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The Y Deletion gr/gr and Susceptibility to Testicular Germ Cell Tumor

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Testicular germ cell tumor (TGCT) is the most common cancer in young men. Despite a considerable familial component to TGCT risk, no genetic change that confers increased risk has been substantiated to date. The human Y chromosome carries a number of genes specifically involved in male germ cell development, and deletion of the *AZFc* region at Yq11 is the most common known genetic cause of infertility. Recently, a 1.6-Mb deletion of the Y chromosome that removes part of the *AZFc* region—known as the "gr/gr" deletion—has been associated with infertility. In epidemiological studies, male infertility has shown an association with TGCT that is out of proportion with what can be explained by tumor effects. Thus, we hypothesized that the gr/gr deletion may be associated with TGCT. Using logistic modeling, we analyzed this deletion in a large series of TGCT cases with and without a family history of TGCT. The gr/gr deletion was present in 3.0% (13/431) of TGCT cases with a family history, 2% (28/1,376) of TGCT cases without a family history, and 1.3% (33/2,599) of unaffected males. Presence of the gr/gr deletion was associated with a twofold increased risk of TGCT (adjusted odds ratio [aOR] 2.1; 95% confidence interval [CI] 1.3–3.6; $P = .005$) and a threefold increased risk of TGCT among patients with a positive family history (aOR 3.2; 95% CI 1.5–6.7; $P = .0027$). The gr/gr deletion was more strongly associated with seminoma (aOR 3.0; 95% CI 1.6–5.4; $P = .0004$) than with nonseminoma TGCT (aOR 1.5; 95% CI 0.72–3.0; $P = .29$). These data indicate that the Y microdeletion gr/gr is a rare, low-penetrance allele that confers susceptibility to TGCT.

Introduction

Testicular germ cell tumor (TGCT [MIM 273300]) is the most common cancer in men aged 15–40 years. The worldwide incidence is 7.5 per 100,000, but rates vary between countries, with the highest incidence among men of European descent (Huyghe et al. 2003; Jemal et al. 2004). The incidence of TGCT has more than doubled over the past 50 years; however, the underlying etiology is unknown (Davies 1981; Adami et al. 1994; Bergstrom et al. 1996; Bosl et al. 1997). Family history is among the strongest known risk factors for TGCT, with multiple studies documenting that brothers and fathers of patients with TCGT have an 8–12-fold and a 4–6-fold increased risk, respectively (Forman et al. 1992; Heimdal et al. 1996; Westergaard et al. 1996; Sonneveld et al. 1999; Hemminki and Li 2004). These relative risks are stronger than those for most other cancer types (Hemminki and Eng 2004), suggesting that there is a substantial genetic contribution to TGCT susceptibility.

Additional risk factors for the development of TGCT include previous TGCT, undescended testes (UDT [MIM 219050]), gonadal dysgenesis (MIM 233430), hypospadius (MIM 146450), hernia, and male infertility (Schottenfeld et al. 1980; Moller and Skakkebaek 1996; Petersen et al. 1998*b*; Akre et al. 1999). Patients with TGCT often present with abnormal semen characteristics beyond those that can be easily explained by localized or systemic effects of the tumor, and TGCT is found at increased frequency among men who showed abnormal results on semen analysis (Petersen et al.

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1998*a*; Jacobsen et al. 2000). It has been documented that patients with TGCT have lower fecundity than that of healthy controls (Moller and Skakkebaek 1999; Richiardi et al. 2004). Given the link between male infertility and TGCT—and the fact that familial aggregation has also been demonstrated for male infertility—genetic factors may exist that contribute to both conditions (Lilford et al. 1994).

Microdeletions of the Y chromosome are the most common known cause of infertility due to spermatogenic failure and account for ~10% of cases (Vogt et al. 1996; Kuroda-Kawaguchi et al. 2001). Male infertility has been associated with specific deletions of Yq11: *AZFa, -b,* and *-c* (MIM 415000). The *AZF* deletions are due to recombination between large palindromic sequences that have >99.9% identity and are composed of long, direct and indirect repeats called "amplicons" (Vogt et al. 1996; Kuroda-Kawaguchi et al. 2001; Skaletsky et al. 2003). The ampliconic portion of the male-specific Y (MSY) region of the human Y chromosome contains a high density of genes from nine gene families, with each gene existing in multiple (2–35) near-identical copies (Skaletsky et al. 2003). Genes within the ampliconic portion of the MSY are expressed predominantly or exclusively in the testis and are believed to contribute to the development and proliferation of germ cells (Reijo et al. 1995). Given the important function of these genes in spermatogenesis, the known deletions in the region, and the link between infertility and TGCT, it has been postulated that the deletions in *AZF* might be associated with TGCT. However, neither *AZF* deletions nor Y-chromosome haplotypes have previously been associated with TGCT case status (Frydelund-Larsen et al. 2003; Quintana-Murci et al. 2003).

