SUPPLEMENTARY MATERIALS

1. SUPPLEMENTARY METHODS

Subjects and Datasets

TGCT cases (n=962) were ascertained from 'The UK Genetics of Testicular Cancer Study' and 'Identification, Epidemiologic and Molecular analyses of families with susceptibility to Testicular Germ Cell Tumour' (**Supplementary Notes 1 and 2**), both coordinated by The Institute of Cancer Research (ICR). All cases had self-reported European ancestry. WES was undertaken in 328 individuals from multiplex TGCT families (289 from the UK, 39 of non-UK Western European ancestry) and 634 unselected cases from the UK (EGAS00001001789). Follow-up genotyping of *BOLL* c.62C>A;p.Ser21Tyr was undertaken in an additional 3,999 unselected cases from the UK.

Written informed consent was obtained from all cases with ethical review board approval (UK National Cancer Research Network Multi-Research Ethics Committee - MREC02/06/66, 06/MRE06/41). This study was conducted in accordance with the declaration of Helsinki.

The controls (n=1644) comprised healthy individuals from the UK 1958 Birth Cohort [1] - 974 from the ICR1000 dataset (EGAS00001000971) and 670 individuals (EGAS00001001667) sequenced at ICR under the same protocol. Follow-up genotyping of *BOLL* c.62C>A;p.Ser21Tyr was undertaken in 4011 controls from two studies; the national study of colorectal cancer genetics (NSCGG) [2] and Genetic Lung Cancer Predisposition Study (GELCAPS) [3]. NSCCG and GELCAP controls were spouses of cancer patients with no personal history of cancer at time of ascertainment.

The subjects/datasets presented in the current study are inclusive of those presented in Litchfield et al. 2016 [4].

Whole exome sequencing

One microgram DNA per individual was fragmented using a Covaris E Series instrument (Covaris Inc. Woburn, Massachusetts, USA). Indexed paired-end libraries were prepared using Illumina TruSeq 62 Mb expanded exome kit (Illumina, San Diego, CA, USA). Forty-nine samples with insufficient input DNA were prepared using Illumina Nextera Rapid Capture 37 Mb exome kit. Sequencing (2x100 bp) was performed using Illumina HiSeq2000 or 2500 technology.

Read mapping and sample QC

Paired-end fastq files were extracted using CASAVA (v.1.8.1, Illumina) and aligned to GRCh37 using Stampy/BWA [5]. Alignments were processed using Genome Analysis Tool Kit (GATK) pipeline (v3) according to best practice [[6],[7]]. Analysis was restricted to the regions in the Truseq 62 Mb bed file (+100 bp padding). Variant Effect Predictor (VEP) was used to provide

transcript-level annotations. Additional annotations included alignability of 100-mers and distance from simple repeats defined by UCSC browser tracks. Mean coverage of 49X was achieved across targeted bases with 83% covered at ≥15X; cases/controls had similar technical sequencing metrics. We excluded 57 subjects (43 TGCT cases, 14 controls) with low-quality data (<50% of bases covered at 15X), or non-European ancestry, as determined from post hoc principal component analysis of sequencing data. Twenty-one controls were excluded due to sex discrepancy or subsequent detection of cancer history.

Variant and gene level quality control

We considered only canonical transcripts, assuming the most deleterious VEP prediction. We imposed GATK internal calling thresholds as per best practice [[6],[7]]: in the 99.5th truth tranche for single nucleotide variants; > 99th tranche for insertion-deletion (indel) variants. Additional filters for inclusion included: genotype quality (GQ) ≥30; for heterozygous calls, alternate depth ≥ 3; χ^2 < 10.83 (*i.e.* p > 0.0001) for observed versus expected alternate:reference alleles (alt-ref-ratio); UCSC alignability =1 (100 bp window size); not in simple repeat or segmental duplication region; Hardy-Weinberg Equilibrium (HWE) test ($p > 1 \times 10^{-8}$); an overall case-control call rate ≥ 75%. Gene level filters removed genes containing only indel variation or genes overlapping with regions of segmental duplication. To ensure a high-quality variant set, reproducibility was verified for a subset of samples (n=162) also genotyped using Illumina HumanCNV370-Duo bead arrays: > 99% concordance was observed between platforms.

