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Variants in *BAK1*, *SPRY4,* and *GAB2* are associated with pediatric germ cell tumors: a report from the Children's Oncology Group

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Abstract

Introduction—Germ cell tumors (GCT) are a rare form of childhood cancer that originate from the primordial germ cell. Recent genome-wide association studies (GWAS) have identified susceptibility alleles for adult testicular GCT (TGCT). Here we test whether these SNPs are associated with GCT in the pediatric and adolescent population.

Methods—This case-parent triad study includes individuals with GCT diagnosed between ages 0–19. We evaluated 26 SNPs from GWAS of adult TGCT and estimated main effects for pediatric GCT within complete trios (N=366) using the transmission disequilibrium test. We used Estimation of Maternal, Imprinting and interaction effects using Multinomial modelling to

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Conflict of Interest

The authors have no financial conflicts of interest to disclose.

evaluate maternal effects in non-Hispanic white trios and dyads (N=244). A Bonferroni correction was used to account for multiple comparisons.

Results—A variant in *SPRY4* (rs4624820) was associated with reduced risk of GCT (OR [95% CI]: 0.70 [0.57, 0.86]). A variant in *BAK1* (rs210138) was positively associated with GCT (OR [95% CI]: 1.70 [1.32, 2.18]), with a particularly strong estimated effect for testis tumors (OR [95% CI]: 3.31 [1.89, 5.79]). Finally, a SNP in *GAB2* (rs948662) was associated with increased risk for GCT (OR [95% CI]: 1.56 [1.20, 2.03]). Nominal associations ($p<0.05$) were noted for eight additional loci. Maternal effects were observed for KITLG SNP rs4474514 (OR [95% CI]: 1.66 [1.21, 2.28]) and rs7221274 (OR [95% CI]: 2.07 [1.43, 2.99]), near TEX14, RAD51C, and PPM1E.

Conclusions—We observed associations between SNPs in *SPRY4*, *BAK1*, and *GAB2* and GCTs. This analysis suggests there may be common genetic risk factors for GCT in all age groups.

Introduction

Germ cell tumors (GCTs) are a form of childhood cancer that is rare in children age 0–14 but accounts for approximately 15% of malignancies diagnosed in adolescents and young adults age 15–19 (Horner MJ). Most GCTs occur in the ovaries or testes; however extragonadal tumors also occur and likely arise from abnormal germ cell migration during fetal development. Although it is presumed that GCTs share a common cell of origin, the primordial germ cell (PGC) (Schneider, et al. 2001; Schneider, et al. 2006), these tumors are heterogeneous and include teratomas and yolk sac tumors (YST) of infants and young children (Type I tumors) and germinomas and nonseminomas of adolescents and young adults (Type II tumors) (Oosterhuis and Looijenga 2005). In addition, tumors can present as a mixture of these types. Their etiology remains poorly understood and evidence suggests that GCTs, including those in adults, are initiated in utero (Henderson, et al. 1979; Schottenfeld, et al. 1980; Sonne, et al. 2008). Thus, alterations in normal embryonic development are likely to be especially relevant to GCT etiology.

Studies of adult testicular GCT (TGCT) have shown strong heritability of these tumors (Bromen, et al. 2004; Forman, et al. 1992; Heimdal, et al. 1996; Sonneveld, et al. 1999; Westergaard, et al. 1996), suggesting a role for genetic factors in their etiology (Crockford, et al. 2006). There are few studies on family history of cancer in pediatric GCTs, and none with a sufficient sample size to specifically evaluate family history of GCT (Johnston, et al. 1986; Shu, et al. 1995; Walker, et al. 1988). Although a major susceptibility gene for adult TGCT has not been identified, genome-wide association studies (GWAS) have found susceptibility loci for TGCT near *KITLG*, *SPRY4*, *BAK1* (Kanetsky, et al. 2009; Rapley, et al. 2009); *DMRT1*, *TERT*, *ATF7IP* (Kanetsky, et al. 2011; Turnbull, et al. 2010); *SLC25A44, UCK2, DAZL, CENPE, PITX1, PRDM14, MFSD1, TEX14, RAD51C, PPM1E, MCM3AP*(Ruark, et al. 2013; Schumacher, et al. 2013); *HPGDS, MAD1L1, RFWD3* (Chung, et al. 2013; Litchfield, et al. 2015b), *HNF1B* (Kristiansen, et al. 2015), *and GSPT1, ZFPM1, GAB2,* (Litchfield, et al. 2015a). Several of these genes are involved in survival (*KITLG* (Runyan, et al. 2006)) or early differentiation of PGCs (*DAZL* (Kee, et al. 2009)), regulation of transcription (*DMRT1* (Krentz, et al. 2009) and *PRDM14* (Tsuneyoshi, et al. 2008; Yamaji, et al. 2008)), and spermatogenesis (*TEX14, RAD51C, PPM1E*

(Greenbaum, et al. 2006; Ihara, et al. 2005; Karlberg, et al. 2004; Kuznetsov, et al. 2007)) and therefore are plausibly involved in TGCT, and potentially pediatric GCT, etiology.