A novel, Y-chromosome 1.6-Mb deletion, designated "gr/gr," was described recently and has been found to be associated with spermatogenic failure (Repping et al. 2003; Machev et al. 2004; de Llanos et al. 2005; Ferlin et al. 2005; Hucklenbroich et al. 2005; Lynch et al. 2005). The gr/gr deletion removes part of the *AZFc* region, including two copies of *DAZ* (deleted in azospermia [MIM 400003]) and one copy of *CDY1* (chromodomain protein, Y-linked 1 [MIM 400016]), as well as several other transcription units. Since father-to-son transmission is observed, the gr/gr deletion likely results in subfertility rather than complete infertility. The deletion was observed to be in association with numerous Y haplotypes, which suggests multiple independent recombination events (Repping et al. 2003). We hypothesized that the gr/gr deletion may play a role in TGCT susceptibility, and we have assessed this genetic factor in a large, international, multicenter study of men with and without TGCT.

Subjects and Methods

Subjects

We studied 4,441 males obtained from numerous sources that we grouped into four categories (table 1), as follows.

Familial studies.—The International Testicular Cancer Linkage Consortium (ITCLC) has obtained genomic DNA from at least one affected individual from 418 pedigrees with two or more members who have TGCT (Rapley et al. 2000). The pedigree structures and the sources of these families are shown in table 2. All cases of TGCT with DNA sampled from TGCT pedigrees in

Table 1

Prevalence of the gr/gr Deletion in TGCT-Affected and Unaffected Males

Subject Group	Prevalence of gr/gr Deletion	
	n	%
Probands of multiple-case families[a]:		
ITCLC family probands	13/396	3.3
Case-series family probands[b]	0/35	0
Total	13/431	3.0
TGCT sporadic case series[c]:		
London	12/419	2.9
Leeds (United Kingdom)	2/263	.8
Rotterdam	4/311	1.3
Toronto	2/14	14.3
Hungary	0/18	0
Other (Germany, Ireland, and Russia)[d]	0/8	0
Total	20/1,033	1.9
TGCT sporadic cases from case-control series:		
Philadelphia	3/99	3.0
Washington State[e]	5/167	3.0
Total	8/266	3.0
Affected individuals:		
With solitary TGCT[f]	0/17	0
With bilateral TGCT[g]	0/61	0
Total	0/78	0
Unaffected males:		
U.K. control series I	3/135	2.2
U.K. control series II	1/514	.2
U.K. control series III	1/225	.4
U.K. control series IV	7/400	1.7
Philadelphia	5/518	.9
Washington State	8/435	1.6
Hungary	8/394	2.0
Total	33/2,599	1.3

[a] Excludes one member from each of 12 MZ twin pairs.
[b] Nine of the case-series probands were part of the ITCLC and are included in table 2.
[c] Individuals with solitary or bilateral TGCT and no family history of TGCT.
[d] These sites are contributors to the ITCLC.
[e] Does not include 23 individuals with unknown family history.
[f] Patients ascertained because of family history of UDT.
[g] Patients ascertained as bilateral cases with no family history of TGCT.

Table 2

Characteristics of Pedigrees of ITCLC Probands with Familial TGCT

Pedigree Characteristic	No. of Pedigrees
Structure:	
Father/son	69
Sibling pairs	182
Sibling trios	9
MZ twins	12
Uncle/nephew pairs (maternal and paternal)	37
Cousin pairs (maternal and paternal)	58
Grandfather/grandson	9
Half-sibling pairs	3
Families with >3 TGCT-affected members	37
Other pedigree structure	2
Proband ascertainment location:	
Australia:	
Melbourne	4
Sydney	19
Canada	24
Czech Republic	1
Denmark	9
France	5
Germany	23
Hungary	15
Ireland	3
Netherlands	7
Norway	34
Russia	1
Switzerland	2
United States[a]:	
Penn and Indiana	21
NCI	18
USC	27
United Kingdom	205

[a] Penn = University of Pennsylvania; Indiana = University of Indiana; NCI = National Cancer Institute; USC = University of Southern California.

the ITCLC were genotyped in the present study; however, only the proband case designated by the local site was counted for statistical analysis. Probands were index cases from which the pedigree was ascertained. The ITCLC has also collected genomic DNA from 61 males affected with bilateral TGCT and 17 males with TGCT and a family history of UDT—both groups without a family history of TGCT. In addition, 35 probands with a family history of TGCT were identified from one of the case series described below, 10 of which were referred to the ITCLC and counted as one of their 418 pedigrees.

