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Large-scale Sequencing of Testicular Germ Cell Tumour (TGCT) Cases Excludes Major TGCT Predisposition Gene

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Abstract

Testicular germ cell tumour (TGCT), the most common cancer in young men, has a significant heritable basis that has long raised questions as to the existence of underlying major high-penetrance susceptibility gene(s). To determine the contribution of rare gene mutations to the inherited risk of TGCT, we analysed germline whole-exome data for 919 TGCT cases and 1609 cancer-free controls. We compared frequencies between TGCT cases and controls of rare (<1%) and low-frequency (1-5%) coding variants (1) individually and (2) collapsed at the gene level via burden testing (T1, disruptive; T2, all deleterious; and T3, all nonsynonymous) using Fisher's exact test with Bonferroni correction of significance thresholds. No individual variant or individual gene showed a significant association with TGCT after correction for multiple testing. In the largest whole-exome sequencing study of testicular cancer reported to date, our findings do not support the existence of a major high-penetrance TGCT susceptibility gene (of odds ratio >10 and allele frequency [combined] > 0.01%). Owing to its power, this study cannot exclude the existence of susceptibility genes responsible for occasional TGCT families or of rare mutations that confer very modest relative risks. In concert with findings from genome-wide association studies, our data support the notion that inherited susceptibility is largely polygenic with substantial contribution from common variation.

Patient summary: In the largest study of its kind, we sequenced ~20 000 genes in 919 men with testicular germ cell tumour (TGCT) and 1609 TGCT-free individuals and found no evidence of a single major gene underlying predisposition to TGCT (in the manner of *BRCA1* for breast cancer). Instead, familial risk of TGCT is likely to be due to varying dosages of hundreds of minor genetic factors.

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Testicular germ cell tumour (TGCT), the most common cancer affecting young men, has a strong heritable basis as evidenced by the four- to eight-fold higher risk of TGCT seen in brothers of TGCT patients [1,2]. This high heritability and the observation of multiplex TGCT families has long fuelled anticipation that there may exist a "major" TGCT susceptibility gene suitable for clinical testing, analogous to *BRCA1* or *BRCA2* in breast cancer. However, early genetic linkage studies proved unfruitful, although they were very much limited in power by the modest size and low frequency of multiplex TGCT families [3].

We previously reported whole-exome case-control segregation analysis of 150 TGCT families focusing on just gene-level analysis of rare (<1%) disruptive (truncating) mutations [4]. No gene was significant according to segregation analysis alone after correcting for exome-wide analysis. However, a range of functional analyses demonstrated an association between familial TGCT and the strongest candidate gene, *DNAAF1*, and related cilia-microtubule genes (CMGs). Nevertheless, mutations in each of those genes were infrequent and none would constitute a "major" TGCT susceptibility gene [4]. Here we present more comprehensive germline whole-exome sequencing analysis of the contribution of rare variants to TGCT susceptibility. We examined multiple different types of rare coding alleles (disruptive, damaging and otherwise) and present a primary analysis of the full cohort of 919 TGCT cases (comprising 613 unselected TGCT cases in addition to the previously reported 306 familial TGCT cases) in comparison to 1609 healthy controls (Supplementary material).

Collection of blood samples and clinical information from all subjects was undertaken after obtaining informed written consent and relevant ethical review board approval at the respective institutions. Case WES data have been deposited at the European Genome-phenome Archive, which is hosted by the European Bioinformatics Institute (accession number EGAS00001001789).

We first examined individual nonsynonymous coding variants, both rare (minor allele frequency [MAF] <1%) and low-frequency (MAF 1–5%), for association with TGCT. A total of 966 695 rare and 4994 low-frequency variants were detected, and a Bonferroni-corrected threshold of $p < 5 \times 10^{-8}$ (i.e. $p < 0.05/\sim 1$ million variants) was imposed. No variant showed an association with TGCT above this significance threshold (Table 1).

We next analysed rare nonsynonymous variants collapsed at the gene level and organised into three groups: T1, disruptive; T2, all deleterious; and T3, all nonsynonymous. A Bonferroni-corrected threshold of $p < 8 \times 10^{-7}$ (ie, $p < 0.05/[20\ 000\ \text{genes} \times 3\ \text{tiers}]$) was imposed. No gene was significant within any variant group at the exome-wide level (Table 2). We assessed the distribution of test statistics compared to a null model using quantile-quantile plots (Supplementary Fig. 1) and found that inflation statistics were in the range $\lambda = 0.75–1.0$, suggesting the data fitted a null distribution overall.