Several susceptibility genes for GCT have been identified among male mice in the 129/Sv mouse model of testicular teratoma (Oosterhuis and Looijenga 2005), including *Dnd1* (Youngren, et al. 2005) and *Dmrt1* (Krentz, et al. 2009). The tumors that arise in 129/Sv mice most closely resemble GCTs that occur in children (Oosterhuis and Looijenga 2005), providing evidence that genetic susceptibility may also be important in pediatric GCTs. Additionally, adult and pediatric GCTs share the primordial germ cell as their common cell of origin and tumors of differing types, including both testicular and ovarian tumors, can be classified together based on their chromosomal complement and developmental potential as proposed by Oosterhuis and Looijenga (Oosterhuis and Looijenga 2005). Due to these shared characteristics, we hypothesized that genetic risk factors of adult TGCT may impact pediatric GCTs of all types. Furthermore, Murray et al. (Murray, et al. 2015) recently showed that all malignant GCTs over-express two microRNA clusters, regardless of patient age, histology, or tumor location, providing additional evidence of common biological characteristics across all types of GCTs. In an independent study, we previously reported associations between SNPs in three of these genes (KITLG, SPRY4, and BAK1) and pediatric GCT (Poynter, et al. 2012), providing further support for a shared genetic etiology. Here, we analyzed 26 SNPs at 21 independent loci identified in GWAS of adult TGCT in a case-parent triad study of pediatric and adolescent GCT to determine whether variation at these loci correlates with risk in the younger age group.

Results

DNA was available for 366 complete trios. Among the children with GCTs, 184 (50%) were male and 182 were female, and 59% were age 11–19 years at diagnosis (Table 1). Most participants were white, non-Hispanic (56%) or Hispanic (35%). Testicular and ovarian tumors represented 25% and 27% of our sample, respectively, while 21% were intracranial and 28% were extragonadal. The most frequent tumor histologies were mixed/other (32%) and germinomas (29%). Twenty two percent of tumors were classified as teratomas and 17% were yolk sac tumors (Table 1).

Among all samples, three loci reached the multiple testing-corrected statistical significance threshold ($p<0.00192$ for 26 tests; Table 2). A variant in *SPRY4* (rs4624820) was associated with GCT in all samples (OR [95% CI]: 0.70 [0.57, 0.86]). Associations were statistically significant among younger children (OR and 95% CI: 0.64 [0.46, 0.89]) and in germinomas (OR [95% CI]: 0.53 [0.36, 0.78]). Point estimates were also decreased with nominally significant p-values ($p<0.05$) for children age ≥11 at diagnosis, ovarian tumors, and tumors of mixed histology (Figure 1).

A variant in *BAK1* (rs210138) was also significantly associated with GCT overall (OR and 95% CI: 1.70 [1.32, 2.18]), with a particularly strong estimated effect for tumors located in the testis (OR and 95% CI: 3.31 [1.89, 5.79]). The estimated association was stronger for male adolescents (OR and 95% CI: 2.71 [1.76, 4.19]) than for younger males (OR and 95%

CI: 1.43 [0.72, 2.83]) or females of any age, although confidence intervals overlap (Figure 2).

Finally, the minor allele of rs948662 (*GAB2*) which tags the originally reported rs7107174 (r^2=0.99) from Litchfield et al (Litchfield, et al. 2015a) was associated with increased risk of GCT overall (OR and 95% CI: 1.56 [1.20, 2.03]). This SNP was also associated nominally significant for both <11 and ≥11 age groups, germinomas, intracranial tumors, ovarian tumors, and males (Figure 3).

Overall and stratified results based on analyses of participants of all races and ethnicities for all variants are presented in supplementary figures. When we restricted analyses to include only non-Hispanic white participants (Table 2), a variant in *MFSD1* (rs8046148) was associated with GCT risk (OR and 95% CI: 0.50 [0.35, 0.72]). Effect estimates for the variants within *SPRY4* and *BAK1* (rs4624820 and rs210138, respectively), but not *GAB2* (rs948662), were stronger and remained statistically significant.

In the analysis of maternal and parent-of-origin effects, we observed maternal effect associations for a variant in *KITLG* (rs4474514; OR and 95% CI: 1.66 [1.21, 2.28]) and a variant near *TEX14, RAD51C,* and *PPM1E (*rs7221274; OR and 95% CI: 1.63 [1.26, 2.12]; Table 3). We observed a maternal parent-of-origin association for the BAK1 variant rs210138 (p=0.0016) and a paternal parent-of-origin association for the variant near *TEX14, RAD51C,* and *PPM1E* (rs7221274; p=0.00019).

Discussion

We observed statistically significant associations between SNPs in *SPRY4*, BAK1, and *GAB2* and pediatric and adolescent GCTs, with a particularly strong association between the variant in *BAK1* and testicular tumors. We also observed maternal associations for SNPs near *KITLG* and the locus containing *TEX14, RAD51C,* and *PPM1E*. We did not observe either direct or maternal effects for the other loci identified by previous studies of adult TGCT, however there were eight other loci for which p-values met the unadjusted but not the adjusted significance threshold. We investigated a relatively small number of SNPs with strong evidence to justify possible associations, and the multiple comparisons correction greatly reduced study power with the limited sample size. Without subsequent validation studies it is not possible to distinguish whether these additional eight loci have real or random associations with pediatric TGCT risk, and we still consider them plausible candidates for future investigation.