Case series.—From the United Kingdom, 682 TGCT-affected patients without a family history were recruited as members of two case series. The first was a consecutive series of patients with TGCT who attended the Royal Marsden National Health Service Trust Hospital testicular cancer clinic (London) from 1996 onward. The second case series was recruited from patients with TGCT who attended the outpatient clinic at Cookridge Hospital (Leeds) during the period from September 1999 to May 2002. In The Netherlands, 311 cases were collected from 12 different hospitals in Rotterdam and surrounding districts between 1991 and 2004. The 18 Hungarian cases were collected from the National Institute of Oncology (Budapest) from 2001 to 2004. The 14 cases from Canada were patients at the Princess Margaret Hospital Testis Clinic (Toronto) collected from 2002 to 2004.

Case-control series.—Consecutive cases of TGCT were ascertained from the University of Pennsylvania Health System (UPHS), and controls were ascertained from UPHS General Medicine and Student Health clinics and were frequency-matched by age and race; 60% of cases are incident, and the remainder are prevalent. The Fred Hutchinson Cancer Research Center (FHCRC) study is a population-based study of patients with first primary TGCT newly diagnosed between 1999 and 2002 among 18–44-year-old residents of three urban counties of western Washington State. Control subjects were frequency-matched by age and were ascertained from the general population of the three counties by use of random-digit telephone dialing. In the FHCRC study, family history of TGCT was determined only among first-degree relatives. Because there were only a few individuals of minority ethnicity in these two studies, and to remain consistent with the assumed predominate ethnicity of the case probands from other ascertainment centers for whom ethnicity is unknown, only non-Hispanic whites from the two case-control studies were included in the analyses.

Unaffected series.—Four series of otherwise healthy white British males were identified. Series I consists of male spouses of patients with cancer who attended the Royal Marsden Hospital National Health Service Trust. Series II consists of spouses of female patients who were recruited as part of the National Cancer Research Network Trial (1999–2002), the Royal Marsden National Hospital Service Trust/Institute of Cancer Research Family History and DNA Registry (1999–2004), or the case-control Genetic Lung Cancer Predisposition Study (1999–2004). Series III consists of male children from the North Cumbria Community Genetics Project from whom umbilical cord blood was obtained. Series IV consists of human male random control DNA panels that were purchased from the European Collection of Cell Cultures. Donors were ethnically matched to cases (U.K. whites) but were not age matched. Unaffected series of healthy Hungarian males were recruited from the Department of Physical Education and Sport at Semmelweis University (Budapest). In addition, 354 race-matched but not age-matched, cancer-free males ascertained through the UPHS General Medicine clinics were genotyped. Regardless of the source, patients with

TGCT and unaffected males donated biological samples and medical information with fully informed consent and local or national ethics review board approval.

Genotyping

We typed the gr/gr deletion by using a multiplex PCR that ensured that a failure of PCR was not designated as a deletion (see fig. 1*A*). The STSs sY1201 (outside the *AZFc* region) and sY1291 (a marker of the gr/gr deletion) (see fig. 1*B*) were amplified together in a 25-μl PCR reaction. The PCR mix included 12.5 pmol of each primer, 2.5 μl of 10 × $NH_4/MgCl_2$ buffer (Taq-Pro DNA Polymerase [Denville Scientific]), 6.25 nmol of each dNTP, 15 ng of genomic DNA, and 1.25 U of Taq-Pro DNA Polymerase. The PCR conditions were an initial cycle at 95°C for 5 min; 40 cycles at 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s; and a final step at 72°C for 10 min (see NCBI UniSTS database for all PCR primer sequences). The same conditions were used to type DNA extracted from white blood cells and buccal swabs. A total of 7 μl of the PCR product was sized on a 1% agarose gel, with the use of a 100-bp ladder as a standard. All samples positive for the deletion were repeated at least twice and were tested for sY1206 and sY1191 to determine whether they carried the larger *AZFc* or the b1/b3 deletion, respectively (fig. 1*B*) (Repping et al. 2004). One unaffected male with an *AZFc* deletion was omitted from further analysis. All gr/gr deletion genotyping was performed at two centers—the Institute of Cancer Research and the University of Pennsylvania. The two centers typed 33 samples in common.