Post-QC, the final dataset comprised 919 TGCT cases, including 306 familial cases (from 150 independent pedigrees) and 613 unselected, and 1,609 controls. An average of 7,126/7,181 non-synonymous variants were identified per case/control sample, consisting of: 5,862/5,877 missense, 1,024/1,058 splice region, 31/31 nonsense, 19/21 splice acceptor, 16/18 splice donor and 74/75 frameshift indel, and 98/100 in-frame indel variants.

Genotyping data

Replication genotyping of *BOLL* c.62C>A;p.Ser21Tyr using KASPar allele-specific primers and analysis of *PALB2* c.2014G>C; p.Glu672Gln in TGCT OncoArray data were performed as previously described [8].

Statistical analyses

Individual variant analyses were performed using a Fisher's Exact Test (FET) comparing allele frequencies between all cases (i.e. sporadic + familial) versus controls, testing both rare (minor allele frequency (MAF) < 1%) and low-frequency (MAF 1-5%) variants for association with TGCT risk. For gene level analyses, all variants were collapsed into three groups: Tier 1 (T1) variants – disruptive mutations (nonsense, frameshift and splice acceptor/donor); Tier 2 (T2) – deleterious mutations (disruptive plus damaging missense variants, as predicted by *in-silico* tool CONDEL [[9],[10]]); and Tier 3 (T3) – all non-synonymous changes. Case-control gene-based variant counts were computed for each. We imposed a maximal MAF threshold of 1%, limiting counts to one individual per pedigree. Gene-level analyses were performed using a FET comparing the proportions of cases versus controls with a variant within a specified group. To account for multiple testing, Bonferroni corrections were applied to significance thresholds.

We modelled the existence of a TGCT predisposition gene with mutant allele frequency combined across pathogenic mutations in the gene ($MAF_{combined}$). Study power to detect was calculated using $MAF_{combined}$ in controls as baseline, while $MAF_{combined}$ in cases was determined by a weighted average of the enrichment found in cases with one, two and three affected first-degree relatives. $MAF_{combined}$ frequencies were then sampled from values between 0.00001 and 0.01 and odds ratios (ORs) between 2 and 10. A FET was then performed for each sampling of cases and controls. This process was performed 10,000 times for all combinations, with frequency of tests surpassing exome-wide significance ($p < 8.0 \times 10^{-7}$) equated to study power. Analyses were implemented in R3.0.2 and Stata (v12) (StataCorp, Lakeway Drive College Station, Texas, USA).

2. SUPPLEMENTARY TABLES

Supplementary Table 1. Collapsed burden testing for 114 cancer susceptibility genes

[Supplementary_Table_1.xlsx]

Supplementary Table 2. Collapsed burden testing for 64 TGCT GWAS loci genes

[Supplementary_Table_2.xlsx]

Supplementary Table 3. Replication genotyping of variant segregating in 4 case pedigree (PED-269)