The SNPs identified in the GWAS of adult TGCT were notable both for their large effect sizes relative to GWAS of other adult cancers and because many were found in or near genes that are important germ cell biology. Only one previous study has examined the association between adult TGCT GWAS loci and pediatric GCT (Poynter, et al. 2012). That study, which used an independent sample set with no overlap in the current study, evaluated four SNPs, three of which were tested in the current analysis (rs4474514, rs210138, and rs755383). In that study the *BAK1* SNP (rs210138) was also associated with increased risk of GCT overall (OR and 95% CI: 1.80 [1.10, 2.95]), particularly for tumors located in the

gonads (OR and 95% CI: 2.28 [1.24, 4.20]). *KITLG* SNP rs4474514 was also associated with tumors located in the gonads (OR and 95% CI: 2.12 [1.09, 4.14] and with GCT in adolescents (OR and 95% CI: 2.28 [1.09, 4.79]) in our previous study. No associations were observed between *DMRT1* SNP rs755383 and pediatric GCT. The final SNP tested in that analysis was in *SPRY4* (rs4324715) and was associated with increased risk of GCT in males (OR and 95% CI: 2.42 [1.01, 5.80]), among adolescents (OR and 95% CI: 2.40 [1.19, 4.83]), and for tumors located in the gonads (OR and 95% CI: 1.84 [1.03, 3.29]). This variant is in moderate to high linkage disequilibrium with the variant typed and analyzed in the present study (rs4624820; r^2=0.84). In this larger study of pediatric GCT, we did not replicate findings for the *KITLG* variant, although we did observe maternal effects for this SNP. This discrepancy in findings may be due to differences in sample composition, as the previous study consisted of 71% female cases. However when we limited the current study to female cases, we observed a null association between the *KITLG* variant and GCT. Alternatively, the association reported may have been a chance finding which was not observed within the larger present dataset. We also observed similar results for the *BAK1* SNP and a variant in *SPRY4* in high LD with the previously evaluated variant. When we combined estimates from both datasets using meta-analytic techniques, the association between the BAK1 variant remained robust (meta-analytic OR and 95% CI: 1.81 [1.57, 2.09]; p-value: 0.00003). Meta-analysis results for the variants within *DMRT1* and *KITLG* remained null; we were not able to combine estimates for the *SPRY4* locus since different variants were used in each study.

Mouse studies show that germ cells originate on the yolk sac then migrate through the dorsal mesentery of the hindgut (Lawson and Hage 1994) before populating the genital ridges (Wilhelm, et al. 2007). In normal fetal development, PGCs that remain in the midline go through a rapid process of cell death through intrinsic apoptosis pathways (Runyan, et al. 2006). Thus it is plausible that genes involved in the survival, differentiation, and apoptosis of PCGs are relevant to germ cell tumor development. rs210138 is located within an intron of *BAK1* (*BCL2*-antagonist/killer 1), which encodes a protein that induces apoptosis by binding to and antagonizing the apoptosis repressor activity of *BCL2* and other anti-apoptotic proteins (Yan, et al. 2000).

The KIT/KITLG pathway is involved in the survival of primordial germ cells during migration to the genital ridge (Runyan, et al. 2006). The *KITLG* pathway also represses expression of *BAK1* in testicular germ cells, and interaction of *BAK1* with anti-apoptotic proteins is implicated in the germ cell apoptosis that occurs in response to blockage of this pathway (Yan, et al. 2000). The mouse orthologue of *KITLG* (Kitl, encoded at the steel locus) is also a modifier of TCGT susceptibility in the 129/Sv mouse (Heaney, et al. 2008). *SPRY4* (Sprouty RTK signaling antagonist 4) is also associated with the *KITLG* pathway (Frolov, et al. 2003). Additionally, it inhibits the mitogen-activated protein kinase (*MAPK*) pathway (Sasaki, et al. 2003). Ovarian germ cell tumors were recently found to harbor somatic mutations in *MAPK1* (Zou, et al. 2016).

GAB2 (GRB2-associated binding protein 2) encodes a docking protein involved in signal transduction from tyrosine kinases. Its role in carcinogenesis is highly plausible given its involvement in cell proliferation and cell transformation. Indeed, it has previously been

shown to function as a proto-oncogene within melanoma, hepatocellular carcinoma, and breast, colorectal, and ovarian cancers (Adams, et al. 2012; Chen, et al. 2016; Matsumura, et al. 2014). Although the association with rs948662 that exceeded the multiple-testing correction threshold was for overall GCT risk, we observed increased point estimates of similar magnitude and nominal associations for this variant and ovarian and intracranial tumors and germinomas.

We observed maternal effects for SNPs near *KITLG* (rs4474514) and the locus containing *TEX14, RAD51C,* and *PPM1E* (rs7221274). In 129/Sv mice, the ortholog of *KITLG* is a modifier of TGCT risk (Heaney, et al. 2008). Although the functional mechanism of *KITLG* in TGCT is not known, studies of knockout mice suggest these genes may prevent germ cell apoptosis (Runyan, et al. 2006). While the role of the maternal genome in modifying risk of pediatric GCT is unclear, a recent study in zebrafish showed that maternal genes are essential in the regulation of PGC properties in the offspring (Forbes, et al. 2015). Furthermore, a study of maternal effects in testicular cancer showed increased risk in offspring associated with interaction between maternal variants in *KITLG* and *SHBG* (Nsengimana and Barrett 2012). Additional support for a role of maternal genetic factors on offspring GCT risk includes evidence that variations in maternal cytochrome p450 gene impact offspring risk of TGCT (Starr, et al. 2005) and the observation of a parent-of-origin effect for *SPRY4* (Karlsson, et al. 2013). In the present study, maternal origin of the variant allele conferred an increased risk of TGCT in offspring.

We detected a maternal parent-of-origin effect of the *BAK1* variant rs210138 and a paternal parent-of-origin effect of the rs7221274 variant near *TEX14, RAD51C, PPM1E*. The mostly likely impact of a parent-of-origin effect is imprinting, an epigenetic event in which gene expression is modified based on the transmitting parent (Guilmatre and Sharp 2012). Our observations of parent-of-origin effects at two loci may indicate that gene expression of these loci is altered in pediatric and adolescent GCT. Molecular evidence for imprinting is necessary to confirm this potential.