To determine whether gr/gr deletions occurred on the same haplotype, we genotyped the markers (in sequence order) *DYS393, DYS19, DYS391, DYS390, DYS385a, DYS385b,* and *DYS392* for cases and controls demonstrating a gr/gr deletion (Ensembl and National Center for Biotechnology Information Web sites). Markers were end-labeled with [γ-^{32}P] ATP by use of T4 polynucleotide kinase, were amplified under standard conditions, and were electrophoresed on standard denaturing polyacrylamide gels, dried, and exposed to x-ray film. All samples were run across a single gel (per marker), to allow easy comparison of alleles between samples.

Statistical Analysis Methods

We classified each patient with TGCT on the basis of the histological diagnosis of his tumor: seminoma or nonseminoma germ cell tumor (NSGCT, including choriocarcinoma, embryonal, teratoma, and mixed cell-type TGCT), and we included only gonadal primaries. Since the Y chromosome is hemizygous, we could determine whether affected family members shared a common Y chromosome by examination of the pedigree. We designated an inheritance pattern of "paternal lineage" for those probands who had at least one affected male relative who shared the same Y chromosome (i.e., identical by descent). In contrast, "maternal lineage" designated probands with affected male relatives who did not share a common Y chromosome.

All analyses were performed using SAS v9.1 (SAS Institute). Using unconditional logistic regression, we determined odds ratio (OR) estimates as measures of the relative risk of TGCT associated with the gr/gr deletion after adjustment for geographic region or ascertainment center (Philadelphia, western Washington State, other North America, Hungary, United Kingdom, and other Europe or Australia; hereafter referred to collectively as "study centers"); 95% CIs around the ORs were estimated using the logarithmic transformation and asymptotic theory. Data on study centers were entered in the model as a series of indicator variables. To estimate and compare the associations for the outcomes of familial versus sporadic TGCT, seminoma versus NSGCT, and paternal versus maternal lineage, we used a multinomial logit model to obtain simultaneously the OR and 95% CI for the association between the gr/gr deletion and each level of outcome, adjusting for study center. Age at diagnosis of TGCT was compared nonparametrically using the Kruskal-Wallis test.

Results

After adjustment for study center, TGCT cases were significantly more likely to carry the gr/gr deletion, compared with unaffected males (aOR = 2.1; 95% CI 1.3–3.6; $P = .005$) (table 3). Among the study centers contributing both TGCT cases and unaffected males to the analysis, the distribution of the gr/gr deletion did not differ ($\chi^2 = 3.3$; 3 df; $P = .35$). The center-specific ORs were 1.7 (western Washington State; 95% CI 0.58–5.0), 1.8 (Hungary; 95% CI 0.37–8.6), 2.3 (United Kingdom; 95% CI 1.1–4.7), and 2.8 (Philadelphia; 95% CI 0.66–12). When analysis was restricted to these centers, the center-adjusted OR was very similar to that which included all data (aOR 2.1; 95% CI 1.3–3.5). In an analysis restricted to the two case-control studies that concurrently enrolled healthy controls and cases without selection for family history, the estimated center-adjusted OR was 2.3 (95% CI 0.89–6.2).

TGCT cases with a family history of TGCT were more likely to carry the gr/gr deletion than those without a family history (3.0% vs. 2.0%, respectively). In comparisons with unaffected males, the center-adjusted OR associated with carriage of the gr/gr deletion was 3.2 (95% CI 1.5–6.7; $P = .0027$) for cases with a family history and 1.9 (95% CI 1.1–3.3; $P = .024$) for cases without a family history. These OR estimates were not statistically different from one another ($P = .16$). Comparable results for analyses were obtained after the 12