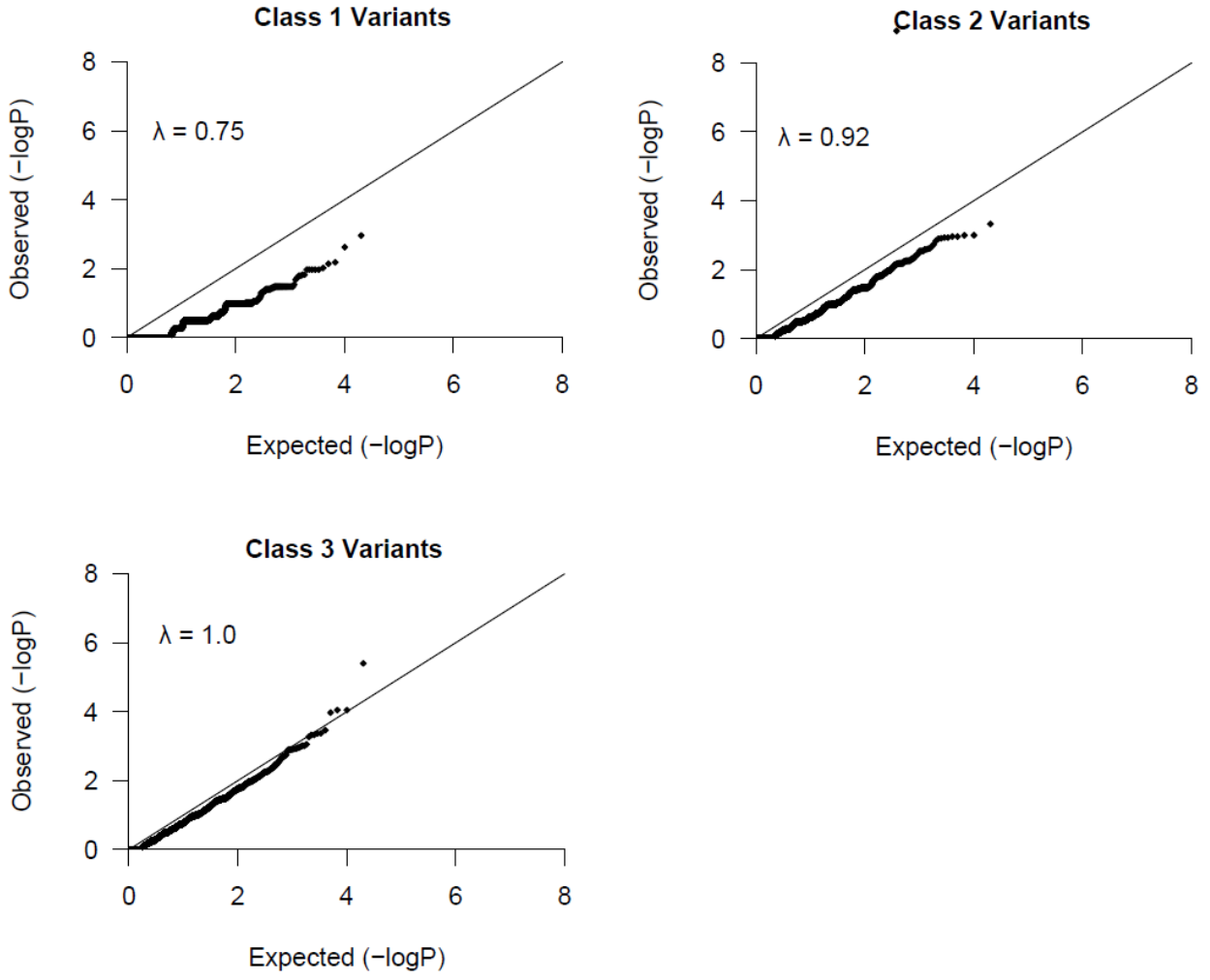
				Case Allele Counts			Control Allele Counts				
Gene	**cDNA**	**Protein**	**Group**	**Alt**	**Total**	**MAF**	**Alt**	**Total**	**MAF**	**OR (95% CI)**	***P* value**
BOLL	c.62C>A	p.Ser21Tyr	3	10	3999	0.03	14	4011	0.04	0.72 (0.32-1.61)	5.4×10^{-1}

Supplementary Table 4. DNAH7 segregating variants in 4 case pedigree (PED-269)

				Pedigrees with full segregation		
Gene	**cDNA**	**Protein**	**Group**	**≥ 3 case (n=12)**	**2 cases (n=138)**	**Total (n=150)**