Pediatric and adolescent GCTs are a heterogeneous group of tumors. Recent studies have shown that the differing histological subtypes not only have separate DNA methylation profiles (Amatruda, et al. 2013; Jeyapalan, et al. 2011) but also exhibit differences in microRNA expression (Murray, et al. 2010) and distinct transcriptome profiles (Palmer, et al. 2008). Furthermore, adult TGCTs arise from PGCs at a different developmental stage than those that occur in children (Oosterhuis and Looijenga 2005; Sievers, et al. 2005). Therefore it is not surprising that our results differed by age group, histology, and tumor location and that we did not observe associations for all SNPs. However, recent evidence suggests that there may be cases of TGCT diagnosed in adulthood that, other than age of onset, meet the criteria of a pediatric GCT (Oosterhuis, et al. 2015). Thus there may be shared etiology between pediatric and adult cases among a subgroup of patients.

This study is the first to use germline DNA in an investigation of genetic risk factors for pediatric GCT. It is also the first comprehensive examination of the association of TGCT-associated variants with risk of pediatric GCT. Strengths of this study include the strong *a priori* hypothesis that adult TGCT and pediatric GCT may share common genetic causes,

including the biological plausibility of variants identified by adult TGCT GWAS. This study had a large sample size overall, although our power was limited in subtype analyses.

We found evidence that GCTs in all age groups may share some genetic risk factors. In addition, ours is the first study to report maternal effects in pediatric GCT for genes associated with adult TGCT. Additional studies are needed to confirm these results. Fine mapping and functional studies are also needed to elucidate the mechanisms driving these associations in GCTs in all age groups.

Materials and Methods

Study participants

Children and adolescents diagnosed with GCT were identified through the Children's Oncology Group Childhood Cancer Research Network (CCRN) (Musselman, et al. 2014) and invited to participate in this case-parent triad study. Children were eligible for the study if they had a primary diagnosis of GCT including germinoma (ICCC code (Steliarova-Foucher, et al. 2005) 9060–9065), teratoma (9080–9084), embryonal carcinoma (9070–9072), yolk sac tumor (9071), choriocarcinoma (9100, 9103, 9104), and mixed GCT (9085, 9101, 9102, 9105) in all sites including the brain between July 1, 2008 and December 31, 2015. Additional eligibility criteria included age < 20 years at diagnosis, the availability of at least one biological parent alive and willing to participate, and ability to complete a questionnaire in English or Spanish. Pathology reports were provided by the participating Children's Oncology Group institutions per the CCRN protocol.

Saliva DNA was collected from the children with GCT and their biological parents for use in genetic analyses, and lifestyle and environmental risk factors were assessed using mailed questionnaires. All participants received a saliva collection kit (Oragene) with instructions and mailer with return postage. Parents and children ≥ 5 years received a standard Oragene saliva DNA kit. Children < age 5 years received the Oragene kit for assisted DNA collection, which uses absorbent sponges. Participants were re-contacted within six months of donation for additional samples if DNA yield was insufficient. This analysis is based on an interim dataset including participants recruited to the study through December 13, 2013.

All study procedures were approved by the University of Minnesota Institutional Review Board.

a priori Variant selection

A literature review of search terms 'germ cell tumor', 'testicular germ cell', 'genetic susceptibility', and 'GWAS' identified 10 articles (Chung, et al. 2013; Kanetsky, et al. 2009; Kanetsky, et al. 2011; Kristiansen, et al. 2015; Litchfield, et al. 2015a; Litchfield, et al. 2015b; Rapley, et al. 2009; Ruark, et al. 2013; Schumacher, et al. 2013; Turnbull, et al. 2010). These manuscripts together identified 31 variants in 25 genes that reached study-wide significance and were therefore considered in the current study. Only the following four variants were not tagged well (r^2>0.80) by any variant on the genotyped array and hence were not assessed in the current study: rs11705932 in *TFDP2*, rs12699477 in *MAD1L1* (though rs3778991 in *MAD1L1* is reported), rs7010162 upstream of *PRDM14*, and

rs2195987 in *RPSAP58*. The final variant excluded from the current study (rs6897876) is in high LD (r^2>0.94) with rs4624820, and both are in the same region of *SPRY4*. As rs6897876 was tagged by rs4624820, we elected to include only the latter variant in this analysis.

DNA extraction

Saliva samples were stored at room temperature and batched for DNA extraction. We performed automated DNA isolation using Autopure LS system (Qiagen) and Puregene chemistry (Gentra Systems) according to the manufacturer's protocols. DNA yield was quantified using 1:10 diluted DNA performed in triplicate by quantitative real-time PCR using absolute quantification on an ABI 7900 Prism real-time instrument and extracted DNA was aliquoted and stored at -20°C until genotyping.

Genotyping

Genotyping was performed by the University of Minnesota Genomics Center (UMGC) using Illumina HumanCoreExome-12 version 1.1_B BeadChips (Illumina, San Diego, CA, USA) according to the manufacturer's specified protocol. Allele cluster definitions for each variant were determined using Illumina GenomeStudio Genotyping Module and the intensity data from those study samples with high quality data (i.e., the 95.4% of samples with a Log R ratio standard deviation < 0.35). The resulting cluster definition file was used on all study samples to determine genotype calls and quality scores. Genotype clusters for all *a priori* selected variants of interest were manually inspected and adjusted as needed using Genvisis (http://www.genvisis.org). For all other variants, genotype calls were made when a genotype yielded a quality score (Gencall value) of 0.25 or higher. The final raw data set contained 542,585 variants that could be used for inferring genetic ancestry, validating putative relationships, and identifying cryptic relatedness.