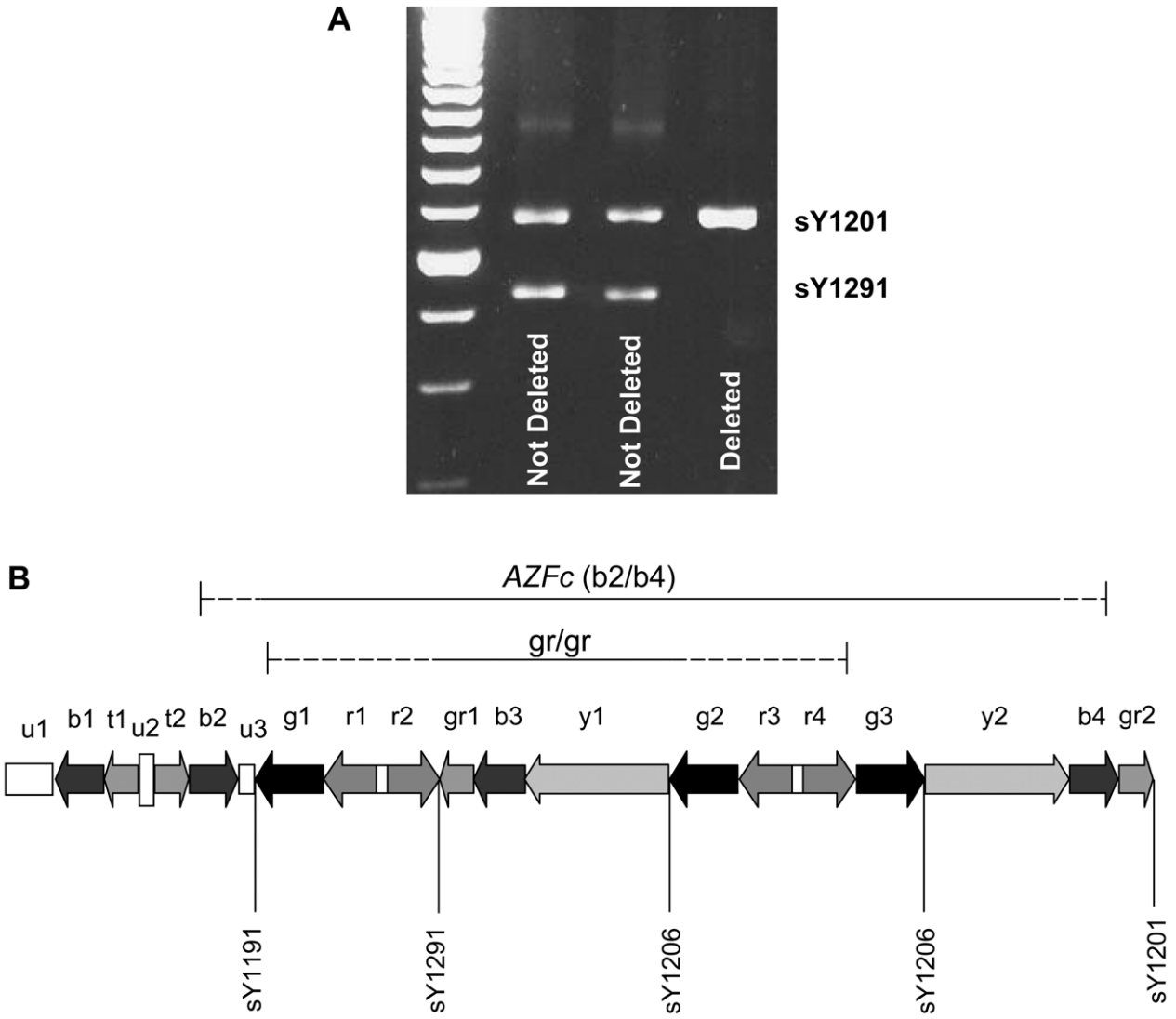

Figure 1 *A*, Genotyping of the gr/gr deletion by multiplex PCR of sY1291 and sY1201. *B*, STSs used to test for the presence of the gr/gr deletion on the Y chromosome and in the ampliconic structure of the *AZFc* region.

MZ twin probands from the case group were included (results not shown). For TGCT cases demonstrating maternal lineage, the prevalence of the gr/gr deletion was 8.6%, whereas, among those with paternal lineage, the prevalence was only 1.7%. The center-adjusted association of the gr/gr deletion was greatly elevated for probands with evidence of maternal lineage (OR 9.8; 95% CI 3.5–27) and was statistically different from that for probands with evidence of paternal lineage (OR 1.6; 95% CI 0.52–4.8; $P = .0019$ for comparison of transmission-specific ORs).