While TGCT has yet to be implicated in any established cancer susceptibility syndrome, it is feasible that association of TGCT with a cancer susceptibility gene (CSG) may have gone undetected owing to its rarity. Gene burden testing of 114 established high- or moderate-penetrance

Table 1 – Top 25 most significant individual variants

Gene	cDNA	Protein	Group	Case alleles			Control alleles			OR (95% CI)	p value*
				Alt no.	Total	MAF	Alt no.	Total	MAF		
PGP	c.859G>A	p.Gly287Arg	T3	98	1718	0.057	97	3088	0.031	1.87 (1.40–2.49)	3×10^{-5}
FAM160A2	c.2195C>T	p.Pro732Leu	T3	11	1692	0.007	55	2594	0.021	0.30 (0.16–0.58)	7×10^{-5}
MLXIP	c.1240G>A	p.Asp414Asn	T3	84	1786	0.047	71	2844	0.025	1.93 (1.40–2.66)	7×10^{-5}
OR1N2	c.709C>T	p.Arg237Cys	T3	34	1846	0.018	20	3162	0.006	2.95 (1.69–5.14)	1×10^{-4}
OR10C1	c.169C>T	p.Pro57Ser	T2	11	1846	0.006	1	3138	0.000	18.8 (2.43–145)	1×10^{-4}
PLK1	c.1388T>A	p.Leu463His	T3	37	1782	0.021	24	3058	0.008	2.68 (1.60–4.50)	2×10^{-4}
ADAMTS18	c.3565G>A	p.Val1189Ile	T3	2	1706	0.001	27	2732	0.010	0.12 (0.03–0.50)	2×10^{-4}
EPB41L5	c.82C>T	p.Arg28Cys	T2	18	1864	0.010	6	3126	0.002	5.07 (2.01–12.8)	2×10^{-4}
JMJD4	c.1024T>C	p.Phe342Leu	T3	103	1848	0.056	101	3036	0.033	1.72 (1.30–2.27)	2×10^{-4}
SKIV2L	c.2749G>A	p.Val917Met	T2	118	1778	0.066	127	3044	0.042	1.63 (1.26–2.11)	2×10^{-4}
ARHGEF17	c.1571C>T	p.Ala524Val	T3	12	1806	0.007	2	3000	0.001	10.0 (2.24–44.8)	3×10^{-4}
SH3TC1	c.2429C>T	p.Thr810Met	T3	2	1640	0.001	27	2702	0.010	0.12 (0.03–0.51)	3×10^{-4}
MPDZ	c.2194T>A	p.Ser732Thr	T2	10	1656	0.006	1	2840	0.000	17.3 (2.21–134)	3×10^{-4}
PALB2	c.2014G>C	p.Glu672Gln	T3	80	1662	0.048	85	3066	0.028	1.77 (1.30–2.42)	3×10^{-4}
EHBP1L1	c.2683A>T	p.Ser895Cys	T2	15	1836	0.008	4	3152	0.001	6.48 (2.15–19.6)	4×10^{-4}
ABCC4	c.1141G>A	p.Val381Ile	T3	11	1676	0.007	2	3060	0.001	10.1 (2.24–45.6)	4×10^{-4}
R3HCC1	c.500C>T	p.Thr167Ile	T2	36	1798	0.020	111	2906	0.038	0.51 (0.35–0.75)	4×10^{-4}
VPS16	c.1561G>A	p.Asp521Asn	T3	10	1802	0.006	1	2970	0.000	16.6 (2.12–129)	4×10^{-4}
P2RX7	c.827G>A	p.Arg276His	T3	56	1792	0.031	50	3160	0.016	2.01 (1.36–2.95)	4×10^{-4}
OR1N1	c.680G>A	p.Arg227Gln	T3	33	1800	0.018	22	3112	0.007	2.62 (1.52–4.51)	5×10^{-4}
DEFB132	c.277G>A	p.Val93Ile	T3	37	1832	0.020	27	3166	0.009	2.40 (1.45–3.95)	6×10^{-4}
ALPK1	c.2042G>A	p.Gly681Asp	T3	47	1870	0.025	38	3164	0.012	2.12 (1.38–3.27)	6×10^{-4}
IYD	c.794G>A	p.Cys265Tyr	T3	78	1824	0.043	77	3108	0.025	1.76 (1.28–2.42)	7×10^{-4}
ZYG11A	c.1027A>G	p.Met343Val	T3	15	1846	0.008	5	3154	0.002	5.16 (1.87–14.2)	7×10^{-4}
POLI	c.1595T>C	p.Phe532Ser	T2	78	1816	0.043	79	3164	0.025	1.75 (1.27–2.41)	7×10^{-4}

MAF = minor allele frequency; OR = odds ratio; CI = confidence interval.
* Bonferroni-corrected threshold of $p\ 5 \times 10^{8}$ for significance.

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