Quality Control

A CEPH Utah HapMap sample was placed on each DNA plate of 96 samples processed together in the laboratory. In addition, 76 blind duplicate samples were distributed among the 16 plates to assess genotyping concordance and to detect plate effects. Blind duplicate reproducibility was 99.80% for all markers, 99.98% for markers that passed QC and 100.00% for those markers used in the present analyses. There was no evidence for plate-specific genotype effects.

Genvisis was used to identify samples with sex aneuploidy. Eight children with GCTs had Klinefelter syndrome (47,XXY karyotype) and one unaffected mother had triple X syndrome (47,XXX karyotype). The affected daughter of the individual with triple X syndrome had a normal 46,XX karyotype. No individuals with Turner syndrome, mosaic Turner syndrome, mosaic Klinefelter syndrome or mosaic triple X syndrome were observed.

Samples having genotypes for at least 98% of the variants were considered for inclusion in analyses. Variants with a call rate of 98% or lower were excluded from further quality control analyses (n=19,782; 3.65%). Variants were removed if: (1) the minor allele frequency of the founders (parents) was less than 0.01 in the data set (n=242,287; 44.65%);

(2) there were differential rates of missing genotypes in the probands versus the parents ($p<0.0001$; n=9) or males versus females ($p<0.0001$; n=1121, 0.21%), (3) allele frequencies differed between males and females ($p<0.0001$; n=143; 0.03%), (4) significant deviation from Hardy–Weinberg equilibrium was observed in the founders ($p<0.00001$; n=8123; 1.5%), or (5) significant deviation from expected when imputing the variant from nearby markers using PLINK's ---mishap test ($p<0.0001$; n=8133; 1.5%). Many markers failed multiple tests. The final data set consisted of 275,996 variants that passed all quality control measures for use in defining ancestry.

Statistical methods and analysis

The transmission disequilibrium test (TDT) as implemented in PLINK was the primary method for estimating main effects, which by design controls for population stratification. Subgroup analyses were conducted by age, sex, histology, and tumor location. Maternal effects and parent of origin effects were tested using a log-linear model in EMIM (Estimation of Maternal, Imprinting and interaction effects using Multinomial modelling). This method requires a homogeneous ancestral population and was therefore restricted to non-Hispanic whites. This status was determined by performing a principal components analysis (PCA) as implemented in EIGENSOFT (Price, et al. 2006)that included HapMap samples (CEPH Caucasian, Yoruba, Han Chinese, Japanese) as anchors. We used a Bonferroni correction to establish a threshold for statistical significance ($p<0.00192$ for 26 tests). The allele frequency of all markers passing quality control was compared between CEU HapMap samples and study samples that self-described as white. Those markers with significantly different frequencies ($p<0.05$; n=9,123) were excluded prior to running the PCA. The first two principal components from the analysis were plotted and those samples clustering tightly with the CEU samples were defined as white non-Hispanic in subsequent EMIM analyses.

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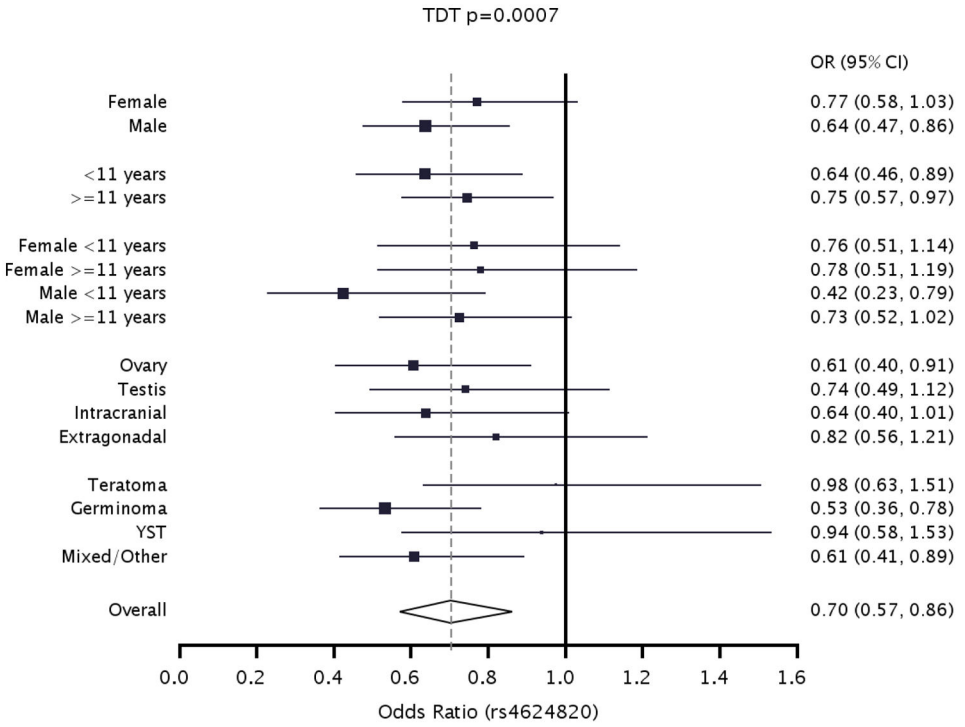

Figure 1.