The association between gr/gr and TGCT was stronger for cases of seminoma than for NSGCT cases (seminoma: aOR 3.0; 95% CI 1.6–5.4; NSGCT: aOR 1.5; 95% CI 0.72–3.0; $P = .041$ for comparison of aORs). Presence of the gr/gr deletion was not associated with an earlier mean age at diagnosis overall (33 vs. 32 years for gr/gr deletion carriers and noncarriers, respectively; $P = .33$) or within histologic type (data not shown). Personal history of UDT could be determined for 1,263 TGCT cases. The proportion of gr/gr deletion carriers was similar among cases with a personal history of UDT (3/123) and those without (32/1,140). The gr/gr deletion was identified in 1 of 92 bilateral TGCT cases without a family history of TGCT and in none of the 39 cases of solitary TGCT with a family history of UDT.

Y haplotyping was performed on 29 TGCT cases and nine unaffected males who demonstrated a gr/gr deletion. Among the TGCT cases, 13 had a family history of disease (7 cases from the United Kingdom, 3 from

Table 3

Associations of the Y Deletion gr/gr and TGCT

Subject Group	gr/gr Deletion *n*	gr/gr Deletion %	OR (95% CI)	aOR[a] (95% CI)	aOR[b] (95% CI)
Unaffected men[c] (*n* = 2,599)	33	1.3	1.0	1.0	1.0
TGCT cases (*n* = 1,842):	42	2.3	1.8 (1.1–2.9)	2.1 (1.3–3.6)	2.1 (1.3–3.5)
Positive family history[d] (*n* = 431)	13	3.0	2.4 (1.3–4.6)	3.2 (1.5–6.7)	2.9 (1.2–6.9)
Negative family history[d] (*n* = 1,376)	28	2.0	1.6 (.97–2.7)	1.9 (1.1–3.3)	1.9 (1.1–3.4)
Paternal lineage[e] (*n* = 345)	6	1.7	1.4 (.57–3.3)	1.6 (.54–5.0)	1.7 (.50–5.9)
Maternal lineage[e] (*n* = 80)	7	8.8	7.5 (3.2–17)	9.8 (3.5–27)	9.4 (3.0–29)
Seminoma[f] (*n* = 827):	27	3.3	2.6 (1.6–4.4)	3.0 (1.6–5.4)	2.8 (1.4–5.2)
Positive family history (*n* = 146)	6	4.1	3.3 (1.4–8.1)	3.3 (1.2–9.4)	2.3 (.51–9.9)
Negative family history (*n* = 660)	20	3.0	2.4 (1.4–4.3)	2.7 (1.4–5.2)	2.8 (1.5–5.5)
Nonseminoma[f] (*n* = 806):	13	1.6	1.3 (.67–2.4)	1.5 (.72–3.0)	1.7 (.79–3.5)
Positive family history (*n* = 145)	5	3.5	2.8 (1.1–7.2)	4.9 (1.7–14)	4.0 (1.1–14)
Negative family history (*n* = 653)	8	1.2	.96 (.44–2.1)	1.3 (.55–3.0)	1.4 (.60–3.3)

[a] Adjusted for study center (Philadelphia, western Washington State, other North America, Hungary, United Kingdom, and other Europe or Australia).

[b] Includes only those study centers that contributed both patients with TGCT and unaffected men (Hungary, Philadelphia, United Kingdom, and western Washington State) and is adjusted for those study centers.

[c] Reference group for all comparisons.

[d] Information on family history of TGCT was not available for 23 cases (17 seminoma and 6 nonseminoma), including 1 gr/gr deletion carrier. In addition, 12 cases (4 seminoma, 2 nonseminoma, and 6 of unknown tumor type) with an affected MZ twin were excluded from analyses of family history.

[e] Information on six TGCT cases with a family history (one from the ITCLC familial studies and five from the case-control studies) was insufficient to determine the type of Y-chromosome transmission.

[f] Information on tumor type was not available for 209 TGCT cases, including 2 gr/gr deletion carriers.

