	**Genotype**	**$N_{Controls}$**	**N_{Cases}**	**OR* (95% CI)**	***P***	**$N_{Controls}$**	**N_{Cases}**	**OR* (95% CI)**	***P***
rs1039900	CC	171	161	1.00		145	146	1.00	
	CT	321	260	0.87 (0.66–1.14)	0.32	284	232	0.82 (0.62–1.10)	0.18
	TT	123	87	0.76 (0.54–1.08)	0.13	99	71	0.72 (0.49–1.05)	0.09
	CT/TT	444	347	0.84 (0.65–1.09)	0.19	383	303	0.79 (0.60–1.05)	0.10
				P_{trend}	0.12			P_{trend}	0.07
rs907141	CC	241	214	1.00		213	198	1.00	
	CG	291	216	0.85 (0.66–1.10)	0.22	261	192	0.81 (0.62–1.06)	0.13
	GG	81	78	1.19 (0.82–1.74)	0.36	53	59	1.19 (0.78–1.81)	0.43
	CG/GG	372	294	0.92 (0.72–1.17)	0.49	314	251	0.87 (0.68–1.13)	0.31
				P_{trend}	0.99			P_{trend}	0.92
rs3731920	GG	463	381	1.00		417	344	1.00	
	AG	107	110	1.30 (0.95–1.76)	0.10	80	92	1.38 (0.99–1.92)	0.06
	AA	15	8	0.85 (0.34–2.12)	0.73	6	4	0.86 (0.24–3.08)	0.81
	AG/AA	122	118	1.26 (0.93–1.69)	0.13	86	96	1.34 (0.97–1.86)	0.08
				P_{trend}	0.45			P_{trend}	0.12
rs2059693	CC	350	256	1.00		308	223	1.00	
	CT	283	262	**1.26 (1.00–1.60)**	**0.05**	241	234	**1.33 (1.04–1.71)**	**0.03**
	TT	55	54	1.36 (0.90–2.05)	0.14	40	47	**1.60 (1.01–2.52)**	**0.05**
	CT/TT	338	316	1.28 (1.02–1.60)	**0.03**	281	281	**1.37 (1.08–1.74)**	**0.01**
				P_{trend}	**0.04**			P_{trend}	**0.008**
rs6729914	TT	182	131	1.00		161	110	1.00	
	CT	299	255	1.18 (0.89–1.56)	0.19	260	230	1.30 (0.96–1.75)	0.09
	CC	136	121	1.22 (0.88–1.71)	0.24	111	108	1.40 (0.98–2.01)	**0.06**
	CT/TT	435	376	1.19 (0.92–1.55)	0.19	371	338	**1.33 (1.00–1.77)**	**0.05**
				P_{trend}	0.22			P_{trend}	0.06

rs20596907141 – rs6729914 haplotype analysis†:

	$\%_{Controls}$	$\%_{Cases}$

		All Subjects *(577 cases, 707 controls)*				White Subjects *(508 cases, 608 controls)*			
SNP	**Genotype**	**$N_{Controls}$**	**N_{Cases}**	**OR* (95% CI)**	***P***	**$N_{Controls}$**	**N_{Cases}**	**OR* (95% CI)**	***P***
	C-T					53	50	1.0	
	T-C					26	32	**1.33 (1.09–1.63)**	**0.005**
	C-C					20	18	0.94 (0.74–1.20)	0.63
								$P_{Global\ test}$	**0.007**

*Findings with $P \leq 0.05$ are in bold type. Odds ratios adjusted for reference age, race (included in analyses of all subjects) and date of serum sample collection

†Haplotypes <1% in frequency excluded from analysis

Table 4

INHA Ex2+2064C>T genotype and risk of TGCT subtypes among White subjects

		***INHA* Ex2+2064C>T Genotype**									
Outcome	**CC (Reference)**	**CT**			**TT**			**CT/TT**			
	N	**N**	**OR* (95% CI)**	**P**	**N**	**OR (95% CI)**	**P**	**N**	**OR (95% CI)**	**P**	**P_{trend}**
Controls	308	241	-		40	-		281	-		-
Cases	223	234	**1.33 (1.04–1.71)**	**0.03**	47	**1.60 (1.01–2.52)**	**0.05**	281	**1.37 (1.08–1.74)**	**0.01**	**0.008**
Seminomas	103	86	1.07 (0.76–1.50)	0.70	21	1.62 (0.90–2.89)	0.10	107	1.14 (0.83–1.58)	0.41	0.19
Nonseminomas	120	148	**1.56 (1.15–2.10)**	**0.004**	26	**1.73 (1.00–3.00)**	**0.05**	174	**1.58 (1.19–2.11)**	**0.002**	**0.003**
Embryonal carcinoma	50	61	**1.56 (1.03–2.35)**	**0.04**	5	0.81 (0.31–2.15)	0.67	66	1.45 (0.97–2.18)	0.07	0.26
Teratoma/Teratocarcinoma	21	26	1.63 (0.89–2.99)	0.11	11	**4.54 (2.00–10.3)**	**0.0003**	37	**2.01 (1.14–3.54)**	**0.02**	**0.0008**
Others	49	61	**1.58 (1.04–2.40)**	**0.03**	10	1.58 (0.73–3.43)	0.25	71	**1.58 (1.05–2.37)**	**0.03**	**0.04**

*Findings with $P \leq 0.05$ are in bold type. Odds ratios adjusted for reference age and date of serum sample collection