Subgroup specific associations between rs4624820 (*SPRY4*) and GCT

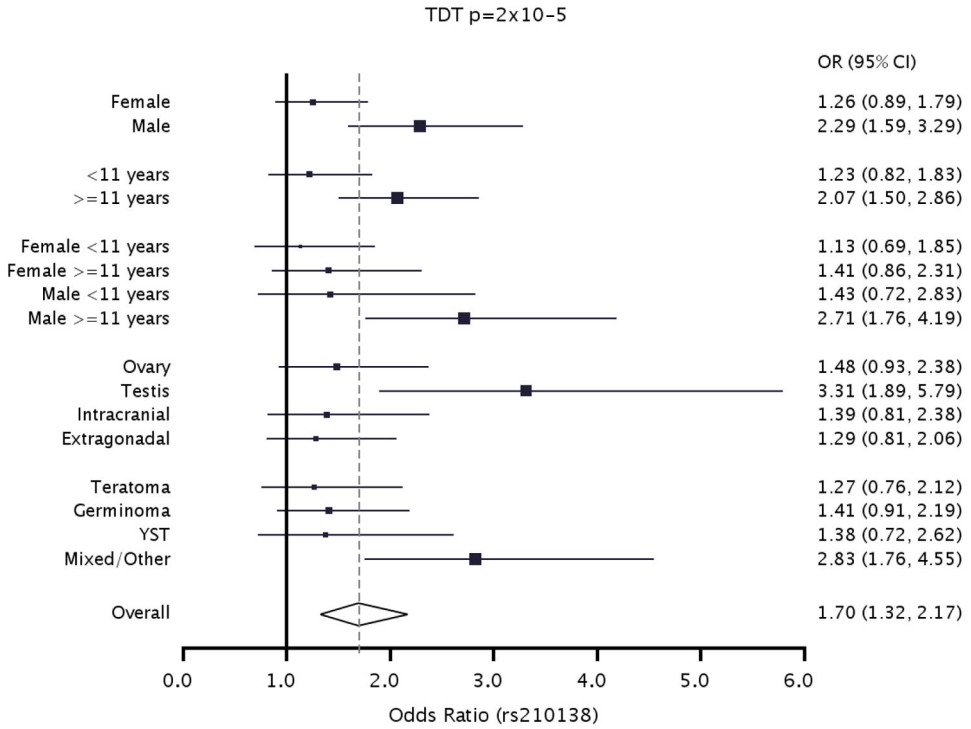

Figure 2.
Subgroup specific associations between rs210138 (*BAK1*) and GCT

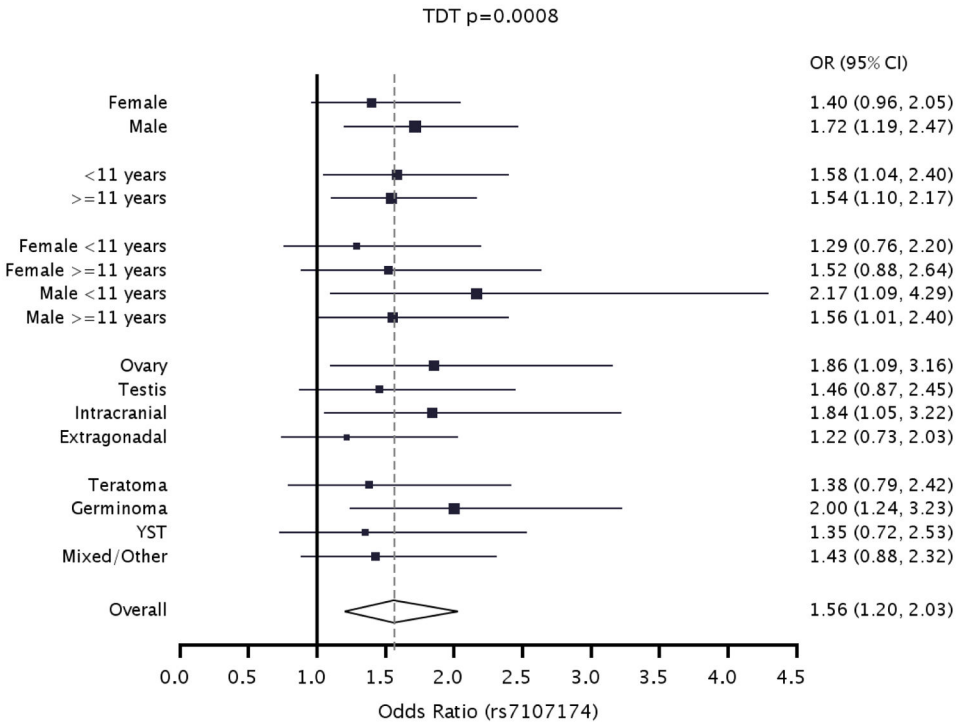

Figure 3.
Subgroup specific associations between rs7107174/rs948662 (*GAB2*) and GCT

Table 1

Demographic and tumor characteristics of study subjects included in TDT and EMIM analyses, respectively

	TDT analysis (direct effects)		EMIM analysis (maternal effects)	
	N	**%**	**N**	**%**
Sex				
Male	184	50.3	121	49.6
Female	182	49.7	123	50.4
Location				
extragonadal	102	27.9	65	26.6
intracranial	75	20.5	47	19.3
ovary	98	26.8	64	26.2
testis	91	24.9	68	27.9
Histology				
YST	61	16.7	41	16.8
germinoma	105	28.7	71	29.1
mixed/other	117	32.0	84	34.4
teratoma	82	22.4	48	19.7
Age at diagnosis				
< 11 years	149	40.7	104	42.6
11–19 years	217	59.3	140	57.4
Ethnicity				
African American	17	4.6	0	0.0
Asian	17	4.6	0	0.0
Hispanic	127	34.7	0	0.0
White	205	56.0	244	100.0
Sample structure				
Trio	366	100.0	205	84.0
Duo	0	0.0	39	16.0