Norway, 2 from Hungary, and 1 from Australia), and 16 had no family history (14 from the United Kingdom and 2 from Canada). All unaffected males were from the U.K. series. Of the 38 samples tested, a total of 23 different haplotypes were seen, with each haplotype differing by at least one microsatellite marker. Six haplotypes were observed twice, and one haplotype was observed in three samples. Of the six shared haplotypes observed twice, two were present in one control and one case sample (all samples from the United Kingdom), three were observed in case samples only (haplotype 3 was seen in two Norwegian cases with a family history, haplotype 4 was present in a U.K. case with a family history and in a single Canadian case, and haplotype 5 was seen in two single U.K. cases), and one was detected in a control sample only. The haplotype observed three times was seen in the two Hungarian cases with a family history and in one U.K. case with no family history. When we examined separately the subgroup of Y haplotypes from U.K. cases and unaffected males, 26 haplotypes were observed that differed by at least one microsatellite. More conservatively, we observed 19 haplotypes in which allele size differed at three or more microsatellites. The allele frequencies for each microsatellite marker did not differ significantly between the U.K. cases with the gr/gr deletion and the U.K. unaffected males with the gr/gr deletion.

Transmission of the gr/gr deletion could be evaluated in only nine cases (six paternal lineage and three maternal lineage) by genotyping other male family members who shared a Y chromosome. In paternal lineage cases, we could demonstrate the transmission of the gr/gr deletion between affected cases (usually fathers or siblings), either by demonstration of the deletion in both affected individuals or by the presence of the deletion in unaffected male relatives in the Y lineage between affected cases. In cases with maternal lineage, transmission of the gr/gr deletion was demonstrated to or from another unaffected male relative who shared a Y chromosome. All cases but one demonstrated transmission of the gr/gr deletion. In one sib pair, one brother carried the gr/gr deletion, and the other did not. DNA was unavailable from the father of these siblings; however, Y haplotyping and genomewide relationship testing showed that these brothers were full siblings, suggesting a de novo origin of the gr/gr deletion. In this case, the brother with the gr/gr deletion was not the proband designated by the referring institution and thus was not counted as part of the statistical analyses.

Discussion

Our study demonstrates that the gr/gr deletion is associated with a twofold increased risk of TGCT, which increases to a threefold risk among patients with a family history of disease. The observed association was stronger

Table 4

Case-Only Associations of the Y Deletion gr/gr and Family History of TGCT

Characteristic of TGCT Cases[a]	gr/gr Deletion			
	n	%	OR (95% CI)	aOR[b] (95% CI)
Negative family history[c] (n = 1,376)	28	2.0	1.0	1.0
Positive family history (n = 431):	13	3.0	1.5 (.77–2.9)	1.7 (.81–3.4)
Paternal lineage[d] (n = 345)	6	1.7	.85 (.35–2.1)	.97 (.38–2.4)
Maternal lineage[d] (n = 80)	7	8.8	4.6 (2.0–11)	5.2 (2.1–13)

[a] Information on family history of TGCT was not available for 23 cases. In addition, 12 cases with an affected MZ twin were excluded from analyses of family history.

[b] Adjusted for study center (Philadelphia, western Washington State, other North America, Hungary, United Kingdom, and other Europe or Australia).

[c] Reference group for all comparisons.

[d] Information on six TGCT cases with a family history (one from the ITCLC familial studies and five from the case-control studies) was insufficient to determine the type of Y-chromosome transmission.

for seminoma than for NSGCT cases. There was no evidence of an association between the deletion and the presence of UDT or bilateral disease. We could not directly determine the relationship between TGCT, infertility, and the gr/gr deletion in our study, because most centers did not collect information about fertility status.

The association between the gr/gr deletion and TGCT case status was seen within each study center from which cases and unaffected males were genotyped, but, because of the rarity of both the disease and the gr/gr deletion, the *P* values for the comparisons were most significant when the sample populations were combined. Although our conclusions are limited to white males of European ancestry, individuals from this population constitute the vast majority of TGCT cases diagnosed around the world (Ferlay et al. 2004). In the United States, white patients account for 94% of all TGCT cases (Ries et al. 2004).

The frequency of the gr/gr deletion was higher among cases with a positive family history of TGCT than among those without a recorded family history. Probands from families demonstrating maternal lineage were at greater risk than probands from families demonstrating paternal lineage. Although this result may be somewhat counterintuitive, the gr/gr deletion impacts male fertility, which may reduce the number of families with the potential for paternal lineage. In addition, the estimate of the gr/gr deletion association among probands with evidence of maternal lineage, though strong, is imprecise because of the small numbers in this subgroup. It is likely that the gr/gr deletion does not act in isolation to increase TGCT risk and that additional genetic and/or environmental factors, which may also cluster in families, operate in concert with the deletion. Since unaffected men are not at risk for becoming a positive-family-history TGCT case, we reran analyses of family history of TGCT and "lineage" among cases only (see table 4). The results support those given in table 3.