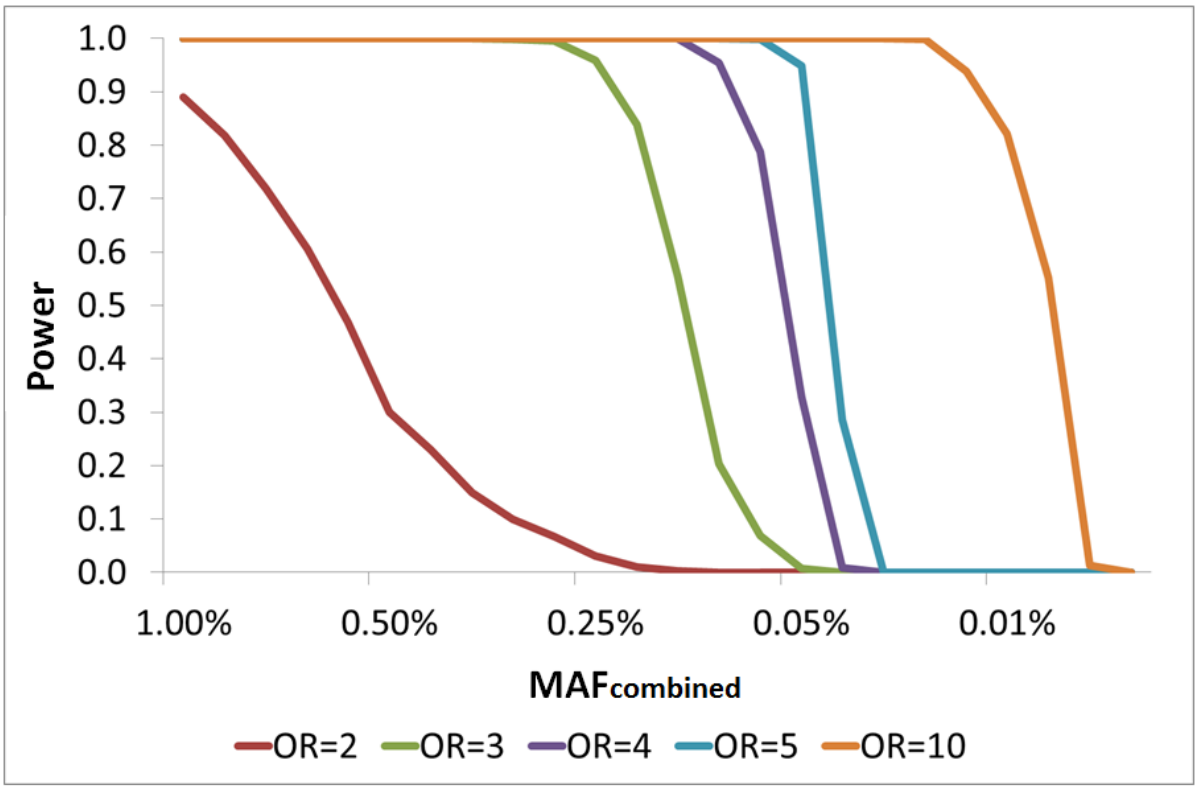
DNAH7	c.1895C>G	p.Ser632Cys	3	2	8	10
DNAH7	c.6340A>G	p.Thr2114Ala	3	2	8	10

3. SUPPLEMENTARY FIGURES



Supplementary Figure 1. Quantile-quantile plots. The negative logarithm of observed (y-axis) versus expected (x-axis) *p* values plotted for each gene (dot) for variants collapsed into three different groups: Class 1 (T1) variants – disruptive mutations (nonsense, frameshift and splice acceptor/donor); Class 2 (T2) – deleterious mutations (disruptive plus damaging missense

variants, as predicted by *in-silico* tool CONDEL [[9],[10]]); and Class 3 (T3) – all non-synonymous changes.



Supplementary Figure 2. Power to detect. Simulations showing power to detect (y-axis) a hypothetical TGCT predisposition gene of different combined allele frequencies (MAFcombined; x-axis) and different effect sizes (coloured lines) in the present study.

4. SUPPLEMENTARY REFERENCES

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5. SUPPLEMENTARY NOTES

Supplementary Note 1. The UK Testicular Cancer Collaboration (UKTCC)

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Supplementary Note 2. International Testicular Cancer Linkage Consortium (ITCLC) centres from which samples were used in this study

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