Table 2

Direct effects of TGCT-associated variants on pediatric GCT risk

									All participants		non-Hispanic whites	
Marker Name	**Original GWAS marker**	**LD with GWAS marker (r^2)**	**Locus**	**Gene**	**Minor allele**	**Major allele**	**MAF**	**Risk allele**	**OR* (95% CI)**	**p-value**	**OR* (95% CI)**	**p-value**
rs2072499	rs2072499	1	1q22	SLC25A44	C	T	0.3326	C	1.21 (0.96, 1.51)	0.10	1.34 (1.00, 1.81)	0.051
rs3790672	rs3790672	1	1q24.1	UCK2	C	T	0.2883	C	1.01 (0.81, 1.25)	0.96	1.24 (0.93, 1.65)	0.14
rs3773832	rs10510452	0.986	3p24.3	DAZL	G	T	0.2908	T	0.92 (0.74, 1.15)	0.47	0.95 (0.70, 1.27)	0.71
rs8336	rs17021463	0.996	4q22.2	HPGDS	A	G	0.4614	G	0.93 (0.75, 1.15)	0.51	0.85 (0.64, 1.12)	0.25
rs2720460	rs2720460	1	4q24	CENPE	G	A	0.3714	G	1.10 (0.89, 1.36)	0.36	1.13 (0.85, 1.50)	0.39
rs2736100	rs2736100	1	5p15	TERT	T	G	0.5469		0.83 (0.68, 1.02)	0.081	1.45 (1.09, 1.91)	0.0094
rs4635969	rs4635969	1	5p15	TERT	T	C	0.1641	T	1.42 (1.09, 1.85)	0.0093	1.43 (1.01, 2.02)	0.044
rs474853	rs3805663	1	5q31.1	PITX1	G	A	0.3959	A	0.75 (0.60, 0.93)	0.0080	0.65 (0.48, 0.88)	0.0044
rs4624820	rs4624820 / rs6897876	1.0 / 0.942	5q31.1	SPRY4	G	A	0.4943	A	**0.70 (0.57, 0.86)**	**0.00074**	**0.62 (0.47, 0.82)**	**0.00063**
exm-rs210138	rs210138	1	6p21.31	BAK1	G	A	0.182	G	**1.70 (1.32, 2.18)**	**0.000020**	**1.82 (1.30, 2.57)**	**0.00047**
rs10275045	rs10275045	1	7p22.3	MAD1L1	T	C	0.4487	T	1.24 (1.01, 1.52)	0.042	1.20 (0.92, 1.59)	0.18
rs3778991	rs3778891	1	7p22.3	MAD1L1	A	G	0.3928	A	1.34 (1.09, 1.65)	0.0061	1.21 (0.92, 1.60)	0.18
exm-rs7040024	rs7040024	1	9p24.3	DMRT1	C	A	0.2373	A	0.74 (0.57, 0.96)	0.022	0.70 (0.49, 1.01)	0.056
rs7553831	rs755383	1	9p24.3	DMRT1	C	T	0.3527	T	0.87 (0.70, 1.09)	0.24	0.94 (0.69, 1.28)	0.70
rs948662	rs7107174	0.99	11q14.1	GAB2	C	T	0.1387	C	**1.56 (1.20, 2.03)**	**0.00083**	1.49 (1.04, 2.14)	0.030
exm-rs2900333	rs2900333	1	12p13	ATF7IP	T	C	0.3416	T	1.15 (0.92, 1.44)	0.21	1.17 (0.88, 1.54)	0.29
exm-rs995030	rs995030	1	12p22	KITLG	A	G	0.2286	G	0.80 (0.61, 1.05)	0.10	0.71 (0.50, 1.02)	0.060
exm-rs4474514	rs4474514	1	12p22	KITLG	G	A	0.2484	A	0.76 (0.59, 0.99)	0.041	0.65 (0.46, 0.91)	0.013
rs4493057	rs4561483	0.987	16q13.13	GSPT1	C	A	0.3655	A	0.81 (0.65, 1.02)	0.074	0.77 (0.58, 1.04)	0.086
rs8046148	rs8046148	1	16q12.1	MFSD1	A	G	0.2296	G	0.69 (0.53, 0.89)	0.0030	**0.50 (0.35, 0.72)**	**0.00015**
rs4888262	rs4888262	1	16q22.3	RFWD3	T	C	0.4631	T	1.07 (0.86, 1.31)	0.56	1.07 (0.81, 1.42)	0.62
rs7188539	rs55637647	0.82	16q24.2	ZFPM1	C	T	0.3468	T	0.94 (0.76, 1.15)	0.52	0.90 (0.68, 1.20)	0.48
exm-rs7501939	rs7501939	1	17q12	HNF1B	T	C	0.436	C	0.77 (0.62, 0.96)	0.018	0.73 (0.55, 0.97)	0.029
rs11656666	rs9905704	0.996	17q22	TEX14, RAD51C, PPM1E	G	A	0.3528	A	0.85 (0.68, 1.07)	0.18	0.85 (0.64, 1.14)	0.28