Several aspects of our study limit the strength of our inferences. First, only 15% of cases and 23% of unaffected males were drawn from designed epidemiologic case-control studies with contemporaneous participant recruitment. Although the comparability of ascertained unaffected males to TGCT cases in the remaining study centers is less certain—and potentially could have introduced bias into our findings—we found similar associations regardless of the source of TGCT cases and the reference group.

Even though it is not possible to rule out the effect of population stratification in our study, it is not likely that this bias greatly impacted our results. Foremost, we successfully reproduced our overall association between the gr/gr deletion and TGCT among several subgroups that were roughly defined by geographic area of ascertainment, the only proxy measure for race/ethnicity that is available for all subjects in the present study. Furthermore, our association was noted separately in the two epidemiological case-control studies.

Although large-scale Y haplotyping of all 4,441 study subjects is beyond the scope of the current analysis, we attempted to determine whether the gr/gr deletion is inherited on a unique background Y haplotype, by evaluating seven microsatellite loci in a proportion of gr/gr deletion–carrying TGCT cases and unaffected males. Despite that microsatellite markers are not as evolutionarily stable as SNP markers, variation at several of these loci would indicate that most gr/gr deletions observed arose on a different Y-chromosome background. Haplotyping of these markers strongly suggested that the majority of deletions arose on different haplotypes and not from a common founder haplotype. Among 38

individuals tested, >23 different Y haplotypes were observed; only 7 were observed more than once and only 1 of those was seen in three individuals. Even after limiting haplotype analysis to a more homogenous group and applying a more stringent criterion to define haplotypic difference (dissimilar allele sizes at three or more markers), we found 19 different haplotypes among the 29 TGCT cases and unaffected controls ascertained in the United Kingdom. Our observation of the gr/gr deletion arising on multiple distinct Y haplotypes is consistent with the data from Repping et al. (2003). We believe that, although bias due to population stratification cannot be completely ruled out, our data suggest that a clear bias introduced by the segregation of Y haplotypic background within the different ethnic groups that comprise white individuals of European descent is unlikely.

Finally, although the sample size was very large (particularly for a relatively rare cancer), the rarity of the Y-chromosome microdeletion led to relatively wide CIs. As such, it will be important to verify this finding in an independent sample and to do so specifically among patients who have TGCT with a positive family history—the group in which we observed the strongest evidence of association. However, because familial TGCT is extremely uncommon and, to our knowledge, there are no other extant TGCT study populations large enough to test our hypothesis, confirmation in the near future seems unlikely.

Our data provide evidence that the gr/gr deletion is a risk factor for TGCT, suggesting a novel role for microdeletions of the Y chromosome in addition to infertility. The gr/gr deletion does not completely eliminate any of the testis-specific genes or transcription-unit families, but it does reduce the copy number of several genes, removing two of the four copies of the *DAZ* gene, one of three copies of *BPY2* (basic charge, Y-linked 2 [MIM 400013]), and one of two copies of *CDY1*. All these genes are specifically expressed in male germ cells and have roles in male germ cell development and differentiation (Reijo et al. 1995, 1996, 2000; Saxena et al. 1996; Kleiman et al. 2003; Ginalski et al. 2004). *DAZ* and *CDY1* have been selectively maintained on the Y chromosome (Dorus et al. 2003) and encode RNA-binding proteins expressed in premeiotic spermatagonia (Reijo et al. 1995; Reynolds and Cooke 2005) and a protein with histone acetyltransferase activity (Lahn et al. 2002), respectively. The function of these genes in the maintenance of normal germ cell activity is incompletely understood, and future work is needed to better characterize the impact of copy loss on germ cell development and differentiation, which may result in a higher risk of neoplasia.

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Web Resources

The URLs for data presented herein are as follows:

Ensembl, http://www.ensembl.org/Homo_sapiens/ (for locus information for *DYS393, DYS19, DYS391, DYS390, DYS385a, DYS385b,* and *DYS392*)

National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/ (for locus information for *DYS393, DYS19, DYS391, DYS390, DYS385a, DYS385b,* and *DYS392*)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for TGCT, UDT, gonadal dysgenesis, hypospadius, *AZF* deletion–associated male infertility, *DAZ, CDY1,* and *BPY2*)

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