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								All participants		non-Hispanic whites	
Marker Name	**Original GWAS marker**	**LD with GWAS marker (r^2)**	**Locus**	**Gene**	**Minor allele**	**Major allele**	**MAF**	**Risk allele**	**OR* (95% CI)**	**p-value**	**OR* (95% CI)**	**p-value**
rs7221274	rs7221274	1	17q22	TEX14, RAD51C, PPM1E	G	A	0.4238	A	0.92 (0.75, 1.13)	0.42	0.95 (0.73, 1.25)	0.73
rs2250213	rs2839186	0.892	21q22.3	MCM3AP	A	G	0.4588	A	1.10 (0.89, 1.35)	0.37	1.05 (0.80, 1.39)	0.72

*Risk of GCT was calculated for carriers of the minor allele

Table 3

Maternal effects of TGCT-associated variants on offspring pediatric GCT risk

Marker Name	Original GWAS marker	LD with GWAS marker (r^2)	Locus	Gene	Minor allele	Major allele	MAF	Risk allele	OR* (95% CI)	p-value	Maternal parent-of-origin p-value	Paternal parent-of-origin p-value
rs2072499	rs2072499	1	1q22	SLC25A44	C	T	0.3326	T	0.93 (0.72, 1.22)	0.61	0.99	0.026
rs3790672	rs3790672	1	1q24.1	UCK2	C	T	0.2883	C	1.12 (0.85, 1.48)	0.43	0.34	0.60
rs3773832	rs10510452	0.986	3p24.3	DAZL	G	T	0.2908	T	0.94 (0.71, 1.25)	0.67	0.85	0.52
rs8336	rs17021463	0.996	4q22.2	HPGDS	A	G	0.4614	A	1.09 (0.84, 1.40)	0.53	0.63	0.39
rs2720460	rs2720460	1	4q24	CENPE	G	A	0.3714	A	0.86 (0.66, 1.12)	0.27	0.72	0.16
rs2736100	rs2736100	1	5p15	TERT	T	G	0.5469	T	1.09 (0.84, 1.40)	0.52	0.29	0.030
rs4635969	rs4635969	1	5p15	TERT	T	C	0.1641	C	0.94 (0.68, 1.29)	0.70	0.58	0.013
rs474853	rs3805663	1	5q31.1	PITX1	G	A	0.3959	G	1.10 (0.84, 1.43)	0.50	0.39	0.0031
rs4624820	rs4624820 / rs6897876	1.0 / 0.942	5q31.1	SPRY4	G	A	0.4943	G	1.04 (0.80, 1.35)	0.76	0.052	0.0072
exm-rs210138	rs210138	1	6p21.31	BAK1	G	A	0.182	G	1.22 (0.90, 1.65)	0.20	0.0016	0.15
rs10275045	rs10275045	1	7p22.3	MAD1L1	T	C	0.4487	T	1.05 (0.81, 1.35)	0.73	0.93	0.26
rs3778991	rs3778891	1	7p22.3	MAD1L1	A	G	0.3928	A	1.10 (0.85, 1.43)	0.47	0.63	0.58
exm-rs7040024	rs7040024	1	9p24.3	DMRT1	C	A	0.2373	A	0.99 (0.72, 1.35)	0.94	0.25	0.17
rs7553831	rs755383	1	9p24.3	DMRT1	C	T	0.3527	T	0.94 (0.72, 1.22)	0.63	1.0	0.39
rs948662	rs7107174	0.99	11q14.1	GAB2	C	T	0.1387	C	1.04 (0.75, 1.45)	0.81	0.099	0.056
exm-rs2900333	rs2900333	1	12p13	ATF7IP	T	C	0.3416	C	0.94 (0.72, 1.23)	0.65	0.85	0.11
exm-rs995030	rs995030	1	12p22	KITLG	A	G	0.2286	A	1.57 (1.14, 2.17)	0.0054	0.80	0.024
exm-rs4474514	rs4474514	1	12p22	KITLG	G	A	0.2484	G	**1.66 (1.21, 2.28)**	0.0015	0.60	0.0057

Marker Name	Original GWAS marker	LD with GWAS marker (r^2)	Locus	Gene	Minor allele	Major allele	MAF	Risk allele	OR* (95% CI)	p-value	Maternal parent-of-origin p-value	Paternal parent-of-origin p-value
rs4493057	rs4561483	0.987	16q13.13	GSPT1	C	A	0.3655	C	1.06 (0.81, 1.39)	0.67	0.39	0.11
rs8046148	rs8046148	1	16q12.1	MFSD1	A	G	0.2296	G	0.90 (0.65, 1.25)	0.53	0.027	0.062
rs4888262	rs4888262	1	16q22.3	RFWD3	T	C	0.4631	T	1.01 (0.78, 1.30)	0.93	0.65	0.66
rs7188539	rs55637647	0.82	16q24.2	ZFPM1	C	T	0.3468	T	0.97 (0.74, 1.26)	0.80	0.87	0.80
exm-rs7501939	rs7501939	1	17q12	HNF1B	T	C	0.436		1.00 (0.77, 1.29)	0.97	0.044	0.24
rs11656666	rs9905704	0.996	17q22	TEX14, RAD51C, PPM1E	G	A	0.3528	G	1.36 (1.04, 1.79)	0.026	0.25	0.0033
rs7221274	rs7221274	1	17q22	TEX14, RAD51C, PPM1E	G	A	0.4238	G	**1.63 (1.26, 2.12)**	0.00023	0.013	0.00019
rs2250213	rs2839186	0.896	21q22.3	MCM3AP	A	G	0.4588	G	0.80 (0.62, 1.03)	0.078	0.19	0.15

* Risk of GCT among offspring was calculated for carriers of the minor allele